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Specific κ opioid receptor agonists

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Abstract

The results of studies on the design of a heterocyclic scaffold for the dynorphin A pharmacophore and on structure–affinity relationships in the MPCB/CCB series are described. The representative ligands provide insights to binding modes of benzomorphan derivatives to the κ opioid receptor.

Keywords: Kappa opioid agonists; (−)-*cis*-*N*-Normetazocine; Peptidomimetics; Kappa opioid receptor

1. Introduction

The biological effects of opioid analgesics are mediated through three different types of opioid receptor, namely μ, κ and δ. Recently, several groups of researchers have reported their molecular cloning, and thus the cloned receptors have allowed studies of individual opioid receptor types with regard to pharmacological profile, structure–function analysis and anatomical distribution [1]. Opioids are clinically used in the management of pain, but their use is limited by a number of undesirable side effects. The use of κ selective agonists involves a low abuse and milder form of dependence in comparison with the prototypic μ opioid ligands [2]. Dynorphin A (H–Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Ile–Arg–Pro–Lys–Leu–Lys–Trp–Asp–Asn–Gln–OH), the principal endogenous ligand of the κ opioid receptor [3], and its shorter homologues, dynorphin A(1–8) and dynorphin A(1–13), while having a high affinity for both natural and cloned κ opioid receptors, also show a modest κ selectivity [4]. Synthetic selective κ agonists belong to the chemical classes of benzomorphans (i.e. ethylketocyclazocine, bremazocine, MPCB and CCB) [5,6] and arylacetamides (i.e. U50,488, U69,593) [2]. Dynorphin A analogues are synthesised in attempts to introduce structural modifications useful for enhanced selectivity for the κ opioid receptor. Recent studies based on κ/δ opioid chimeric receptors showed that κ peptide ligands have multiple ligand-binding domains and that the extracellular regions play an important role in the selective interaction of dynorphin A with the κ opioid receptor [7]. This implies that the design of a peptidomimetic of dynorphin A should benefit from the presence of the 'message' sequence and, at the same time, from the essential chemical fragments of the C-terminal region.

2. Drug design and results

In the last few years we designed and synthesised MPCB [5] and its *p*-chlorophenyl analogue (CCB) [6], the first (−)-*cis*-*N*-normetazocine agonist derivatives with high affinity and specificity for the κ opioid receptor (Fig. 1). Our proposed idea is that the cyclopropylmethyl-normetazocine (CPM) nucleus represents a scaffold able to support the κ pharmacophoric elements (phenol ring, basic nitrogen, carbonyl group and phenyl ring) in a suitable conformation, which could mimic the 'message' fragment of dynorphin A. The subsequent chemical modifications have regarded the possibility of developing a peptidomimetic of dynorphin A. Then, we synthesised a series of hybrid ligands of MPCB and CCB, in which the CPM nucleus was linked to various C-terminal fragments of dynorphin A(1–8) [8]. MPCB–GRRI and MPCB–RRI compounds (Fig. 1) showed high affinities to both cloned and native κ

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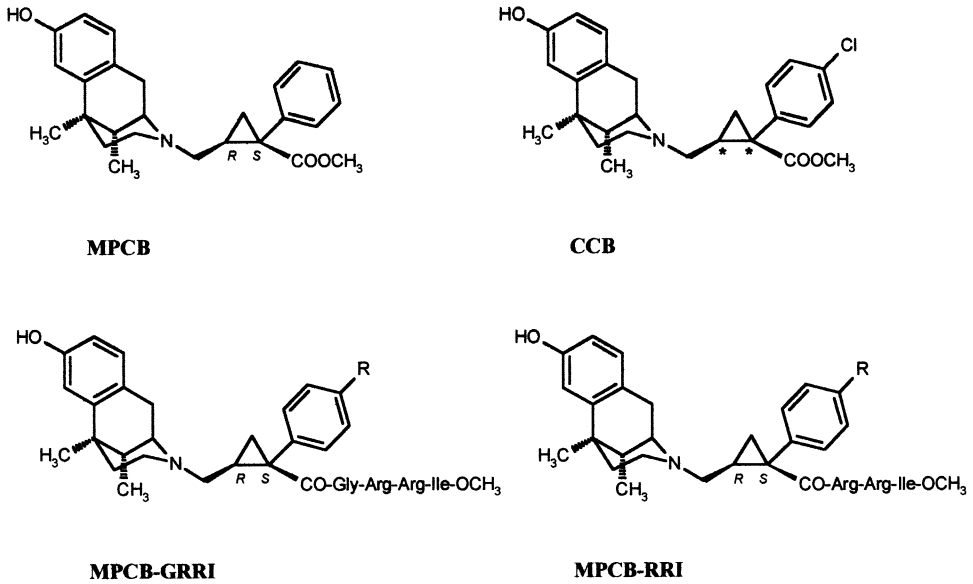


Fig. 1. MPCB, CCB and hybrid compounds.

opioid receptors, further supporting the hypothesis that MPCB and CCB are appropriate mimetics of the *N*-terminal fragment of dynorphin A. Moreover, hybrid compounds could be considered mimetic of dynorphin A(1–8) and confirmed that the peptide sequence represents the 'address' fragment of selective endogenous ligands.

Recently, we designed and synthesised two novel series of analogues of MPCB–GRRI and MPCB–RRI with non-peptide replacement at the C-terminus [9]. The compounds MPCB–Gly–Leu–NH–$(CH_2)_n$–NH–C(=NH)–C_4H_9 ($n = 5, 6$) (D5AB and D6AB, respectively) showed high affinity and selectivity for κ opioid receptors (Fig. 2).

The binding affinities of all compounds synthesised in comparison with those of standard compounds (U50,488 and dynorphin A(1–8)) are reported in Table 1.

The compounds derived from non-peptide ligands MPCB and CCB, i.e. hybrid compounds MPCB–GRRI and MPCB–RRI and compounds D5AB and D6AB, all have μ/κ and δ/κ selectivity ratios higher than those showed by κ endogenous ligands. The D5AB and D6AB compounds have an agonist profile with $ED_{50} = 0.88$ and 1.1 mg/kg, respectively, in the mouse abdominal constriction test after subcutaneous administration. These data confirm the idea that these molecules might interact with κ opioid receptors in a very close way to that of endogenous ligands. Finally, contrary to other benzomorphan and arylacetamide derivatives, all compounds showed negligible affinity for σ_1 sites and did not induce psychotomimetic effects by interacting with binding sites other than opioid receptors.

Therefore, the aim of more recent research has been to verify the possibility that the phenol functionality present in the (−)-*cis*-*N*-normetazocine nucleus might not be absolutely necessary for an efficient binding of these analogues of dynorphin A(1–8) to the κ opioid receptor. It is evident that, in the class of arylacetamide compounds, the phenol hydroxyl is not necessary for high κ opioid affinity, whereas in the benzomorphan series this group is beneficial. Since we have proposed that a similar docking in the κ opioid receptor of the benzomorphans and arylacetamides [10] should be possible, we synthesised two novel analogues of dynorphin A(1–8) (**1** and **2**) lacking this functionality (Fig. 3). In binding assays these compounds have been shown to maintain relevant κ affinity and selectivity. Thus, with respect to other benzomorphan derivatives, this has permitted us to demonstrate that the additional binding site of their 'address' fragment with the extracellular regions of the κ opioid receptor could be beneficial in the docking of these ligands.

In order to obtain some insight on the structure of MPCB and CCB, we synthesised deoxy-CCB, (*S*,*R*)-CCB and (*R*,*S*)-CCB (Fig. 4). Binding assays demon-

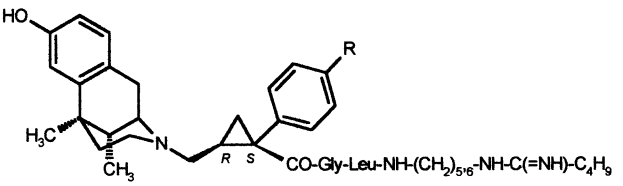


Fig. 2. D5AB and D6AB.

Table 1
Binding data[a] of the synthesised and standard compounds

Compound	$Ki \pm$ SEM (nM)[b,c]			Selectivity ratios	
	κ	μ	δ	μ/κ	δ/κ
MPCB[d]	240 ± 39	>25 000	>25 000	nc	nc
CCB[e]	0.41 ± 0.19	>25 000	>25 000	nc	nc
MPCB–GRRI[f]	54.3 ± 8.5	2300 ± 356	>25 000	42.36	nc
MPCB–RRI[f]	78.4 ± 6.4	4600 ± 817	>25 000	58.67	nc
D5AB[g]	6.7 ± 0.4	2530 ± 136.6	2750 ± 145.7	375.0	407.8
D6AB[g]	5.3 ± 0.3	2150 ± 111.8	2230 ± 113.7	408.0	423.8
U50,488[g]	5.01 ± 0.3	716 ± 37.9	8100 ± 162.0	142.9	1616
Dyn A(1–8)[g]	123.8 ± 6.2	1350 ± 71.5	240.7 ± 12.5	10.9	1.9

[a] nc: not calculated.
[b] Values represent the mean of three separate experiments each carried out in duplicate.
[c] K_i values were obtained as [^{3}H]U69,593 displacement for κ receptor and [^{3}H]Diprenorphine displacement for μ and δ receptors.
[d] Ronsisvalle et al. [5].
[e] Ronsisvalle et al. [6].
[f] Ronsisvalle et al. [8].
[g] Ronsisvalle et al. [9].

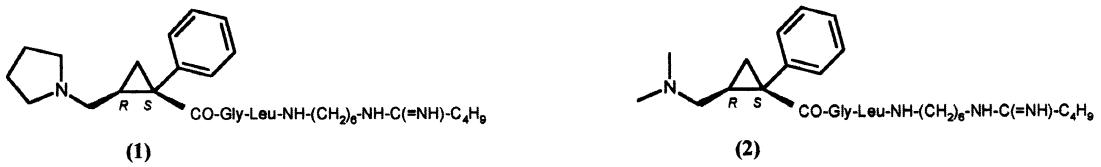


Fig. 3. No-benzomorphan analogues of dynorphin A(1–8).

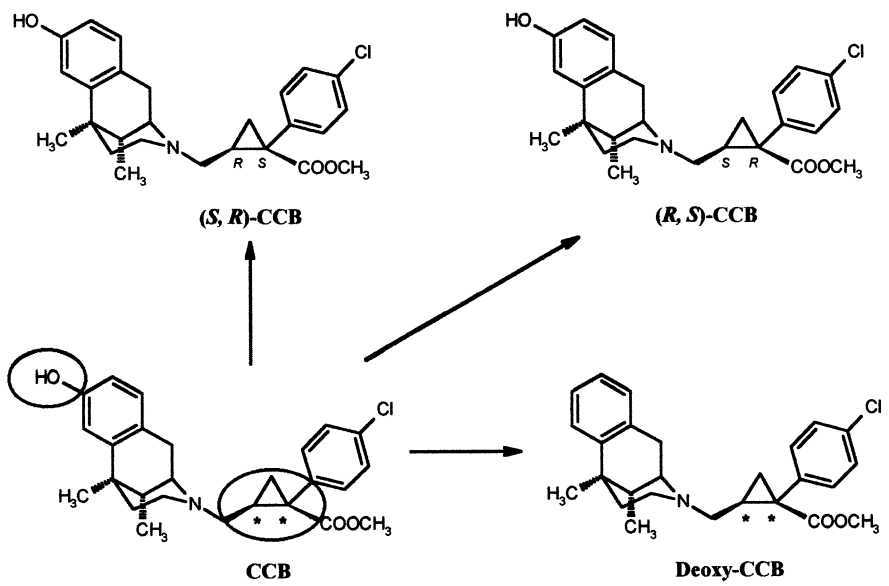
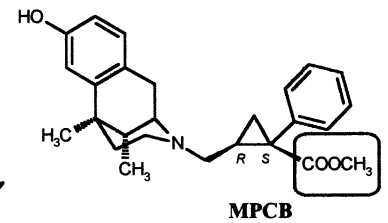


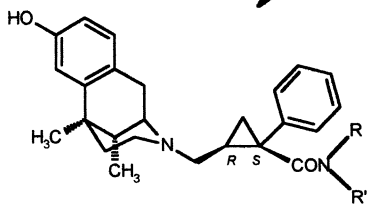
Fig. 4. Analogues of CCB.

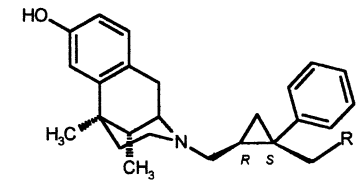
strated that removing phenol hydroxyl in the deoxy-CCB compound resulted in a complete loss of κ affinity, whereas the separation of the diasteroisomeric mixture of CCB showed a κ affinity profile of (*S*,*R*)-CCB and (*R*,*S*)-CCB similar to that of the diasteroisomers of MPCB. In the receptor–ligand interaction the benzene ring probably cannot allocate in the domain occupied by the phenol ring, preventing the ammonium moiety from forming a salt bridge with the Asp138 carboxylate group.

A number of analogues of MPCB were synthesised in order to develop structure–affinity relationships



MPCB





R	R'	Compounds
H	H	3
H	CH_3	4
CH_3	CH_3	5
H	C_2H_5	6
C_2H_5	C_2H_5	7
$-(CH_2)_4-$		8
$-(CH_2)_5-$		9
$-(CH_2)_2-O-(CH_2)_2-$		10
H	C_6H_5	11
H	$CH_2-C_6H_5$	12

R	Compounds
Pyrrolidin-1-yl	13
Piperidin-1-yl	14
Morpholin-4-yl	15
OH	16
$OCOCH_3$	17

Fig. 5. Analogues of MPCB.

(SARs) in this series of compounds with the aim to optimise the receptor–ligand interaction and, then, to improve the κ opioid receptor binding. By replacing opportunely the ester group we synthesised amides (**3**–**12**), amines (**13**–**15**), alcohol (**16**) and reversed ester (**17**) (Fig. 5). The introduction of amide groups was detrimental except for the benzylamide derivative, whereas the amine and reversed ester substitutions led to maintain significant κ binding affinity [11].

3. Conclusions

The present studies have identified the functionalities capable of mimicking relevant groups of the C-terminal region of dynorphin A(1–8) and have provided the basis to improve the design of a heterocyclic scaffold for the dynorphin A pharmacophore. Moreover, synthetic ligands, lacking in the benzomorphan nucleus, probably activate the receptor by a two-step mechanism as proposed by Coward et al. [7]. With respect to MPCB/CCB SARs, the deoxy-CCB compound reinforced the importance of the OH group in the morphine congeners, showing that the hydroxyl group could have a pivotal role in this series. The separation of the two diastereoisomers confirmed the importance of the stereochemistry on the cyclopropylmethyl group in the interaction with κ opioid receptor. The good κ affinities of reversed ester and amine compounds confirmed the hypothesis that the H-bonding group is a crucial feature in the interaction between ligand and κ receptor.

Acknowledgements

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