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∇^EPhe^4-enkephalin analogs

Delta receptors in rat brain are different from those in mouse vas deferens

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Conformationally restricted enkephalin analogs containing *E*-cyclopropylphenylalanine (∇^EPhe), [D-Ala^2, (2*R*,3*S*)-∇^EPhe^4,Leu^5]enkephalin and its (2*S*,3*R*) isomer, were evaluated in receptor-binding assays using rat brain and in assays using muscle preparations. The (2*S*,3*R*) isomer was almost completely inactive in all assays. In contrast, the (2*R*,3*S*) isomer showed a very high affinity for the δ and a very weak affinity for the μ receptors in rat brain . The extent of δ affinity and the selectivity of this isomer were almost equal to those of [D-Pen^2,D-Pen^5]enkephalin. However, the (2*R*,3*S*) isomer was inactive in both the mouse vas deferens and guinea pig ileum assays, and showed no antagonistic activity in these tissues. These results indicate that the (2*R*,3*S*) isomer interacts with the δ receptors in rat brain, but not with those in the mouse vas deferens, and they suggest that the δ receptors in the central and peripheral nervous systems are different from each other.

Enkephalin analog; Conformational restriction, Opiate receptor; Receptor heterogeneity

1. INTRODUCTION

The incorporation of conformational constraints into the opioid peptide enkephalins has been recognized as a useful structural modification to assess the stereochemical influence on the resulting biological profiles [1]. For example, simple structural modification of an amino acid residue in a peptide sequence can elicit physicochemical and conformational changes such as increased rigidity and hydrophobicity, restricted orientation of side chains, and possible induction of folding. The usefulness of such local conformational restriction has been well demonstrated by the synthesis of the *Z* and *E* isomers of dehydrophenylalanine^4-enkephalins [2,3]. This line of study has also strongly suggested that receptor-specific and very selective enkephalin analogs can be designed to elucidate structure-activity relationships based on the existence of multiple opioid peptides and opiate receptors [1]. The presence of δ and μ opiate receptors in brain has been demonstrated by examining the independent binding affinities using specific radiolabeled ligands [4,5]. Also, the heterogeneity of opiate receptors was confirmed in the peripheral tissues [6]; i.e. the mouse vas deferens (MVD) contains predominantly δ receptors, and the guinea pig ileum (GPI) contains μ receptors.

1-Aminocyclopropanecarboxylic acids, namely, cyclopropylamino acids (∇AA: the inverted triangle, ∇, is used to designate 'cyclopropyl', and superscript, ∇^E, indicates the configuration about the cyclopropane ring), are structurally constrain-

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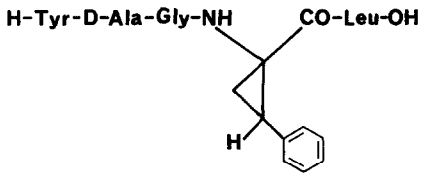


Fig.1. Chemical structure of [D-Ala^2, ∇^EPhe^4,Leu^5]-enkephalin.

ed amino acids. The orientation of the β-substituents in ∇AA is restricted rigidly to only two possible values (0 and 120°) of the χ_1 angle, corresponding to the Z and E configurations, respectively. Incorporation of ∇AA into a peptide chain will effectively constrain its conformation due to the small ϕ and ψ angles at ∇AA conformational energy minima. We have previously reported the synthesis of enkephalin analogs containing cyclopropylphenylalanine (∇Phe) in the E-(2R,3S) and E-(2S,3R) configurations (fig.1); namely, [D-Ala^2,(2R,3S)-∇^EPhe^4,Leu^5]- and [D-Ala^2,(2S,3R)-∇^EPhe^4,Leu^5]enkephalins [7]. It was expected that the incorporation of ∇^EPhe into enkephalin would fix several of its conformational parameters thereby restricting severely its molecular shape and allowing it to recognize and bind selectively to one of the multiple opiate receptors. Unfortunately, these analogs were completely inactive in both the MVD and GPI biological assays [7]. Surprisingly, however, in a preliminary binding assay using rat brain membranes, we have found that the (2R,3S) isomer exhibits a high δ receptor affinity with a very high selectivity. Here, we thus wish to report in detail the receptor-binding results of these ∇^EPhe^4-enkephalins in rat brain, and the results of biological assays for the evaluation of possible antagonistic activity in MVD and GPI.

2. MATERIALS AND METHODS

2.1. *Peptides*

The synthesis of [D-Ala^2,(2R,3S)- or (2S,3R)-∇^EPhe^4,Leu^5]enkephalin was reported previously [7,8]. [D-Ala^2,D-Leu^5]enkephalin (DADLE) and [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAGO) were purchased from Sigma (St. Louis, MO). [D-Pen^2,D-Pen^5]enkephalin (DPDPE) was purchased from Bachem (Bubendorf, Switzerland) and was utilized only for the δ receptors in both binding and biological assays because of its extremely low affinity for the μ receptors.

2.2. *Receptor-binding assays*

Receptor-binding assays using rat brain membrane preparations were carried out essentially as in [9]. [^{3}H]DADLE (41.8 Ci/mmol, Amersham) and [^{3}H]DAGO (42 Ci/mmol, Amersham) were used as tracers specific for the δ and μ receptors, respectively, at a final concentration of 0.25 nM. [^{3}H]DPDPE (35 Ci/mmol) was obtained from Amersham. Incubations were carried out for 60 min at 25°C in 50 mM Tris-HCl buffer (pH 7.4) containing bacitracin (100 μg/ml) as an enzyme inhibitor.

2.3. *Biological assays*

Biological assays using the electrically stimulated smooth muscle preparations of MVD and GPI were performed essentially as in [10]. Standard compounds (DADLE for MVD, and normorphine for GPI) were assayed in each preparation to permit estimation of relative potencies.

In order to examine the effectiveness of (2R,3S)-∇^EPhe^4-enkephalin (0.01–1 μM) as an antagonist against DADLE in MVD and normorphine in GPI, the assay was carried out essentially as described in [11].

3. RESULTS AND DISCUSSION

The (2R,3S) and (2S,3R) isomers of [D-Ala^2, ∇^EPhe^4,Leu^5]enkephalin were firstly re-examined for their inhibitory effects on the electrically stimulated contractions of MVD and GPI using DADLE as a standard (table 1). Both isomers were found to be almost completely inactive as reported in [7]. When they were evaluated in the radioligand-binding assays using rat brain membrane preparations, the (2S,3R) isomer, as in the biological assays, showed no activity, but the (2R,3S) isomer exhibited a very high affinity for the δ receptors (K_i = 13 nM) using [^{3}H]DADLE as tracer (table 1), although it was almost 10-fold less active than DADLE. Since the affinity of this isomer for the μ receptors was extremely weak (K_i = 3290 nM) using [^{3}H]DAGO as tracer (table 1),

Table 1

Biological activities of enkephalin analogs in the mouse vas deferens (MVD) and guinea pig ileum (GPI) assays, and receptor-binding affinities in rat brain

	Biological assays IC_{50} (nM)		Binding assays K_i (nM)	
	MVD	GPI	[^{3}H]DADLE	[^{3}H]DAGO
(2*R*,3*S*) isomer	2000	3700	13.0	3290
(2*S*,3*R*) isomer	5800	14000	2560	1960
DADLE	0.51	46.1	1.37	12.9
DPDPE	3.78 (2.19)[b]	ND[a] (6930)[b]	10.7	ND[a]

[a] Not determined
[b] Values from [12]

the receptor selectivity ratio, K_i([^{3}H]DAGO)/K_i([^{3}H]DADLE), was calculated to be about 250. This ratio is very high as compared with that of the δ ligand DADLE (ratio = 9.5, calculated from table 1). It is also of note that the selectivity of the (2*R*,3*S*) isomer for the δ receptors in rat brain is almost equal to the reported selectivity of the standard δ ligand DPDPE [12].

The results in table 1 raise a fundamental question about the activity of the (2*R*,3*S*) isomer, since there is a distinct discrepancy between the results for the δ receptors in rat brain and in MVD. The (2*R*,3*S*) isomer is active in rat brain, but inactive in MVD. Since it is generally thought that the δ receptors in rat brain and MVD are similar [6], we thought that the (2*R*,3*S*) isomer might have antagonistic activity at the δ receptors in MVD; however, it showed no antagonistic activity in either the MVD or the GPI assays. The (2*R*,3*S*) isomer had no effects on the agonist action of DADLE in MVD and of normorphine in GPI at any concentration examined (0.01–1 μM). Thus, it is more likely that the (2*R*,3*S*) isomer of ∇^EPhe^4-enkephalin clearly distinguishes between the δ receptors in the central and peripheral nervous systems, and that the δ receptors in rat brain differ from those in MVD.

For comparison, the most frequently utilized δ ligand to date, DPDPE, was tested together in the [^{3}H]DADLE and MVD assays. In the binding assay, DPDPE was almost 10-fold less active than DADLE (table 1) as reported by others [12]. However, DPDPE displayed a concentration-response curve very similar to that of the (2*R*,3*S*) isomer, showing almost the same affinity as the (2*R*,3*S*) isomer for the δ receptors in rat brain. On the other hand, DPDPE was highly active in the MVD assay (IC_{50} = 3.78 nM) despite the inactivity of the (2*R*,3*S*) isomer in this tissue. Obviously, DPDPE does not discriminate between the δ receptors in rat brain and MVD.

The (2*R*,3*S*) isomer and DPDPE were also evaluated in rat brain using [^{3}H]DPDPE as a tracer. It was found that the (2*R*,3*S*) isomer (IC_{50} = 16.8 nM) and DPDPE (12.6 nM) were almost equally active also in this δ-binding assay, confirming the result of the [^{3}H]DADLE-binding assay described above.

In conclusion, the present study strongly suggests that the δ receptors in rat brain and MVD possess slight structural differences that only [D-Ala^2,(2*R*,3*S*)-∇^EPhe^4,Leu^5]enkephalin can recognize. These conclusions raise a number of questions, such as (i) what is the biological activity of this analog in rat brain? (ii) Why is the compound active only in brain? Further studies to answer these and other questions are in progress in our laboratory.

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