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RAPID COMMUNICATION

ADAMANTANE AS A BRAIN-DIRECTED DRUG CARRIER FOR POORLY ABSORBED DRUG: ANTINOCICEPTIVE EFFECTS OF [D-Ala^2]Leu-ENKEPHALIN DERIVATIVES CONJUGATED WITH THE 1-ADAMANTANE MOIETY

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1-Aminoadamantane hydrochloride (Amantadine hydrochloride) has been administered orally for the treatment of Parkinson's disease. The 1-aminoadamantane analogue memantine (1-amino-3,5-dimethyladamantane), an antiparkinsonism drug, has been reported to distribute to the brain and liver predominantly after systemic and oral administration [1]. From a physico-chemical point of view, the tricyclic structure of adamantane would be expected to have high thermodynamic activity relating to the lipophilicity [2]. Based on these pharmaceutical and physico-chemical properties of adamantane derivatives, we were interested in the possibility of the adamantane moiety serving as a tool for drug delivery to the central nervous system (CNS) of poorly absorbed drugs, that cannot cross the blood-brain barrier (BBB) easily.

In this study, we chose the Leu-enkephalin analogue, [D-Ala^2]Leu-enkephalin, as a model of a poorly absorbed compound. Leu-enkephalin shows antinociceptive activity after direct intracisternal injection, but is inactive after peripheral administration [3]. This is due mainly to its instability towards proteolytic enzymes and its poor permeability through the BBB. We expected that the introduction of the adamantane moiety to [D-Ala^2]Leu-enkephalin through a covalent bond would increase the lipophilicity, resulting in enhanced permeability across the BBB, based on the well-known principle that the permeability of drugs across the BBB corresponds to their lipophilicity [4,5].

METHODS

Peptide synthesis

Four kinds of [D-Ala^2]Leu-enkephalin derivatives conjugated with 1-adamantane (Ada) at the N- or C-terminus of the peptide (Fig. 1) were prepared by solution-phase peptide synthesis.

Determination of partition coefficients

The partition coefficients of [D-Ala^2]Leu-enkephalin and its derivatives in the n-octanol/water system were determined on HPLC according to a method in the literature [6].

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In vitro bioassay

The inhibitory effect on the electrically evoked contraction of isolated guinea pig ileum was measured according to the method of Kosterlitz and Waterfield [7].

In vivo bioassay

The antinociceptive activity of each compound was determined by the tail-pressure method in ddy male mice (approximately 20 g) after subcutaneous administration according to the method of Hata et al. [8]. All tested derivatives were dissolved in a mixture of N,N-dimethylacetamide and saline (4:6) and injected in a volume of 100 μL of solution (1-50 mg/kg body wt).

NH_2-Tyr-(D-Ala)-Gly-Phe-Leu-COOH

(Ⅰ) NH_2-Tyr-(D-Ala)-Gly-Phe-Leu-CO-O-Ada

(Ⅱ) NH_2-Tyr-(D-Ala)-Gly-Phe-Leu-CO-NH-Ada

(Ⅲ) Ada-O-CO-NH-Tyr-(D-Ala)-Gly-Phe-Leu-COOH

(Ⅳ) Ada-CO-NH-Tyr-(D-Ala)-Gly-Phe-Leu-COOH

Ada = 1-Adamantane

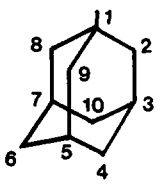

Fig. 1. [D-Ala²]Leu-enkephalin and its derivatives (Ⅰ-Ⅳ).

RESULTS AND DISCUSSION

Chemistry

Four [D-Ala²]Leu-enkephalin derivatives conjugated with the 1-adamantane moiety via ester (Ⅰ), amide (Ⅱ) and (Ⅳ), and carbamate (Ⅲ) bonds were prepared by solution-phase peptide synthesis. The purity of each derivative was ascertained on the basis of elemental analysis, amino acid analysis after acid hydrolysis, and fast-atom-bombardment mass spectrometry (FAB-MS). The reversed-phase HPLC exhibited excellent purity (98%).

The partition coefficient (log P) of each derivative in the n-octanol/water system is listed in Table 1. The derivatives were much more lipophilic than [D-Ala²]Leu-enkephalin (-0.22), as expected. It is noteworthy that the derivatives that conjugated with 1-adamantane at the C-terminus of the peptide, (Ⅰ) and (Ⅱ), were highly lipophilic.

Biological activity

In the in vitro assay (Table 1), the derivatives that conjugated at the C-terminus of the peptide, (Ⅰ) and (Ⅱ), inhibited the electrically evoked contraction of isolated guinea pig ileum as actively as [D-Ala²]Leu-enkephalin and morphine hydrochloride, whereas the derivatives that conjugated at the N-terminus of the peptide, (Ⅲ) and (Ⅳ), were inactive. This result reflects the fact that the free amino-group of Tyr is involved in the interaction with opiate receptors [9].

In the in vivo bioassay, [D-Ala²]Leu-enkephalin and the derivatives (Ⅲ) and (Ⅳ) showed no antinociceptive effect (maximum doses; 50 mg/kg), whereas (Ⅰ) and (Ⅱ) exhibited significant antinociceptive effects (Fig. 2). The effect of (Ⅰ) was dose-dependent in the range of 5-50 mg/kg in mice and reached a maximum level at 60-90 min after subcutaneous injection. The effect of (Ⅱ) was less potent than that of (Ⅰ). Although this result is inconsistent with that of the in vitro study, it seems to be due to the differences in affinity for the receptor. Another possibility is that these derivatives may behave as prodrugs and convert to the parent drug,

enkephalin, in the brain. As controls, 1-adamantanol, 1-aminoadamantane, and the dissolvent were tested and showed no effects. The responses of (I) and (II) in both in vitro and in vivo assays were naloxone-reversible.

Table 1. Opioid activity on the isolated guinea pig ileum

Compound	ED_{50} (M)	N	Relative potency	log P
Morphine HCl	$3.2 \pm 0.43 \times 10^{-8}$	17	1.00	----
[D-Ala^2]Leu-enkephalin	$3.6 \pm 1.17 \times 10^{-8}$	7	0.89	-0.22
Derivative (I)	$9.4 \pm 4.82 \times 10^{-8}$	7	0.34	2.12
Derivative (II)	$1.5 \pm 0.71 \times 10^{-8}$	5	2.13	2.73
Derivative (III)	----	-	----	0.96
Derivative (IV)	----	-	----	0.46

Each ED_{50} value (mean ± SEM) is compared to that of morphine ($5.24 \pm 0.63 \times 10^{-8}$M, N = 37) and Met-enkephalin ($3.35 \pm 1.17 \times 10^{-8}$M, N = 21) reported in Ref. 10.

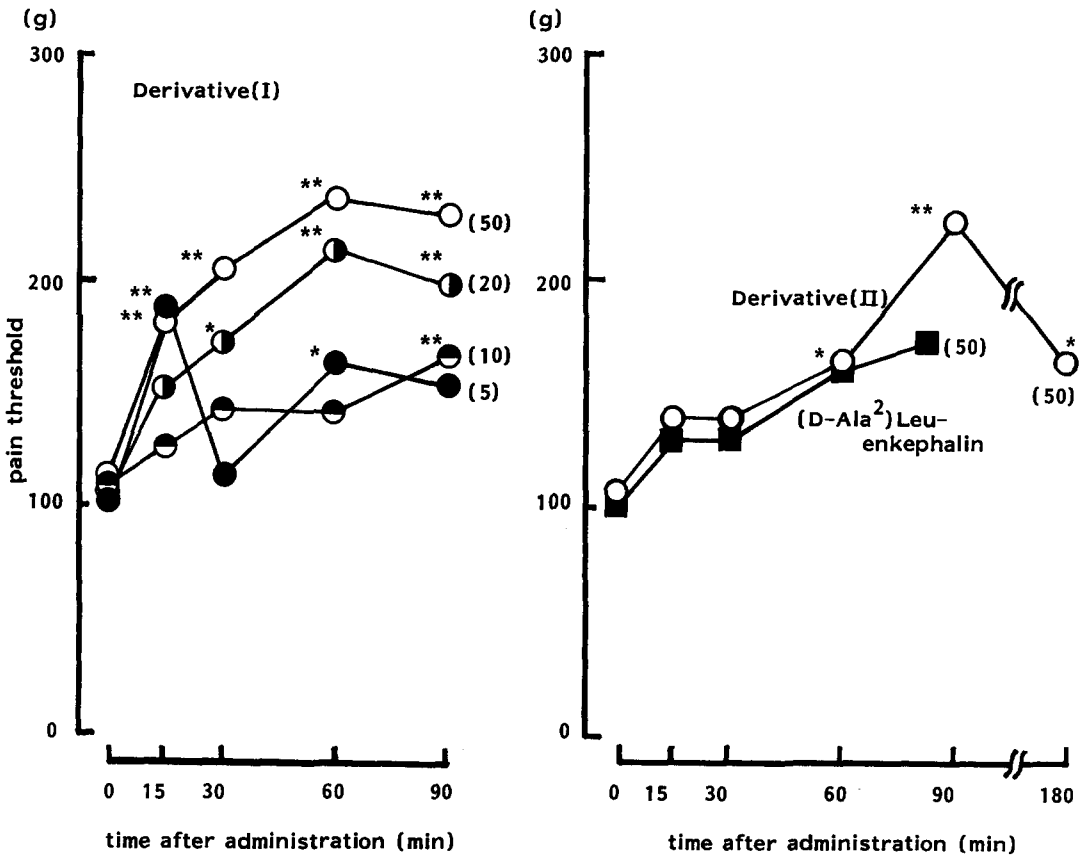

Fig. 2. Effects of derivative (I) and derivative (II) on antinociceptive activity. Activity was determined by the tail-pressure method in mice. The values in parentheses are the doses in mg/kg s.c. Each point is the mean value of pain threshold from ten mice. Key: (★) $P < 0.05$ against pre-drug value, and (★★) $P < 0.01$ against pre-drug value.

Results from these preliminary experiments indicate that the antinociceptive effects of (Ⅰ) and (Ⅱ) with the 1-adamantane moiety at the C-terminus of [D-Ala^2]Leu-enkephalin could be exerted due to the enhanced lipophilicity of the peptides, which enabled (Ⅰ) and (Ⅱ) to cross the BBB. To enhance the lipophilicity enough to pass across the BBB and also to obtain the *in vivo* stability, the incorporation of the sterically hindered amino acid with long alkyl side chains or expanded ring systems (fatty amino acids) into the peptide chain was reported and led to the highly potent enkephalin analogues [11]. Our approach described here may be effective in exploring the brain-directed prodrug candidates, as these derivatives were conjugated with a highly lipophilic moiety through enzymatically labile ester (Ⅰ) or amide (Ⅱ) linkages. The antinociceptive activities of (Ⅰ) and (Ⅱ) are still far weaker than that of morphine; however, the effectiveness and possibilities of the utility of the adamantane moiety as a drug carrier to the CNS for biologically active peptides and other poorly absorbed compounds should be considered. Based on these preliminary experiments, more effective and smoothly biodegradable prodrug-type compounds for CNS could be designed. The high lipophilicity and low toxity of the adamantane moiety promise a possible application as a drug carrier in the CNS drug delivery.

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