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A highly selective ligand for brain δ opiate receptors, a ∇^{E}Phe^{4}-enkephalin analog, suppresses μ receptor-mediated thermal analgesia by morphine

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[D-Ala^{2},(2*R*,3*S*)-∇^{E}Phe^{4},Leu^{5}]enkephalin (CP-OH) [∇denoting cyclopropyl; superscript *E* indicating the *E*-configuration about the cyclopropane ring], a highly selective opioid ligand for δ receptors in rat brain, but not for those in the mouse vas deferens, was examined for in vivo biological activities by intracerebroventricular administration. CP-OH (5–20 μg) showed no analgesic activity in the hot plate (51°C) test using rats. However, it suppressed completely the analgesic effects of intraperitoneally administered morphine (3 mg/kg rat) in a dose-dependent manner. CP-OH showed no binding affinity for brain κ receptors to which dynorphin, an opioid peptide that inhibits morphine analgesia, binds predominantly. These results suggest that, besides the conventional δ receptors which mediate analgesia, the rat brain contains another δ-like receptor which has a modulatory role to attenuate morphine-induced analgesia mediated through the μ receptors, and that this modulatory receptor does not exist in the mouse vas deferens.

Opiate receptor; Analgesia; Antagonism; Enkephalin analog; Receptor heterogeneity

1. INTRODUCTION

The synthetic opioid peptide, [D-Ala^{2},(2*R*, 3*S*)-∇^{E}Phe^{4},Leu^{5}]enkephalin (CP-OH), contains a conformationally restricted amino acid *E*-(2*R*, 3*S*)-cyclopropylphenylalanine (∇^{E}Phe) (fig.1) [1]. We have recently reported that CP-OH can discrimate between the δ opiate receptors in rat brain and in the mouse vas deferens (MVD), interacting with the former, but not with the latter [2]. Thus, it was suggested that the δ receptors in rat brain are different from those in the MVD.

[D-Pen^{2},D-Pen^{5}]enkephalin (DPDPE) and [D-Ala^{2},D-Leu^{5}]enkephalin (DADLE) are very potent and selective for the δ receptors in rat brain [3]. They are also active in the peripheral tissue of the MVD. There is a distinct difference between the biological profiles of these peptides and of CP-OH, since CP-OH has no agonist or antagonist activities in the MVD in spite of its high affinity for rat brain δ receptors [2]. These results inevitably raised a fundamental question, i.e. what is the activity of CP-OH in rat brain? In the present study, we have examined the possible thermal analgesic activity of CP-OH by intracerebroventricular (i.c.v.) administration. We also examined the ability of CP-OH to potentiate morphine-induced analgesia, since such an effect has been described for DADLE [4,5]. Herein we report the results of these in vivo biological assays for ∇^{E}Phe^{4}-enkephalin, and discuss a possible new role of brain δ receptors in the multiple opiate receptor system.

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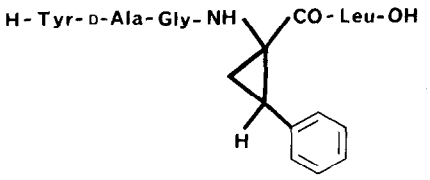

Fig.1. Chemical structure of [D-Ala^2,(2*R*,3*S*)-γ^EPhe^4,Leu^5]-enkephalin.

2. MATERIALS AND METHODS

2.1. *Drugs*

CP-OH was synthesized by the solution method, the synthesis having been described in [1]. [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAGO), DADLE and bremazocine were purchased from Sigma (St. Louis, MO). DPDPE was purchased from Bachem (Bubendorf, Switzerland). Morphine sulfate was obtained from Takeda (Osaka).

2.2. *In vivo biological assays*

Chronic cannulas (A 0.8, L 1.4, V 3.3, Bregma) were implanted in the cerebral ventricle of a separated group of male Wistar rats (220–270 g) anesthetized with intraperitoneally injected sodium pentobarbital (40 mg/kg rat). These rats were housed for a week to recover from surgery. Drugs were administered i.c.v. (5 μl) and rats were tested at 15 min postdrug administration. Each rat was placed on the hot plate (51 ± 0.5°C) and the time (s) required to lick the rear paw was recorded.

In order to examine the effect of the peptide CP-OH on morphine-induced analgesia, morphine (3 mg/kg rat) was first injected i.p. and the peptide was administered i.c.v. (5 μl) after 15 min. Rats were then tested at 15 min postdrug administration on the hot plate to record the latency (s) to lick the rear paw.

2.3. *Receptor-binding assay*

Receptor-binding assays using rat brain membrane preparations were carried out essentially as in [6]. (−)-[9-^{3}H(n)]Bremazocine (41.4 Ci/mmol, New England Nuclear) was used as a tracer specific for the κ receptors. Incubations were carried out for 90 min at 25°C in 50 mM Tris-HCl buffer (pH 7.4) containing DADLE (100 nM) and DAGO (100 nM) as masking ligands of the δ and μ receptors, respectively, and also bacitracin (100 μg/ml) as an enzyme inhibitor.

3. RESULTS AND DISCUSSION

It has been reported that DADLE and DPDPE produce significant analgesia in the hot plate test after i.c.v. administration in rats [7]. Since DPDPE is a highly δ-selective ligand in the central nervous system (CNS) and produces analgesia, it was suggested that both δ and μ receptors in brain mediate analgesia [7]. CP-OH is also highly selective for δ receptors in the CNS. In the binding assays using rat brain homogenate and the tracer [^{3}H]DADLE or [^{3}H]DPDPE, CP-OH and DPDPE showed very similar dose-response curves and exhibited similar potencies [2]. However, in the present study, CP-OH showed no analgesic effects at all in the hot plate test using rats. The i.c.v. administration of CP-OH (5, 10, 20 μg) produced no increases in hot plate (51°C) latencies (s) (fig.2), indicating that the peptide lacks anti-nociceptive activity. This striking result indicates that equally potent ligands in the binding assay showed completely different in vivo effects on analgesia, i.e. DPDPE was active, whereas CP-OH was inactive.

Vaught and Takemori [4] and Barrett and Vaught [5] reported that Leu^5-enkephalin, DADLE, and [D-Ser^2,Leu^5]enkephalyl-Thr^6, putative δ-selective ligands, administered i.c.v. at subanalgesic doses potentiated morphine-induced analgesia in the tail-flick assay using mice and, thus, it was suggested that the δ receptors play an indirect modulatory role in analgesia. Here, we also examined the effect of CP-OH on morphine-induced analgesia, by injecting CP-OH (0, 10, 20 μg i.c.v.) into rats after administration of morphine (3 mg/kg i.p.). Surprisingly, CP-OH antagonized the thermal analgesic activity of morphine very effectively in a dose-dependent manner (fig.3). This suppressive effect of CP-OH on morphine analgesia is in sharp contrast with the reported morphine potentiation described for other δ-selective ligands. Since it is difficult to accept the idea that these opposite in vivo effects are mediated through the same opiate receptors, we performed further investigations.

Friedman et al. [8] reported that dynorphin-(1–13), itself devoid of analgesic activity, significantly suppressed analgesia induced by morphine in the mouse tail-flick test. Since dynorphin is known to interact with κ receptors in the brain [9], we suspected that CP-OH might be attenuating morphine analgesia by interacting with the κ receptors. Thus, we examined the binding ability of CP-OH for κ receptors in rat brain using [^{3}H]bremazocine as a κ tracer. Due to considerable binding of bremazocine to δ and μ receptors, incubations were carried out in the presence of DADLE (100 nM) and DAGO (100 nM) to mask δ and μ receptors, respectively. As shown in fig.4, unlabeled bremazocine displayed a concentration-

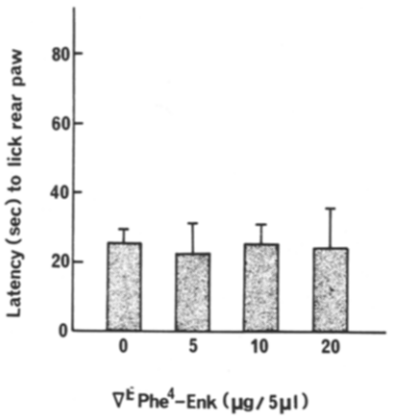

Fig.2. Analgesic effect of i.c.v. administered ∇^{E}Phe^4-enkephalin in the hot-plate (51°C) assay at 15 min postdrug injection. Bars represent means ± SE of responses of 4–5 rats per dose.

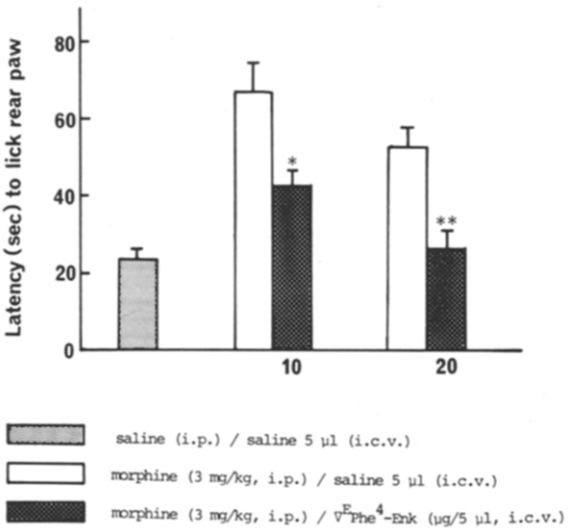

Fig.3. Effect of ∇^{E}Phe^4-enkephalin on morphine-induced analgesia in the hot plate (51°C) test at 15 min postdrug injection. Morphine (3 mg/kg rat) was injected i.p. and after 15 min the peptide dissolved in saline (5 μl) was administered i.c.v. For control experiments (CT), saline was injected i.p. instead of morphine. Bars represent means ± SE of responses of 7–9 rats per dose. * Significantly different from control (* $P<0.05$, ** $P<0.01$).

dependent displacement curve with $IC_{50} = 1.13$ nM, while DADLE and DAGO showed very weak affinity with IC_{50} values of >10 and 2.4 μM, respectively. Clearly, CP-OH and DPDPE exhibited no binding at all in this assay (fig.4). These results indicate that the suppression of morphine-induced analgesia by CP-OH is not mediated through the $\varkappa$ receptors.

These results clearly show that CP-OH, namely [D-Ala^2,(2*R*,3*S*)-∇^{E}Phe^4,Leu^4]enkephalin, belongs to a new class of highly receptor-selective opioid ligands. In the CNS, CP-OH exhibited a high affinity for δ receptors, but not for μ and $\varkappa$ receptors and, moreover, suppressed morphine-induced analgesia. In the peripheral tissues, CP-OH exhibited no agonist or antagonist activities. Although there are limitations to the use of data obtained from an in vivo procedure for differentiation of receptor functions, these results strongly suggest that the brain δ receptors play a negative modulatory role in μ receptor-mediated morphine analgesia.

At this point, however, there is still a fundamental and unresolved discrepancy between the in vivo activities of CP-OH and DPDPE, both of which are equally δ-selective in the brain. One possible explanation is that the analgesic effect of DPDPE is not through the δ receptors in the brain, but through those in the spinal cord, as suggested by Ohlsson et al. [10] when they found that significant fractions of i.c.v. injected peptides can reach the spinal cord. Indeed, DPDPE was reported to be a potent agonist for hot-plate analgesia at the spinal cord level [11]. In this case, higher doses of CP-OH, which we could not achieve because of its solubility, might produce similar thermal analgesia by interacting with the spinal δ receptors. Alternatively, or more likely, the brain may have another 'δ-like' receptor to which CP-OH binds predominantly. In this receptor model, δ-like receptors play a modulatory role in morphine-induced analgesia and the 'conventional' δ receptors only mediate analgesia. CP-OH, then, interacts with the δ-like receptors, but not with the δ receptors, while DPDPE and DADLE bind to both.

Relating to the latter hypothesis, several biological and analytical studies suggesting an allosteric interaction between δ and μ receptors have been reported recently. Met^5-enkephalin per se has an analgesic activity by i.c.v. administration. However, it was found that this peptide also exhibits inhibitory activity against morphine-

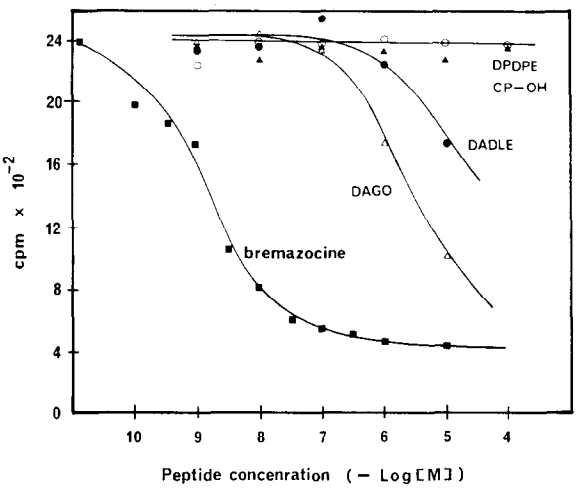

Fig.4. Dose-response curves of opioid peptides and opiate in the radioligand receptor-binding assay using rat brain and [^{3}H]-bremazocine.

induced analgesia when a subanalgesic dose is injected i.c.v. [12,13]. Since Met^5-enkephalin attenuates whereas Leu^5-enkephalin potentiates morphine analgesia [4,5], Vaught et al. [13] postulated an allosteric coupling model between morphine (μ) and enkephalin (δ) receptors, suggesting a modulatory role of δ receptors in μ-receptor-mediated analgesia. Furthermore, Rothman and Westfall [14,15] analyzed this allosteric model by detailed receptor-binding studies and formulated the hypothesis that morphine and enkephalin allosterically modulate each other. In this one-site allosteric model, δ receptor functions to mediate both agonist and antagonist messages. However, as mentioned above, it is unlikely that the opposite messages were transmitted through the same opiate receptors; Rothman et al. [16,17] then postulated a two-site allosteric model, based on the finding of the two distinct sites of δ receptors. They showed that [^{3}H]DADLE apparently labels two binding sites [16] and the binding to one site, a low-affinity [^{3}H]DADLE-binding site, is inhibited noncompetitively by the μ-agonist oxymorphone, suggesting an allosteric coupling between this site and μ receptors [17]. Taking these results into consideration, it is most likely that our δ-like receptor is the one coupled with μ receptors in a two-site allosteric model by Rothman et al. [16,17] and CP-OH should be a highly selective and specific ligand for this site. We tentatively designate this δ-like receptor as the δ-1 receptor and the conventional δ receptor as the δ-2 receptor, which may correspond to the type I and II δ receptors, respectively, described by Rothman et al. [16]. Apparently, rat brain contains both δ-1 and δ-2 receptors, while mouse vas deferens contains only δ-2 receptor.

In conclusion, by utilizing a highly specific enkephalin analog having conformational constraints, the present study strongly suggests the existence of a receptor which modulates μ-mediated morphine analgesia. CP-OH will become a very useful peptide compound in studies aimed at clarifying the molecular mechanism and functional role of this receptor in a complicated and multiple opiate ligand-receptor system. Further biological and pharmacological studies are in progress in our laboratory.

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