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Analgesic activity and selectivity for opioid receptors of enantiomers of ohmefentanyl[1]

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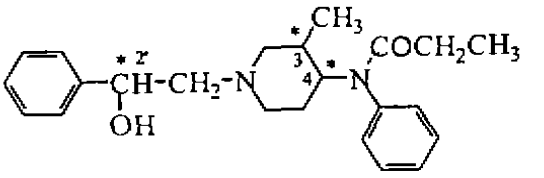
KEY WORDS ohmefentanyl; stereoisomers; pain measurement; mu opioid receptors; delta opioid receptors; radioligand assay; structure-activity relationship

AIM: To study analgesic activity and selectivity for opioid receptor subtypes of 8 enantomers of ohmefentanyl (Ohm). **METHODS:** Analgesic activity was evaluated using the hot plate test in mice. Selectivity for opioid receptors was determined in binding assay and bioassay. **RESULTS:** Among the 8 enantiomers of Ohm, the most potent isomer was F-9204, (+)-*cis*-(3*R*,4*S*,2′*S*)-Ohm, with an analgesic ED_{50} (ip) value of 1.1 (0.8 − 1.3) μg · kg^{-1}. These enantiomers selectively acted on μ opioid receptors. K_i values (μ) of enantiomers F-9204 and F-9202, (−)-*cis*-(3*R*,4*S*,2′*R*)-Ohm were 4.0 ± 2.0 and 5 ± 4 pmol · L^{-1}, respectively. Their $K_i(\delta)/K_i(\mu)$ ratios were 22 500 and 22 800, respectively. On guinea pig ileum and mouse vas deferens F-9202, F-9204, F-9205, F-9206, F-9207, and F-9208 exhibited very potent inhibitons, which were antagonized by naloxone. In rabbit vas deferens these enantiomers had no effect. **CONCLUSION:** Eight enantiomers of Ohm selectively acted on μ opioid receptors. F-9204 showed the strongest analgesic activity and the highest selectivity for μ opioid receptors.

Ohmefentanyl (Ohm), *N*-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenyl-propanamide is a new and potent opioid analgesic agent found in our laboratory. The analgesic activity of *cis* isomers of Ohm is extremely potent[1]. In opioid receptor binding assay Ohm is a highly potent and selective ligand for μ opioid receptors[2,3]. In bioassay Ohm is a potent and selective μ-agonist[4]. Compared with a typical μ-receptor agonist DAGO, [(D-Ala^2, MePhe^4, Gly-ol^5) enkephalin], Ohm possesses other advantages, such as stable structure and easy crossing blood-brain-barrier.

Due to presence of 3 chiral centers in the structure of Ohm, there are 8 enantiomers of Ohm. In order to study the relationship between stereostructure of Ohm and pharmacological actions, 8 enantiomers of Ohm were evaluated in the present paper.



Ohmefentanyl (Ohm)

MATERIALS AND METHODS

Chemicals Eight enantiomers of Ohm were synthesized in the 5th Department in our Institute. The absolute configurations (F9201-F9208) were determined by χ-ray crystallographic study[5]. Morphine and naloxone were obtained from Qinghai Pharmaceutical Factory and Shanghai Medical University, respectively. [^{3}H](D-Ala^2, MePhe^4, Gly-ol^5) enkephalin {[^{3}H]DAGO, 2.22 TBq · mol^{-1}} and [^{3}H](D-Pen^2, D-Pen^5)enkephalin {[^{3}H]DPDPE, 1.04 TBq · mol^{-1}} were purchased from Amersham and New England Nuclear Co, respectively.

Analgesic test Kunming strain ♀ mice (ZKD-005) weighing 18－22 g, were supplied by the Shanghai Experimental Animal Center, Chinese Academy of Sciences. Analgesic activity was evaluated using the hot plate test[6]. The efficiency of analgesic activity was defined by the pain threshold doubled after medication. Analgesic ED_{50} and 95 % confidence limits were calculated according to Bliss method[7].

Receptor binding assay Homogenate of brain was prepared[8]. The bindings in μ and δ receptors were

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determined with the highly selective μ ligand, [^{3}H]DAGO, and the highly selective ligand, [^{3}H]DPDPE, respectively. To determine nonspecific binding, DAGO or DPDPE 10 μmol · L^{-1} were included in the incubation mixture. Aliquots (containing 1 mg protein in the final volume of 1 mL) of the crude membrane preparation were incubated with tested drugs and [^{3}H] ligand at 30 ℃ for 45 min. The mixture was cooled in ice bath, and filtered through Whatman GF/B filter in Millipore 1225 filter. The filters were washed 3 times with 4 mL aliquots of ice-cold Tris-HCl buffer, and transferred to scintillation vials. Hydrophilic scintillation cocktail 4 mL was added and stayed overnight. Radioactivity was counted by a Beckman LS6500 liquid scintillation analyzer. IC_{50} values, the concentrations produced a 50 % inhibition of the specific binding of radioligand, were estimated by linear regression from concentration-probit semi-logarithmic plot. The K_i (dissociation constant of inhibitor) was calculated from $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the concentration and K_d is the dissociation constant of [^{3}H]ligand.

Bioassay in isolated organs The myenteric plexus-longitudinal muscle from guinea pig ileum[9] and the vas deferens of mouse[10] or rabbit[11] were suspended in Krebs solution at 37.0 ± 1.0 ℃ and gassed with 95 % O_2 + 5 % CO_2. After equilibration for 30 min, longitudinal contractions were evoked by field stimulation through Pt-electrodes at the two ends of the preparation. For the guinea pig ileum and the rabbit vas deferens, single pulses were used (50 V, 1.0 ms duration, 15 s interval). For the mouse vas deferens, the trains were consisted of 3 pulses at intervals of 200 ms (40 V, 1.0 ms duration, 15 s interval). The contractions were recorded by a force displacement transducer and auto-equilibrium recorder. The concentrations of the compounds that reduced the height of the contractions by 50 % (IC_{50}) were obtained from concentration-response curves.

RESULTS

Analgesic activity Except F-9203, all enantiomers showed potent analgesic activity. The most potent isomer was F-9204, (+)-*cis*-(3*R*,4*S*,2′*S*)-Ohm. Its analgesic ED_{50} was 1.1 (0.8 - 1.3) μg · kg^{-1}, being 6182 times more active than morphine [ED_{50} 6.8 (5.5 - 8.4) mg·kg^{-1}]. But its enantiomer F-9203, (−)-*cis*-(3*S*,4*R*,2′*R*)-Ohm, had no analgesic activity in dose up to 10 mg · kg^{-1}. The order of analgesic potency of these enantiomers was F-9204 > F-9202 > F-9208 > F-9205 > F-9206 > F-9207 ≫ F-9201 (Tab 1).

Affinities for μ and δ opioid receptors All enantiomers strongly inhibited the specific binding of [^{3}H]DAGO with μ opioid receptors (Tab 2).

Tab 2. K_i value (nmol · L^{-1}) and selectivity of ohmefentanyl enantiomers for μ and δ opioid receptors. n = 3 homogenates (each was pooled from 10 mice), $\bar{x} \pm s$.

Compound	[^{3}H]DAGO (μ)	[^{3}H]DPDPE (δ)	$K_i(\delta)/K_i(\mu)$
F-9204	0.004 ± 0.002	90 ± 10	22 500
F-9202	0.005 ± 0.004	114 ± 14	22 800
F-9208	0.06 ± 0.03	200 ± 13	3 333
F-9205	0.08 ± 0.07	>1 000	>12 500
F-9206	0.13 ± 0.02	150 ± 10	1 153
F-9207	0.15 ± 0.05	230 ± 16	1 533
F-9201	2.9 ± 0.4	>10 000	>3 448
F-9203	5.58 ± 0.21	>10 000	>1 710

The two most active isomers were F-9204 and F-9202 with K_i values of 4.0 ± 2.0 pmol · L^{-1} and

Tab 1. Analgesic ip ED_{50} of 8 enantiomers of ohmefentanyl (mouse hot plate).

Compound	Absolute *configuration*	Analgesic ED_{50}* / mg·kg^{-1}	Analgesic potency
F-9204	(+)-*cis*-(3R,4S,2′S)	0.0011 (0.0008 - 0.0013)	6 182
F-9202	(−)-*cis* (3R,4S,2′R)	0.0046 (0.0031 - 0.0066)	1 478
F-9208	(−)-trans-(3R,4R,2′S)	0.0097 (0.006 - 0.150)	701
F-9205	(+)-trans-(3S,4S,2′S)	0.014 (0.011 - 0.017)	486
F-9206	(−)-trans-(3R,4R,2′R)	0.072 (0.057 - 0.091)	94
F-9207	(+)-trans-(3S,4S,2′R)	0.075 (0.064 - 0.087)	91
F-9201	(+)-*cis*-(3S,4R,2′S)	10 (4/10)	
F-9203	(−)-*cis*-(3S,4R, 2′R)	>10 (0/10)	
Morphine		6.8 (5.5 - 8.4)	1

*(95 % confidence limits or the number of effective mice)

5 ± 4 pmol·L^{-1}, respectively. These enantiomers exhibited very weak inhibitory effect for specific binding of [^{3}H]DPDPE with opioid receptor. Their $K_i(\delta)/K_i(\mu)$ ratios were very large. The $K_i(\delta)/K_i(\mu)$ ratios for F-9204 and F-9202 were 22 500 and 22 800, respectively. There was a correlation ($r = 0.95$) between analgesic ED_{50} and K_i values of [^{3}H]DAGO for these enantiomers. Some isomers with extremely potent analgesia such as F-9204 and F-9202 displayed strong inhibitions on the specific binding of [^{3}H]DAGO; some isomers with weak analgesia such as F-9201 displayed a weak inhibition for the [^{3}H]DAGO binding; some isomers with moderate analgesic activity such as F-9206 and F-9207 displayed a moderate inhibition for the [^{3}H]DAGO binding (Fig 1).

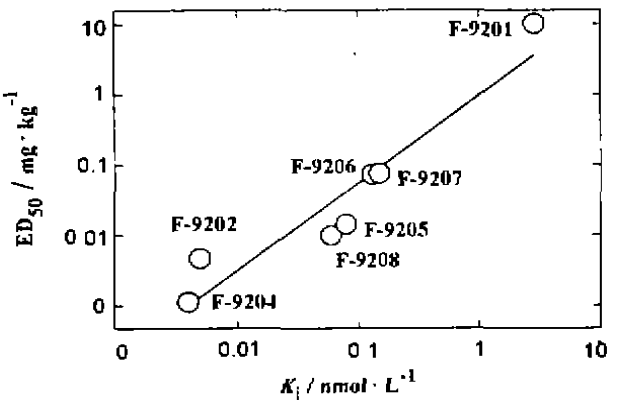

Fig 1. Linear correlation between K_i and analgesic ED_{50} values of ohmefentanyl enantiomers ($r = 0.95$).

Effects on isolated organs Except F-9201 and F-9203, all enantiomers exhibited strongly inhibitory actions on the electrically evoked contractions of the guinea pig ileum and the mouse vas deferens (Tab 3).

The most potent isomer was F-9204. Its IC_{50} values in the guinea pig ileum and the mouse vas deferens were 0.08 ± 0.01 nmol·L^{-1} and 0.10 ± 0.03 nmol·L^{-1}, respectively.

The inhibitory effects were antagonized by naloxone (Fig 2).

In the rabbit vas deferens which contains κ-receptorsf exclusively, these enantiomers did not exhibit inhibitory effects.

Tab 3. IC_{50} value (nmol·L^{-1}) of ohmefentanyl enantiomers in the guinea pig ileum, mouse, and rabbit vas deferens. $n = 3$ animals. $\bar{x} \pm s$.

Compound	Guinea pig ileum	Mouse vas deferens	Rabbit vas deferens
F-9204	0.08 ± 0.01	0.10 ± 0.03	>100
F-9202	0.42 ± 0.07	0.59 ± 0.16	>100
F-9208	0.8 ± 0.4	1.64 ± 0.23	>1 000
F-9205	0.92 ± 0.11	1.15 ± 0.09	>1 000
F-9207	2.58 ± 0.22	4.9 ± 0.7	>1 000
F-9206	4.3 ± 0.6	4.9 ± 0.9	>1 000
F-9201	>1 000	>1 000	>1 000
F-9203	>1 000	>1 000	>1 000

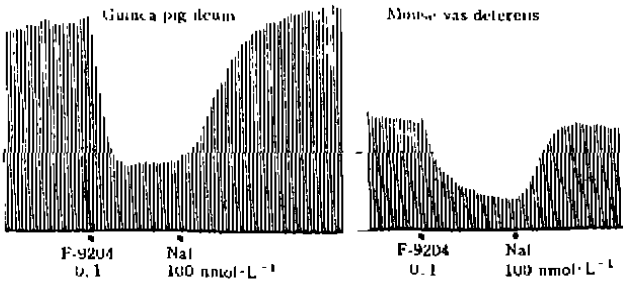

Fig 2. Naloxone reversed inhibitory action of F-9204.

DISCUSSION

The effects of stereostructure of Ohm on pharmacological actions were studied in this paper. By comparing analgesic actions of *cis*-isomers of Ohm, it was shown that (3*R*,4*S*) configuration at piperidine 3- and 4-carbon was very important for analgesic action. For example, isomers F-9204, (+)-*cis*-(3*R*,4*S*,2′*S*)-Ohm and F-9202, (−)-*cis*-(3*R*,4*S*,2′*R*)-Ohm exhibited extremely potent analgesic activity. But their antipodes F-9203, (−)-*cis*-(3*S*,4*R*,2′*R*)-Ohm and F-9201, (+)-*cis*-(3*S*,4*R*,2′*S*)-Ohm had no or very weak analgesic activity, suggesting strict stereo-specificity of the structure to opioid receptors. By comparing analgesic actions of 8 enantiomers of Ohm, it was also seen that analgesic activity of the isomers with 2′S-hydroxyl group was more potent than that of respondent isomers with *R* configuration.

These isomers showed high affinity and very high selectivity for μ opioid receptors. Among these isomers the two most active isomers were

F-9204 and F-9202. It indicated that (3*R*,4*S*) configuration of piperidine ring in *cis*-isomers is very important for selectivity of μ opioid receptors. Rothman *et al*[12] had reported that $K_i(\delta)/K_i(\mu)$ ratio of (±)-*cis*-Ohm (RTI-4614-4) was 26 909, but analgesic activity of (−)-*cis*-(3*S*,4*R*,2′*R*)-isomer was more potent than that of (+)-(3*R*,4*S*,2′*S*)-isomer[13], which is contradictory with our results.

The analgesic activities of isomers F-9201 and F-9203 were very weak. Although they inhibited specific binding of [^{3}H]DAGO to μ opioid receptors in nmol·L^{-1} level, both isomers in concentration up to 1 μmol·L^{-1} were found to be inactive in the guinea pig ileum and the mouse vas deferens. Other 6 isomers had stronger inhibitory actions on the electrically evoked contractions of the guinea pig ileum and the mouse vas deferens. The actions could be antagonized by naloxone, indicated that they acted mainly on μ opioid receptors. There is a good parallel correlation between the inhibitory potency in the guinea pig ileum and the mouse vas deferens and the inhibitory potency of specific binding of [^{3}H]DAGO to μ receptors for these isomers. It also indicated their actions on isolated organs were related to the affinity on μ opioid receptors. In the rabbit vas deferens contained κ-receptors exclusively these enantiomers had no inhibitory effect, suggested that they had no activity for κ-receptors.

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羟甲芬太尼对映异构体的镇痛活性及对阿片受体的选择性

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关键词 羟甲芬太尼；立体异构体；痛测定；μ阿片受体；δ阿片受体；放射配位体测定；结构-活性关系

镇痛

A **目的**：研究羟甲芬太尼对映异构体的镇痛活性及对阿片受体亚型的选择性. **方法**：小鼠热板法测痛 受体结合和离体生物试验测定对阿片受体的选择性 **结果**：8个羟甲芬太尼对映异构体中作用最强的异构体是F-9204，(+)-顺-(3*R*,4*S*,2′*S*)-羟甲芬太尼，其镇痛ED_{50}(ip)为1.1(0.8−1.3) μg·kg^{-1}. 它们选择性作用于μ阿片受体. 异构体F-9202，F-9204，F-9205，F-9206，F-9207和F-9208对豚鼠回肠和小鼠输精管有很强的抑制作用，并被纳洛酮拮抗，但对兔输精管没有作用. **结论**：羟甲芬太尼8个对映异构体选择性作用于μ阿片受体，其中F-9204的镇痛作用最强，对μ受体的选择性最高.

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