ELSEVIER

European Journal of Pharmacology 424 (2001) 195–198

ejp

www.elsevier.com/locate/ejphar

Analgesic activity and opioid receptor selectivity of stereoisomers of ohmefentanyl isothiocyanate

Bi-Yi Chen [1], Wen-Qiao Jin *, Xin-Jian Chen, You-Cheng Zhu, Zhi-Qiang Chi

2nd Department of Pharmacology, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

Received 19 February 2001; received in revised form 27 June 2001; accepted 29 June 2001

Abstract

Ohmefentanyl is a very potent and highly selective agonist for μ-opioid receptors. We now study analgesia, in vitro activity and opioid receptor affinity of the stereoisomers of ohmefentanyl isothiocyanate. We found that some isomers of ohmefentanyl isothiocyanate had a potent analgesic effect and that all isomers except (3*R*,4*S*,2′*S*)-ohmefentanyl isothiocyanate had a more potent inhibitory action on the electrically evoked contractions of mouse vas deferens than of guinea pig ileum. The inhibitory actions could be antagonized by naloxone. However, compared with the activity of the corresponding stereoisomers of ohmefentanyl, these ohmefentanyl isothiocyanates had significantly reduced analgesia and in vitro activity. They also inhibited the binding of [^{3}H]DPDPE ([D-Pen^2,D-Pen^5]enkephalin) and [^{3}H]DAGO ([D-Ala^2,Mephe^4,Gly-ol^5]enkephalin) to opioid receptors in mouse brain membranes. The inhibitory effect of stereoisomers of ohmefentanyl isothiocyanate at μ-opioid receptors was markedly lower than that of their parent compounds. The affinity of stereoisomers of ohmefentanyl isothiocyanate for δ-opioid receptors was, however, greater than or equal to that of their corresponding stereoisomers of ohmefentanyl. The results showed that the introduction of an isothiocyanato group into the phenyl ring in position-1 of ohmefentanyl reduced bioactivity and affinity to μ-opioid receptors but that the selectivity of these compounds for δ-opioid receptors was enhanced. Isomer (3*R*,4*S*,2′*R*)-ohmefentanyl isothiocyanate showed highest selectivity for δ-opioid receptors ($K_i(\mu)/K_i(\delta) = 13.6$) and potent analgesic activity ($ED_{50} = 0.25$ mg/kg).

Keywords: Ohmefentanyl isothiocyanate; Stereoisomer; Opioid receptor; Analgesia; Bioassay; Receptor binding

1. Introduction

We have previously reported that ohmefentanyl is a very potent analgesic agent and a highly selective agonist for μ-opioid receptors (Jin et al., 1981, 1987; Xu et al., 1988; Goldstein and Naidu 1989). Ohmefentanyl not only has a 6300 times more potent analgesic activity than that of morphine, but it has relatively lower physical dependence and psychic dependence liabilities than morphine (Chen et al., 1995a,b). Due to presence of three chiral centers in the structure of ohmefentanyl, there are eight enantiomers of ohmefentanyl. We had studied analgesic activity, physical dependence and selectivity for opioid receptors of these enantiomers (Jin et al., 1996; Guo et al., 2000). Several studies have shown that non-peptide δ-opioid receptor agonists have clinical potential as safe and effective pain relieving agents (Clark and Dondio, 1997). In the study of structure–activity relationship, introducing an isothiocyanato-group (SCN-group) into the phenyl ring in position-1 of some fentanyl derivatives may shift receptor selectivity to a δ-selective property (Rice et al., 1983; Burke et al., 1984, 1986; Kim et al., 1989). Therefore, the eight enantiomers of ohmefentanyl isothiocyanate were synthesized, and the analgesia, in vitro activity and receptor selectivity of these stereoisomers are reported here.

2. Materials and methods

2.1. Drugs

Eight stereoisomers of ohmefentanyl isothiocyanate were synthesized in the Chemical Group, 2nd Department of Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences. The absolute configurations

* Corresponding author. Tel.: +86-21-6431-1833x510; fax: +86-21-6437-0269.

E-mail address: wqjin@mail.shcnc.ac.cn (W.-Q. Jin).

[1] Present address: Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China.

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01172-4

of eight stereoisomers of isothiocyanato-ohmefentanyl were determined by X-ray crystallography. Naloxone was obtained from Shanghai Medical University. [^{3}H]DAGO ([D-Ala^2,Mephe^4,Gly-ol^5]enkephalin, 54 mCi/mmol) and [^{3}H]DPDPE ([D-Pen^2,D-Pen^5]enkephalin, 36 mCi/mmol) were purchased from Amersham-Pharmacia and Dupont, respectively (Fig. 1).

2.2. Animals

Male or female Kunming strain mice and guinea pigs (ZKD-005) were supplied by the Shanghai Experimental Animal Center, Chinese Academy of Sciences. The animals were housed in plastic cages with food and water ad libitum. All experiments were conducted during the light period of a 12:12-h light–dark cycle.

2.3. Antinociceptive assay

Antinociceptive activity of the compounds was measured in the mouse hot-plate assay (Zhao and Zhu, 1956) 5 min after subcutaneous injection. Animals were placed on a zinc plate heated to 55 ± 1 °C. The pain response was defined as licking of hind paws. The efficiency of antinociceptive activity was defined as doubling of the pain threshold after medication (Huang et al., 1984). The analgesic duration of the compounds was determined at the ED_{95} dose of the compounds. The number of animals in each dose group was 10. Analgesic ED_{50} values were calculated using the method of Bliss (1938).

2.4. Bioassay

The mouse vas deferens and the myenteric plexus-longitudinal muscle of guinea pig ileum were prepared according to the methods described by Hughes et al. (1975) and Kosterlitz et al. (1970), respectively. All preparations were suspended in an organ bath containing 4 ml Krebs solution. The bath fluid was kept at 37 ± 1 °C and gassed continuously with 95% O_2 and 5% CO_2. The resting tension was maintained at 250 mg for mouse vas deferens and 500 mg for guinea pig ileum. The composition of the Krebs solution was (in mmol/l): NaCl 119, KCl 4.7, $CaCl_2$ 2.55, KH_2PO_4 1.6, $NaHCO_3$ 25, glucose 11 and mepyamine maleate 0.00013. $MgSO_4$ 1.18 was included for guinea pig ileum. After equilibration for 45 min, longitudinal contractions were evoked by field stimulation through Pt-electrodes at the upper and lower ends of the bath. The parameters of stimulation were as follows. For mouse vas deferens, the trains consisted of four pulses at intervals of 200 ms (40 V, 1.0-ms duration, 15-s interval). For guinea pig ileum, single pulses of 1 ms were used (30 V, 1.0-ms duration, 15-s interval). The contractions were recorded with a force displacement transducer and an auto-equilibrium recorder. The agonist potencies of the compounds were obtained from dose–response curves by calculating the concentration of the compounds that reduced the height of the contractions by 50% (IC_{50}).

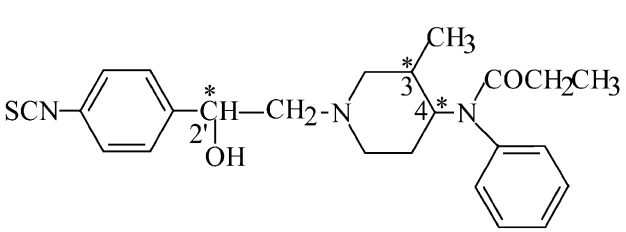

Fig. 1. Chemical structure of ohmefentanyl isothiocyanate (* asymmetric carbon atom).

2.5. Binding assay

Mouse brain homogenates were prepared as described by Jin et al. (1981). The binding for μ- and δ-opioid receptors was determined with the highly selective μ-opioid receptor ligand, [^{3}H]DAGO and the highly selective δ-opioid receptor ligand, [^{3}H]DPDPE, respectively. Non-specific binding was measured by incubation in the presence of 10 μmol/l naloxone. The binding assays were conducted at 30 °C in 0.05 mol/l Tris–HCl buffer (pH 7.4). Aliquots (containing 1.5 mg protein in the final volume of 1 ml) of crude membrane preparation were incubated with the drugs and tritiated ligand at 30 °C for 40 min. After the incubation, the mixture was immediately cooled in an ice bath and then filtered rapidly through Whatman GF/B glass fiber filters in a Brand harvester. The filters were washed three times with 4-ml aliquots of ice-cold 0.05 mol/l Tris–HCl buffer, and transferred to counting vials. Four ml hydrophilic scintillation cocktail was added and allowed to stand overnight. The radioactivity was counted with a Beckman LS6500 liquid scintillation analyzer. The IC_{50} values of the drugs tested, defined as the concentrations that produced 50% inhibition of the specific binding of radioligand, were estimated by linear regression from a concentration-probit semi-logarithmic plot. The K_i values, the equilibrium dissociation constant of the inhibitor was calculated from the formula $K_i = IC_{50}/[1 + (L)/K_d]$, where L is the concentration of labelled ligand and K_d the dissociation constant of labelled ligand (Cheng and Prusoff, 1973).

3. Results

3.1. Analgesic activity

Except for isomers (3*S*,4*R*,2′*S*), (3*S*,4*R*,2′*R*) and (3*R*,4*R*,2′*R*)-ohmefentanyl isothiocyanate, the enantiomers showed potent analgesic activity. The most potent isomer was (+)-*cis*-(3*R*,4*S*,2′*S*)-ohmefentanyl isothiocyanate. Its analgesic ED_{50} was 0.113 mg/kg, being 34 times more active than morphine (ED_{50} 3.86 mg/kg, s.c.). Its enan-

Table 1
Analgesic ED_{50} (mg/kg, s.c.) and duration (min) of action of eight enantiomers of ohmefentanyl isothiocyanate (mouse hot-plate)

Absolute configuration	Analgesic ED_{50}[a]	Analgesic potency	Duration[b]
(+)-*cis*-3*S*,4*R*,2′*S*	10 (inactive)		
(−)-*cis*-3*R*,4*S*,2′*R*	0.256 (0.212–0.308)	15	50 ± 26
(−)-*cis*-3*S*,4*R*,2′*R*	10 (inactive)		
(+)-*cis*-3*R*,4*S*,2′*S*	0.113 (0.097–0.132)	34	43 ± 31
(+)-*trans*-3*S*,4*S*,2′*S*	1.95 (1.56–2.54)	2	35 ± 14
(−)-*trans*-3*R*,4*R*,2′*R*	10 (inactive)		
(−)-*trans*-3*S*,4*S*,2′*R*	9.27 (7.39–11.6)	0.42	15 ± 10
(+)-*trans*-3*R*,4*R*,2′*S*	7.22 (5.30–9.84)	0.53	69 ± 20
Morphine	3.86 (2.82–5.27)	1	64 ± 13

[a] 95% confidence limits.
[b] Mean ± S.D.

tiomer, (−)-*cis*-(3*S*,4*R*,2′*R*)-ohmefentanyl isothiocyanate, however, had no analgesic activity in doses up to 10 mg/kg. The order of analgesic potency of these enantiomers was (3*R*,4*S*,2′*S*) > (3*R*,4*S*,2′*R*) > (3*S*,4*S*,2′*S*) > (3*R*,4*R*,2′*S*) > (3*S*,4*S*,2′*R*)-ohmefentanyl isothiocyanate (Table 1).

3.2. Effects on isolated organs

Except for isomers (3*S*,4*R*,2′*S*)-ohmefentanyl isothiocyanate and (3*S*,4*R*,2′*R*)-ohmefentanyl isothiocyanate, the other six stereoisomers of ohmefentanyl isothiocyanate exhibited potent inhibitory actions on the electrically evoked contraction of guinea pig ileum and mouse vas deferens (Table 2). For guinea pig ileum, the most potent isomer was (3*R*,4*S*,2′*S*)-ohmefentanyl isothiocyanate. For mouse vas deferens, the most potent isomer was (3*R*,4*S*,2′*R*)-ohmefentanyl isothiocyanate. The effects of these compounds on guinea pig ileum and mouse vas deferens were reversed by naloxone. Except for isomer (3*R*,4*S*,2′*S*)-ohmefentanyl isothiocyanate, the other stereoisomers showed more potent activity on mouse vas deferens than on guinea pig ileum. The activities of these isomers were, however, markedly lower than those of their corresponding stereoisomers of ohmefentanyl (Jin et al., 1996).

Table 2
The inhibitory actions (IC_{50}, μmol/l) of stereoisomers of ohmefentanyl isothiocyanate on electrically evoked contraction of guinea pig ileum (GPI) and mouse vas deferens (MVD) (mean ± S.D., $n = 3$)

Absolute configuration	GPI	MVD	GPI/MVD
(+)-*cis*-3*S*,4*R*,2′*S*	> 10	> 10	
(−)-*cis*-3*R*,4*S*,2′*R*	2.26 ± 1.62	0.17 ± 0.08	13.3
(−)-*cis*-3*S*,4*R*,2′*R*	> 10	> 10	
(+)-*cis*-3*R*,4*S*,2′*S*	0.30 ± 0.03	0.36 ± 0.07	0.83
(+)-*trans*-3*S*,4*S*,2′*S*	2.6 ± 0.18	1.05 ± 0.26	2.5
(−)-*trans*-3*R*,4*R*,2′*R*	2.6 ± 0.15	1.36 ± 0.27	1.9
(−)-*trans*-3*S*,4*S*,2′*R*	11.0 ± 1.4	1.56 ± 0.15	7.1
(+)-*trans*-3*R*,4*R*,2′*S*	0.70 ± 0.23	0.40 ± 0.17	1.7

Table 3
The inhibitory constant (K_i, μmol/l) of stereoisomers of ohmefentanyl isothiocyanate for opioid receptors in mouse brain homogenates (mean ± S.D., $n = 3$)

Absolute configuration	(^{3}H)DAGO (μ)	(^{3}H)DPDPE (δ)	K_i(μ)/K_i(δ)
(+)-*cis*-3*S*,4*R*,2′*S*	1.04 ± 0.02	3.28 ± 0.95	0.32
(−)-*cis*-3*R*,4*S*,2′*R*	1.63 ± 0.76	0.12 ± 0.07	13.6
(−)-*cis*-3*S*,4*R*,2′*R*	2.45 ± 0.57	3.25 ± 1.13	0.75
(+)-*cis*-3*R*,4*S*,2′*S*	0.08 ± 0.04	0.18 ± 0.13	0.44
(+)-*trans*-3*S*,4*S*,2′*S*	0.29 ± 0.07	0.03 ± 0.02	9.7
(−)-*trans*-3*R*,4*R*,2′*R*	0.51 ± 0.12	0.21 ± 0.07	2.4
(−)-*trans*-3*S*,4*S*,2′*R*	0.36 ± 0.05	0.23 ± 0.08	1.6
(+)-*trans*-3*R*,4*R*,2′*S*	0.17 ± 0.10	0.21 ± 0.14	0.80

3.3. Affinity to μ- and δ-opioid receptors

The affinities of all enantiomers of ohmefentanyl isothiocyanate to μ- and δ-opioid receptors are shown in Table 3. As compared with that of the corresponding stereoisomers of ohmefentanyl (Jin et al., 1996), their affinity to μ-opioid receptors was markedly low, but their affinity to δ-opioid receptors was almost equal to or more potent than those of their corresponding stereoisomers of ohmefentanyl. The most active of these isomers at δ-opioid receptors was (+)-*trans*-(3*S*,4*S*,2′*S*)-ohmefentanyl isothiocyanate with a K_i value of 30 nmol/l; the most selective isomer for δ-opioid receptors was (−)-*cis*-(3*R*,4*S*,2′*R*)-ohmefentanyl isothiocyanate, which had a $K_i(\mu)/K_i(\delta)$ value of 13.6.

4. Discussion

The analgesia, in vitro activity, and opioid receptor selectivity of stereoisomers of ohmefentanyl isothiocyanate are described in this paper. By comparing analgesic actions of *cis*-isomers of ohmefentanyl isothiocyanate, we showed that the (*3*R,*4*S) configuration at the piperidine 3- and 4-carbon is very important for analgesic activity. For example, isomers (+)-*cis*-(3*R*,4*S*,2′*S*)-ohmefentanyl isothiocyanate and (−)-*cis*-(3*R*,4*S*,2′*R*)-ohmefentanyl isothiocyanate exhibited potent analgesic activity. However, their antipodes (−)-*cis*-(3*S*,4*R*,2′*R*)-ohmefentanyl isothiocyanate and (+)-*cis*-(3*S*,4*R*,2′*S*)-ohmefentanyl isothiocyanate had no analgesic activity, suggesting strict stereospecificity of the structure for opioid receptors. Comparing the analgesic actions of six active enantiomers of ohmefentanyl isothiocyanate also showed that the analgesic activity of the isomers with a 2′*S*-hydroxyl group was more potent than that of corresponding isomers with the 2′*R* configuration.

Consistent with the results of the analgesic assay, the isomers (3*S*,4*R*,2′*S*)-ohmefentanyl isothiocyanate and (3*S*,4*R*,2′*R*)-ohmefentanyl isothiocyanate were not found to be active in guinea pig ileum and mouse vas deferens. Isomer (3*R*,4*S*,2′*R*)-ohmefentanyl isothiocyanate and

(3*R*,4*S*,2′*S*)-ohmefentanyl isothiocyanate had the most potent inhibitory effect on the electrically evoked contractions of guinea pig ileum or mouse vas deferens. Except for isomer (3*R*,4*S*,2′*S*)-ohmefentanyl isothiocyanate, the five other active isomers were more potent on mouse vas deferens (GPI) than on guinea pig ileum (MVD). It is remarkable that the IC_{50}(GPI)/IC_{50}(MVD) ratio for isomer (3*R*,4*S*,2′*R*)-ohmefentanyl isothiocyanate was 13.3. The selectivity of this isomer for δ-opioid receptors was markedly higher than that of superFIT (*cis*-(+)-3-methylfentanyl isothiocyanate), a partial agonist of δ-receptors (Burke et al., 1986).

Our previous study had shown that the two most active isomers (3*R*,4*S*,2′*S*)-ohmefentanyl and (3*R*,4*S*,2′*R*)-ohmefentanyl showed very potent affinity and very high selectivity for μ-opioid receptors (Jin et al., 1996). However, a corresponding isomer of ohmefentanyl isothiocyanate, (3*R*,4*S*,2′*R*)-ohmefentanyl isothiocyanate showed more potent affinity and higher selectivity for δ-opioid receptors. Another corresponding isomer of ohmefentanyl isothiocyanate, (3*R*,4*S*,2′*S*)-ohmefentanyl isothiocyanate showed markedly low affinity for μ-opioid receptors as compared with that of (3*R*,4*S*,2′*S*)-ohmefentanyl.

The present study demonstrated that introducing an isothiocyanato-group into the phenyl ring in the position-1 of ohmefentanyl may enhance the selectivity for δ-opioid receptors, but the bioactivity and affinity for μ-opioid receptors of these compounds were significantly reduced. The most interesting of these stereoisomers was (−)-*cis*-(3*R*,4*S*,2′*R*)-ohmefentanyl isothiocyanate which exhibited potent analgesic activity (ED_{50} = 0.25 mg/kg) and highest selectivity for δ-opioid receptors [$K_i(\mu)/K_i(\delta)$ = 13.6]. Further study of the physical and psychic dependence for these stereoisomers is necessary for exploration of the mechanism of opioid dependence and the development of new drugs.

Acknowledgements

This study was supported by Grant G (1998) 051126 from the Ministry of Science and Technology of China and Grant No. 29790123 from the Natural Science Foundation of China.

References

Bliss, C.I., 1938. The determination of the dosage-mortality curve from small numbers. Q. J. Pharm. Pharmacol. 11, 192–196.

Burke Jr., T.R., Bajwa, B.S., Jacobson, A.E., Rice, K.C., Streaty, R.A., Kell, W.A., 1984. Probes for narcotic receptor mediated phenomena: 7. Synthesis and pharmacological properties of irreversible specific ligands for μ and δ opiate receptors. J. Med. Chem. 27, 1570–1574.

Burke Jr., T.R., Jacobson, A.E., Rice, K.C., Silverton, J.V., Simonds, W.F., Streaty, R.A., Klee, W.A., 1986. Probes for narcotic receptor mediated phenomena: 12. *cis*-(+)-3-methylfentanyl isothiocyanate, a potent site-directed acylating agent for opioid receptors: synthesis, absolute configuration, and receptor enantioselectivity. J. Med. Chem. 29, 1087–1093.

Chen, J., Li, X., Zheng, J.W., 1995a. The discrimination effect of three opiate analgesics. Chin. Pharm. Bull. 11, 32–35.

Chen, J., Xie, L., Wang, W.P., Zheng, J.W., 1995b. Systematical appraisal of psychic dependence for ohmefentanyl. Chin. Bull. Drug Depend. 4, 9–11.

Cheng, Y.C., Prusoff, W.H., 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Clark, G.D., Dondio, G.M., 1997. Recent advances in the development of a non-peptide δ opioid analgesic. Pharmacol. Rev. Commun. 9, 49–60.

Goldstein, A., Naidu, A., 1989. Multiple opioid receptors: ligand selectivity profiles and biding site signatures. Mol. Pharmacol. 36, 265–272.

Guo, G.W., He, Y., Jin, W.Q., Zou, Y., Zhu, Y.C., Chi, Z.Q., 2000. Comparison of physical dependence of ohmefentanyl stereoisomers in mice. Life Sci. 67, 113–120.

Huang, Z.M., Zhou, J., Chen, X.J., Zheng, W.J., Zhang, H.P., Chi, Z.Q., 1984. Analgesic activity and toxicity of potent analgesics ohmefentanyl and mefentanyl. Acta Pharmacol. Sin. 5, 153–158.

Hughes, J., Kosterlitz, H.W., Leslie, F.M., 1975. Effect of morphine on adrenergic transmission in the mouse vas deferens, assessment of agonist and antagonist potencies of narcotic analgesics. Br. J. Pharmacol. 53, 371–381.

Jin, W.Q., Hu, H., Zhu, Y.C., Fang, S.N., Xia, X.L., Huang, Z.M., Ge, B.L., Chi, Z.Q., 1981. Studies on synthesis and relationship between the analgesic activity and receptor affinity for 3-methylfentanyl derivatives. Sci. Sin., Ser. B 24, 710–720.

Jin, W.Q., Chen, X.J., Chi, Z.Q., 1987. The choice of opioid receptor subtype in isolated preparations by ohmefentanyl. Sci. Sin., Ser. B 30, 176–181.

Jin, W.Q., Wang, Z.X., Chen, J., Chen, X.J., Chi, Z.Q., 1996. Analgesic activity and selectivity for opioid receptors on enantiomers of ohmefentanyl. Acta Pharmacol. Sin. 17, 421–424.

Kim, C.H., Rothman, R.B., Jacobson, A.E., Mattson, M.V., Bykov, V., Streaty, R.A., Klee, W.A., George, C., Long, J.B., Rice, K.C., 1989. Probes for narcotic receptor mediated phenomena: 15. (3*S*,4*S*)-*trans*-3-methylfentanyl isothiocyanate, a potent site-directed acylating agent for the δ opioid receptors in vitro. J. Med. Chem. 32, 1392–1398.

Kosterlitz, H.W., Lydon, R.J., Watt, A.J., 1970. The effects of adrenaline, noradrenaline and isoprenaline on inhibitory α- and β-adrenoceptors in longitudinal muscle of the guinea pig ileum. Br. J. Pharmacol. 39, 398–413.

Rice, K.C., Jacobson, A.E., Burke Jr., T.R., Bajwa, B.S., 1983. Irreversible ligands with high selectivity toward δ or μ opioid receptors. Science 220, 314–316.

Xu, H., Chen, J., Chi, Z.Q., 1988. Ohmefentanyl—a new agonist for μ opioid receptor. Sci. Sin., Ser. B 28, 504–511.

Zhao, Y., Zhu, X.Y., 1956. A modified "hot-plate" method for the evaluation of analgesics. Acta Pharmacol. Sin. 4, 97–105.