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Comparison of physical dependence of ohmefentanyl stereoisomers in mice

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Abstract

Stereo-structural difference of ohmefentanyl stereoisomers on analgesic action and receptor affinity has been studied. To assess the difference of ohmefentanyl stereoisomers in physical dependence, the potency of physical dependence was quantified by estimating the ED_{50} value of ohmefentanyl stereoisomers in the naloxone-precipitated jumping test in mice. Morphine was used to assess the method and as a drug of comparison. The results indicate that the degree of physical dependence of morphine can been quantified by estimating the ED_{50} value of morphine withdrawal jumping induced by naloxone. A significant difference was observed in withdrawal jumping ED_{50} values among ohmefentanyl stereoisomers. Of these isomers, F9202 and F9204 had similarly potent analgesic action, but very significant difference in naloxone precipitated withdrawal response. Dependent potency index of F9204 was 618-fold weaker than that of F9202. It is concluded that a stereo-structural difference in physical dependence is found to exist among ohmefentanyl stereoisomers. Compound F9204 displayed a strong analgesic action and weak physical dependent potency.

Keywords: Ohmefentanyl; Stereoisomer; Morphine; Withdrawal; Physical dependence

Introduction

Ohmefentanyl, [N-1 (β-hydroxy-β-phenylethyl)-3-methyl-4-piperityl]-N-phenylpropanamide, which is derived from fentanyl and synthesized through the introduction of methyl group in the 3-position and replacement of the phenylethyl group in the 1-position of piperidine ring of fentanyl by a hydroxyl phenylethyl group, is a novel and potent analgesic agent. As an analgesic, ohmefentanyl is 28 times more potent than fentanyl and 6300 times more potent than morphine in mice (1). As all opioids, repeated administration of analgesic dose of ohmefentanyl also can result in the development of physical dependence. The potential ability

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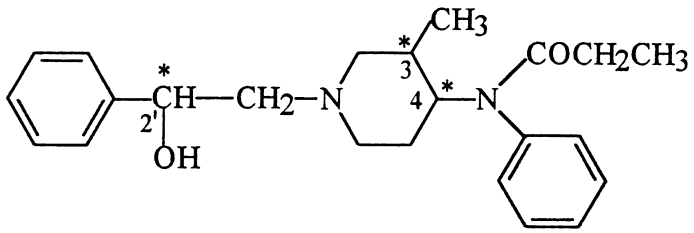
of the compound to induce dependence has been preclinically evaluated in different animal species (2). The ability to develop physical dependence of ohmefentanyl is lower compared with that produced by morphine, qualitatively similar to that of fentanyl in animals, although it has been demonstrated that ohmefentanyl is a potent and highly selective μ-agonist (3–8). Due to the presence of three chiral centers in chemical structure of ohmefentanyl, it has eight stereoisomers. The analgesic activities and selectivity for opioid receptor of these stereoisomers had been studied in our laboratory (9). It was showed that there was a large difference in analgesic activity among ohmefentanyl stereoisomers. However, the relationship between stereo-structure and dependence of ohmefentanyl had not been described. In the present study, we established a new quantitative index to estimate the degree of physical dependence, and determined the physical dependence of ohmefentanyl stereoisomers in mice.

Materials and Methods

Chemicals

Ohmefentanyl stereoisomers were synthesized by the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China). The absolute configurations of eight stereoisomers (F9201–9208) of ohmefentanyl were determined by X-ray crystallographic study. Morphine was purchased from Qinghai Pharmaceutical Factory (Xining, China). Naloxone was obtained from Shanghai Medical University (Shanghai, China).



Chemical Structure of Ohmefentanyl

Animals

Kunming strain male mice (ZKD-005) weighting 18–22g, were supplied by the Animal Center, Shanghai Medical University. Mice were maintained on a 12/12 hr light/dark cycle and fed and watered *ad libitum* before the experiment procedures. The mice were transferred to the laboratory environment after a final administration of morphine or ohmefentanyl stereoisomers.

Treatment of morphine and ohmefentanyl stereoisomers

All drugs were administered s.c. in an injection volume of 10ml/kg, injections were delivered at 8:00 a.m. and 4:00 p.m., and solutions were freshly made prior to each injection. To develop morphine dependence, mice were treated with morphine twice a day for 6 days. The doses were started at 20mg/kg (2×analgesic ED_{95}) and increased by 20mg/kg every time to

remain finally a dose of 100mg/kg ($10\times ED_{95}$). On the different days of morphine administration, the animals received a final injection of morphine. Naloxone was challenged and withdrawal signs (jumping and other parameters) were observed for 30min. For study of appropriate precipitated-time and doses of naloxone, withdrawal was precipitated at various times (1, 2, 4 and 6hr) after a final administration of morphine by injection of various doses of naloxone (0.25, 0.5, 1, 2, and 4 mg/kg). Ohmefentanyl stereoisomers (F9201–9208) were administered at the similar schedule as for morphine. The initial doses of these isomers were administered on the basis of analgesic ED_{95} dose level as follows: F9201 10mg/kg, F9202 0.25μg/kg, F9203 5.0mg/kg, F9204 5.0μg/kg, F9205 15μg/kg, F9206 165μg/kg, F9207 120μg/kg and F9208 25μg/kg twice daily. The dose was increased by 1× initial dose every day until a maintenance dose (5× initial dose) was attained. All drugs were freshly prepared by dissolving in normal saline and doses were calculated on the basis of the drug salts.

Assessment of physical dependence

The degree of physical dependence was evaluated by the cumulative dose of tested drugs inducing 50% positive jumping response (ED_{50}) in naloxone precipitated jumping test. The jumping response can be measured by determining the percentage of mice that jump vertically within 30 min after injection of naloxone. The mice were placed singly into 30×30 cm glass cylinders immediately after i.p. injection of naloxone. The number of vertical jumping was recorded during a 30 min observation period. The positive jumping response was defined as jumping more than 4 times within a 30 min period.

Statistics

ED_{50} and 95% confidence limited for dose-response curves were calculated according to the method of Litchfield and Wilcoxon (10) with the aid of a computer program. The line was obtained by linear regression analysis of the dose-response curve. The correlationship between dosage of morphine and jumping response rate (number of positive animal/number of total animal tested) was analyzed by using Pearson's two-tailed test.

Results

Quantitative determination of physical dependence in morphine-dependent mice

To explore an appropriate dose and administration time of naloxone, effects of different dose and precipitated time of naloxone on jumping response were studied. The results showed that a dose of 2mg/kg naloxone induced vertical jumping beyond 4 times in 95% morphine-dependent mice, the jumping response rate reached maximum at 2hr after a final morphine administration (Fig. 1). This dose and injection time of naloxone were used in subsequent experiments. Fig. 2 showed the curve of percentage of positive jumping and cumulative dose of morphine in naloxone precipitated jumping test. A good linear relationship was obtained between dosage of morphine and jumping response rate with a correlation coefficient of 0.96 ($P<0.01$).

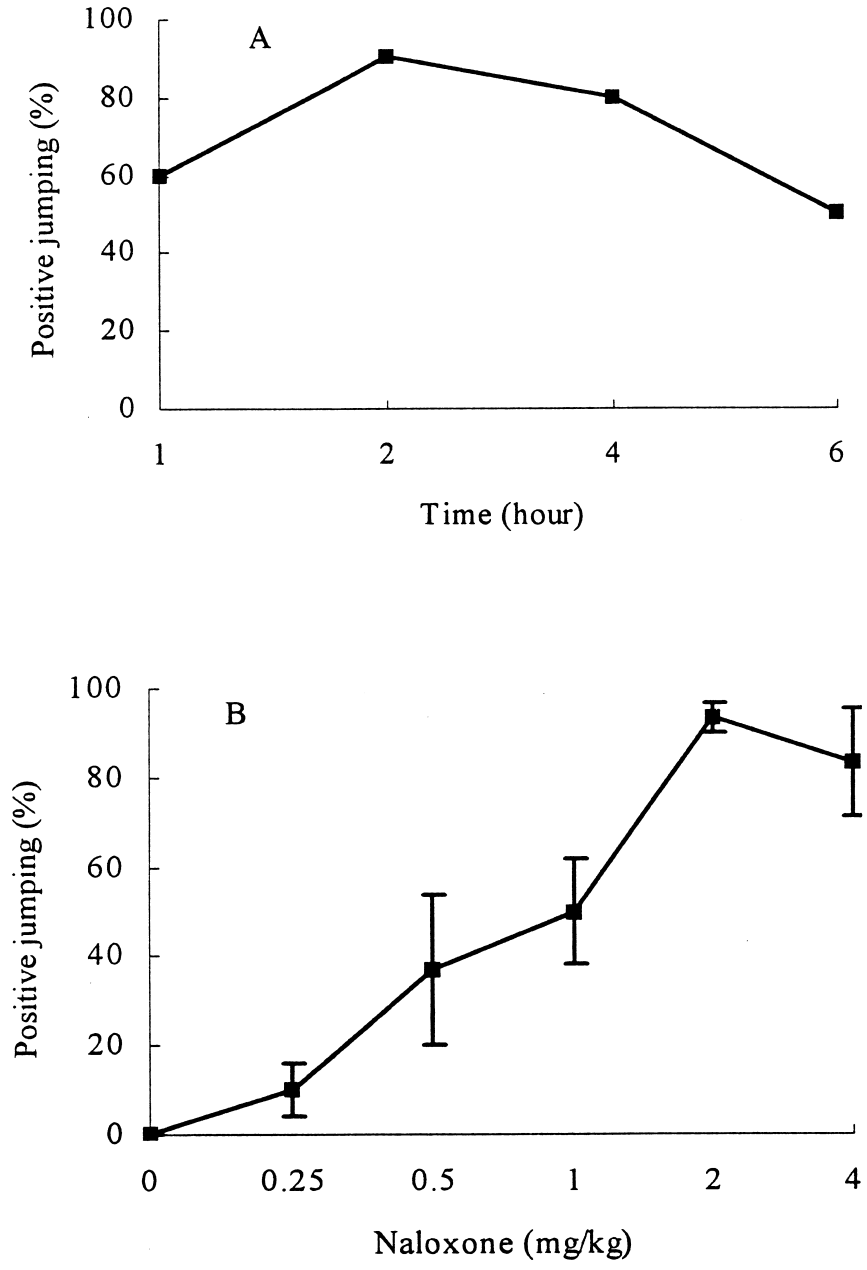


Fig. 1. Withdrawal jumping precipitated by naloxone in morphine-dependent mice. The positive jumping response rate denote the number of mice jumped more than 4 times over the total number of mice tested, expressed as percent rate (%). A: naloxone (2mg/kg) was injected at various time (1, 2, 4 and 6 hr) after a final morphine administration; B: naloxone was injected at various doses (0.25, 0.5, 1.0, 2.0 and 4.0 mg/kg) at 2 hr after a final morphine administration. Data were expressed as mean±SEM, n=3.

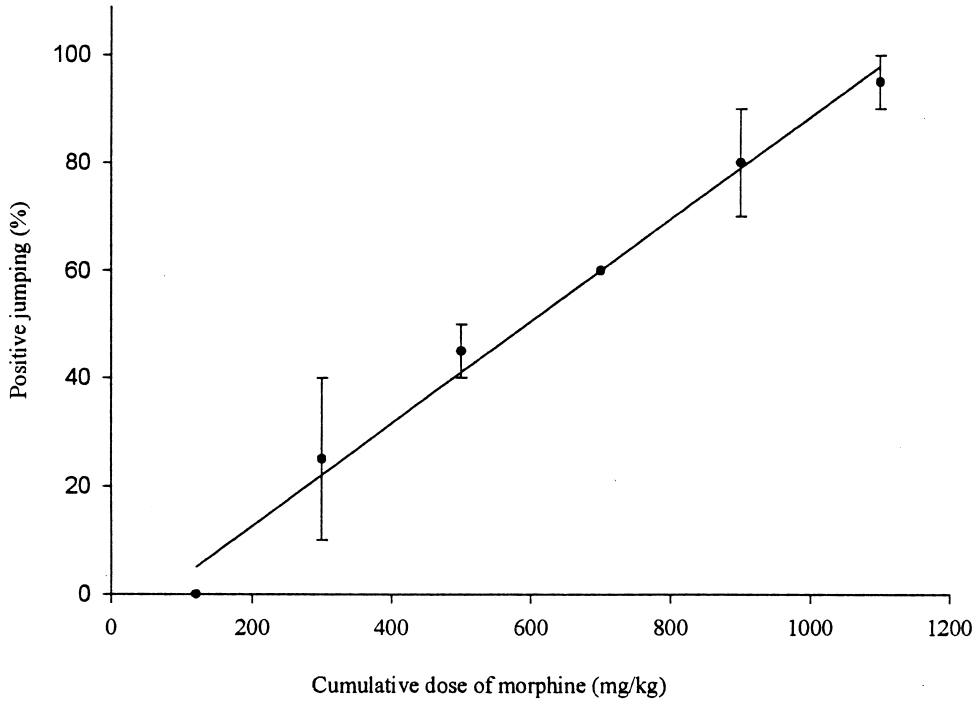


Fig. 2. Dose-response curve of morphine dependence mice in naloxone precipitated jumping test, each point represents the mean±SEM of three experiments for 10 mice in each group.

Liability of physical dependence of ohmefentanyl stereoisomers

Except F9201, All isomers showed the physical dependence liability. The syndrome elicited by the antagonist naloxone in ohmefentanyl dependent mice resembles that in morphine dependent mice in many respects. On basis of ED_{50} value, the order of physical dependent potency of these isomers was F9202>F9205>F9208>F9204>F9206>F9207>F9203> morphine. Withdrawal jumping of mice pretreated with F9201 was not observed in the dosage up to 60mg/kg, which elicited severely toxic signs, and death of a few mice. To assess relative dependence capacity, dependence potency index (DPI) was calculated as withdrawal jumping cumulative ED_{50}/analgesic ED_{50}. Higher DPI indicates relatively lower dependent capacity. DPI of F9204 is maximal, over 618-fold higher than that of F9202, another extremely potent analgesic. Fig. 3 showed the curve of percentage of positive jumping and cumulative dose of F9202 and F9204 in naloxone precipitated jumping test We have reported on the analgesic activity of ohmefentanyl stereoisomers (9), these data were collected in the present study (Table 1).

Discussion

Morphine withdrawal syndrome were typically produced by either terminating chronic morphine exposure or by administering an opiate antagonist to morphine pretreated animals.

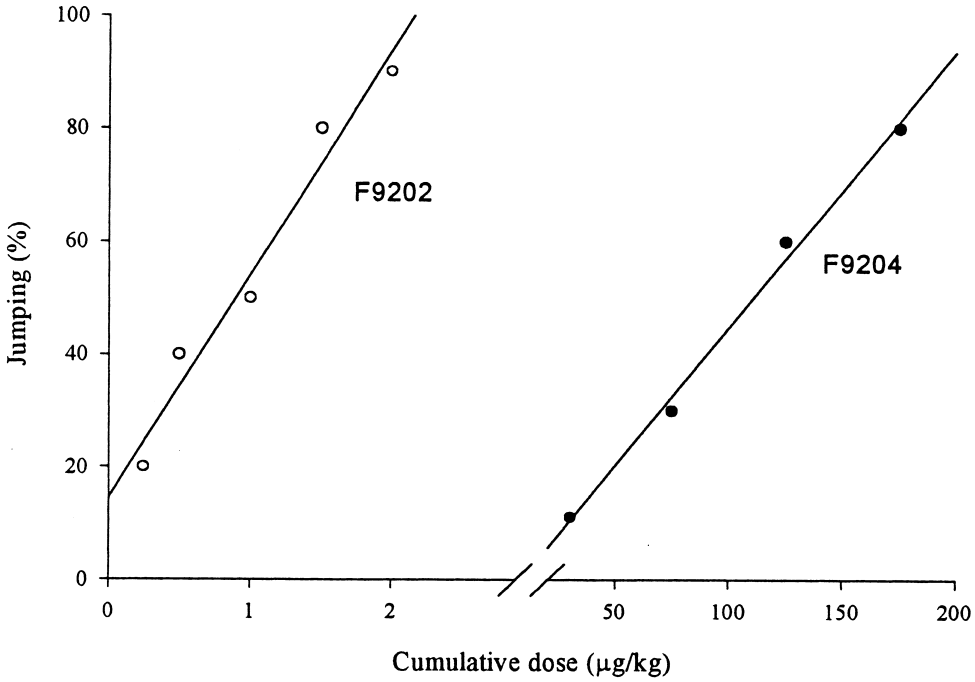


Fig. 3. Dose-response curve of withdrawal jumping induced by the i.p. administration (2mg/kg) of naloxone in mice chronically pretreated with a progressively increasing dose of ohmefentanyl stereoisomers (F9202 and F9204). Data are values obtained from 10 mice in each group, as described in the Method section.

The stereotyped jumping response has been shown to be a highly characteristic sign of morphine withdrawal syndrome (11), and the vertical jumping test has been used to estimate the degree of physical dependence (12). Generally, the degree of physical dependence was assessed by the intensity of withdrawal signs, or by the amount of naloxone required to precipitate the withdrawal jumping (11–13). However, the former is only used to compare relatively with the dependence of morphine, the later is limited by the dose range of naloxone. Therefore, the quantification of the degree of physical dependence is not direct, it is difficult for comparing physical dependence capacity among several drugs. To quantify the physical dependence capacity of morphine, and to compare the difference of ohmefentanyl stereoisomers in the physical dependence, Cumulative dose of morphine and ohmefentanyl stereoisomers inducing 50% positive jumping response in naloxone precipitated jumping test were measured. Our study indicated that there was a good correlationship between the dosage of morphine and jumping response rate, ED_{50} value of morphine can be measured according to the dose-response curve. Ohmefentanyl has been reported to produce tolerance quickly (2). In this study, the tolerance of ohmefentanyl stereoisomers has not been compared.

Numerous attempts have been made to study the role of various opioid receptor types in development of morphine phisical dependence, mu opioid receptors are known to play a major role in the development of morphine dependence (14, 15). Ohmefentanyl is an effective analgesic agent derived from fentanyl and exhibits a good analgesic efficacy comparable to

Table 1
Analgesic and withdrawal jumping ED_{50} values of ohmefentanyl stereoisomers

Compound	Absolute Configuration	Analgesic ED_{50} (95% C.L., mg/kg)[a] (9)	Withdrawal Jumping Cumulative ED_{50} (95% C.L.,mg/kg)	Dependence Potency Index[b]
F9204	(+)-*cis*-(3R,4S,2′S)	0.00106 (0.0008–0.0013)	0.0984 (0.0684–0.141)	92.8
F9207	(+)-trans-(3S,4S,2′R)	0.075 (0.064–0.087)	0.383 (0.233–0.629)	5.1
F9206	(−)-trans-(3R,4R,2′R)	0.072 (0.057–0.091)	0.361 (0.211–0.617)	5.0
F9208	(−)-trans-(3R,4R,2′S)	0.0097 (0.006–0.15)	0.0445 (0.0282–0.0701)	4.6
F9205	(+)-trans-(3S,4S,2′S)	0.014 (0.011–0.017)	0.0303 (0.0197–0.0466)	2.1
F9202	(−)-*cis*-(3R,4S,2′R)	0.0046 (0.0031–0.0066)	0.0007 (0.0004–0.0010)	0.15
F9203	(−)-*cis*-(3S,4R,2′R)	>10 (0/10)	9.941 (6.111–16.17)	<1
F9201	(+)-*cis*-(3S,4R,2′S)	10 (4/10)	>60	>6
Morphine		6.8 (5.5–8.4)	513 (424–620)	75.4

[a] C.L. = confidence limits.
[b] Dependence potency index was obtained by withdrawal jumping cumulative ED_{50}/analgesic ED_{50}.

morphine and fentanyl in animal experiments. In the bioassay, ohmefentanyl also showed to be a potent μ-receptor agonist with high selectivity and affinity (9). This study reveals that physical dependence induced by ohmefentanyl stereoisomers displays a significant difference as with their analgesic activity, it was indicated that there were perhaps stereo-structural requirements for physical dependence at mu receptor levels. By comparing the analgesic and withdrawal jumping ED_{50} of ohmefentanyl stereoisomers, it is shown that analgesic action and physical dependent capacity of ohmefentanyl stereoisomers have great difference. Isomer F9204 and F9202 exhibited extremely potent analgesic activity and high affinity and selectivity for mu receptors. It was considered that (3R, 4S) configuration at piperidine 3- and 4-carbon was very important for analgesic action. In the earlier study, Brine et al. (16) also reported on marked differences in the functional properties of four ohmefentanyl stereoisomers that are quite consonant with the present study. However, it is interesting that F9204 and F9202 have similar stereo-structure, mu and delta receptor affinity and analgesic action, but withdrawal jumping ED_{50} value of F9204 is 140-fold higher than that of F9202. DPI of F9204 is 618-fold higher than that of F9202. The results indicate that physical dependent potency of F9204 is low relatively. Similarly to the properties of the fentanyl isomer F9204, the potent opioid analgesics, etorphine and dihydroetorphine, also show much higher analgesic action and much lower physical dependence than morphine both in rodents (17–20) and monkeys (21). In addiction, we found that the physical dependent capacity was observed by administration of low dosage of compound F9203, which exhibited no analgesic action at dose of 10mg/kg. It is suggested that there is a different mechanism between analgesic and dependence of these compounds.

In conclusion, it is convenient and feasible to use the ED_{50} value of drugs to assess and compare the dependence liability in mice. There are significant differences for the degree of physical dependence of ohmefentanyl stereoisomers. Two most potent isomers, F9204 and F9202, which had significant difference in physical dependence, may be proposed as useful tools in the study of the mechanism of opioid dependence.

Acknowledgments

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