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Potent 3-methylfentanyl analogs: morphine-like catalepsy and receptor binding characteristics

XU Heng, YAO Yi-He, ZHU You-Cheng, CHEN Jie, CHI Zhi-Qiang

(Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031)

ABSTRACT Ohmefentanyl and analogs caused morphine-like catalepsy. The cataleptic activity was correlated with the binding affinity for μ opioid receptor, but there was no correlation between cataleptic activity of the compounds and their δ receptor affinity. Among these compounds, F8608 displayed the best binding selectivity for μ opioid receptor. This new compound may be proposed as an useful tool in studying of μ opioid receptor. Present studies confirm the earlier observation that ohmefentanyl is a new agonist selective for μ opioid receptor.

KEY WORDS 3-methylfentanyl; catalepsy; endorphin receptors; radioligand assay

The opioid receptors of μ, δ and κ types were found in the brain[1,2]. Locomotor depression and catalepsy may be mediated by μ opioid receptor, whereas locomotor stimulation is due to an action on δ type receptor[3,4]. We have studied the relationship between analgesic activity and receptor binding affinity for 3-methylfentanyl derivatives[5]. Most of the compounds possess morphine-like analgesic activity. Compound ohmefentanyl (Ohm, *N*[1-(β-hydroxy-β-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropionamide) is of particular interest because it is a potent and selective ligand for μ opioid receptor[6-8].

In this paper, we describe the cataleptic activity and receptor binding characteristics of 3-methylfentanyl analogs, in order to have an insight into the structures required for biological activity and binding selectivity, and to confirm the assumption that catalepsy is mediated by μ opioid receptor.

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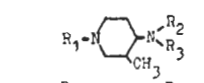
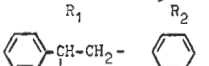
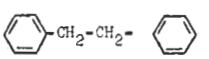
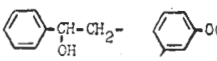
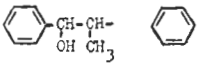
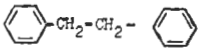
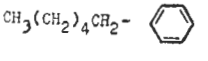
MATERIALS AND METHODS

cis-A F7302 (Ohm), *cis*-F7209 (3-methylfentanyl), F 7207, F 7533, F 8608 and F 8518 were synthesized in our laboratory (Tab 1). Fentanyl citrate was produced by Yichang Pharmaceutical Factory of Hubei. [^{3}H](D-Ala^2, MePhe^4, Gly-ol^5)-enkephalin ([^{3}H]DAGO, 2.22 TBq/mmol) and [^{3}H](D-Pen^2, D-Pen^5) enkephalin ([^{3}H]DPDPE, 1.04 TBq/mmol) were purchased from Amersham.

Tab 1. Structures of 3-methylfentanyl derivatives



	R_1	R_2	R_3
F 7302			COEt
F 7209			COEt
F 7533			COEt
F 7207			COEt
F 8608			COOEt
F 8518			COEt

Male rats (198±SD 22 g) and mice (20.8±2.5 g) were decapitated and the

brains without cerebellum were homogenized in 1:10 (wt:vol) of ice-cold sucrose 0.32 mol/L. The brain homogenates were centrifuged for 10 min at 1000 × g. The supernatants were incubated for 30 min at 30℃ and centrifuged for 30 min at 36 000 × g (4℃). The pellets were.washed with 0.05 mol/L Tris-HCl buffer (pH 7.4), recentrifuged for 30 min at 36 000 × g and suspended in ice-cold 0.05 mol/L Tris-HCl buffer and homogenized. The concentration of protein was determined by the method of biuret reaction[9].

Opioid receptor binding assays were made[6]. The binding of [^{3}H]DAGO (1.0-1.5 nmol/L) and [^{3}H]DPDPE (2.5-3.0 nmol/L) to rat (or mouse) brain membranes were used as the specific labels for μ- and δ-sites, respectively. The specific binding of [^{3}H]DPDPE and [^{3}H]DAGO were determined as the difference in the counts obtained in the presence as well as absence of unlabelled levorphanol (2 μmol/L) or etorphine (1 μmol/L). All samples were tested in triplicate determinations from pooled membranes. Data represented the mean of triplicate determinations and the error was less than 10%. Each experiment was repeated 2-3 times with similar results.

The cataleptic activity of 3-methylfentanyl analogs in rats (♂ and ♀, 204±21 g) was determined[10]. The catalepsy test was performed in mice (♂ and ♀, 19.1±0.9 g)[4]. The intracerebroventricular injection were operated in conscious mice. Drugs in 10 μl saline was injected (icv) in approximately 5 s. To assess whether the mouse presented an immobility state, mouse was placed with its head downward on a wire netting in an inclined (45°) plane. It was positive if the mouse stayed in this position for at least 30 s.

RESULTS

Analgesic activity and receptor binding affinity of compound F 8608 and F 8518 Structure-activity relationship study (Tab 2) showed that F8608 and F 8518 possessed a morphine-like analgesic activity. The analgesic ED_{50} (mice, hot plate test) of F8518 and F8608 were 268 and 110 μg/kg, respectively. Binding assay showed that these 2 compounds specifically displaced [^{3}H]etorphine binding to mouse brain membranes, displaying binding affinity for opioid receptors.

Tab 2. Analgesic activity (mice, ip hot plate) and receptor binding affinity of compounds F8518 and F8608.

	ED_{50} (mg/kg, 95% fiducial limits)	[^{3}H]etorphine (0.35 nmol/L) IC_{50} nmol/L n = 2 expts
F8518	0.268 (0.191—0.376)	50.1 54.9
F8608	0.110 (0.087—0.142)	56.2 55.6

Comparison of the relative activity of 3-methylfentanyl analogs in rats Ohm and analogs caused morphine-like catalepsy in rats. A dyskinesia and muscular rigidity in rats was observed after ip these compounds. The rank order of potencies in producing catalepsy was: F7302>F7209>F7533>F 7207>Fentanyl>F 8608>F 8518 and was the same as that in their analgesic activity[5]. The cataleptic activity of these compounds was correlated with their binding affinity for μ opioid receptors (Tab 3). When IC_{50} of these compounds in inhibiting [^{3}H]DAGO binding was plotted against their cataleptic ED_{50}, a good linear relationship was obtained (r = 0.92) (Fig 1).

Comparison of the relative activity of 3-methylfentanyl analogs in mice The cataleptic activity and receptor binding affinity of 3-methylfentanyl analogs in mice were shown in Tab 3. Though opioid-induced catalepsy in mice was not so obvious as in rats, an immobility state verging on the catalepsy syndrome developed in rats was observed after icv these compounds. The

Tab 3. Cataleptic activities and receptor binding affinities of 3-methylfentanyl analogs in rats and mice.

	Rats				Mice			
	ED_{50}ip 100 nmol/kg(95% fiducial limits)	[^{3}H]DAGO IC_{50} nmol/L	[^{3}H]DPDPE IC_{50} nmol/L	$\frac{IC_{50}\ DPDPE}{IC_{50}\ DAGO}$	ED_{50} icv 100 pmol/mouse(95% fiducial limits)	[^{3}H]DAGO IC_{50} nmol/L	[^{3}H]DPDPE IC_{50} nmol/L	$\frac{IC_{50}\ DPDPE}{IC_{50}\ DAGO}$
F7302	0.23 (0.18—0.27)	0.19	89.1	481	0.28 (0.26—0.31)	0.22	79.4	363
F7209	0.65 (0.60—0.69)	0.49	56.2	114	2.91 (2.65—3.21)	0.59	30.0	51
F7533	1.53 (1.43—1.63)	0.98	126	129	4.25 (3.80—4.75)	1.78	141	79
F7207	4.20 (3.94—4.46)	1.07	—	—	7.16 (6.49—7.90)	6.31	—	—
Fentanyl	9.82 (8.63—11.0)	3.47	>100	>30	13.9 (12.3—15.8)	6.31	631	100
F8608	12.0 (10.8—14.7)	1.26	>1000	>700	6.33 (5.72—7.01)	3.16	3160	1000
F8518	15.6 (14.6—16.7)	3.16	1070	339	17.9 (16.8—19.1)	7.41	1050	142

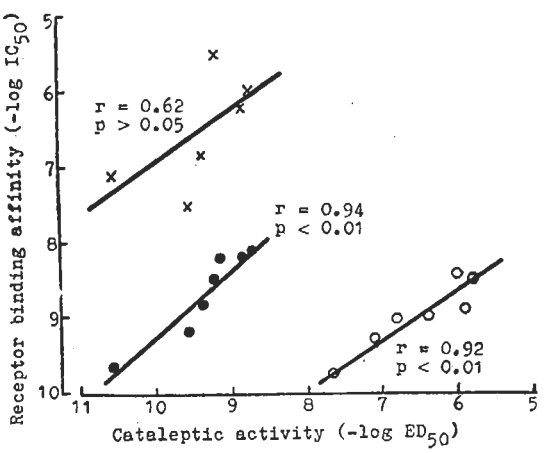


Fig 1. Correlation between cataleptic activity and receptor binding affinity for [^{3}H]DAGO (μ) in rats (○), for [^{3}H]DAGO in mice (●), and for [^{3}H]DPDPE (δ) in mice (×).

data in Tab 3 showed a good correlation between cataleptic activity and binding affinity for μ opioid receptors (r = 0.94) (Fig 1).

DISCUSSION

These results confirm our earlier observations[6-8] that ohmefentanyl is a new agonist selective for μ opioid receptors. This conclusion is supported by the data from Goldstein's laboratory that ohmefentanyl is more μ-selective than sufentanyl, about the same as DAGO (personal communication, 1986). Ohmefentanyl and analogs caused morphine-like catalepsy. The cataleptic activity was correlated with the binding affinity for μ opioid receptors, but there was no correlation between cataleptic activity and δ receptor affinity (Fig 1). The results showed that cataleptic activity produced by 3-methylfentanyl analogs is mainly mediated by μ opioid receptors. However, participation of other subtypes of opioid receptors in mediating cataleptic effect are still possible.

Binding assay showed that these compounds have higher affinities for μ sites than δ sites, but the μ-selectivity is different. Ohmefentanyl is more μ-selective than fentanyl and 3-methylfentanyl. The inhibitory potency of F8608 (*N*-[1-(2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylurethan) on [^{3}H]DAGO is at least 700 times greater than that on [^{3}H] DPDPE,

showing its best binding selectivity for μ sites. It may be related to the structural features. The compound F8608 possesses fundamental structure requirements for μ opioid receptors. This new compound may be proposed as an useful tool for the studies of μ opioid receptors.

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强的 3-甲基芬太尼类似物：吗啡样的僵住症以及受体结合特性

徐 珩、姚一禾、朱友成、陈 洁、池志强 （中国科学院上海药物研究所，上海 200031）

提要 羟甲芬太尼及其类似物产生吗啡样的僵住症，这种僵住活性相关于对 mu 阿片受体的结合亲和力，但是化合物的僵住活性与它们对 δ 受体的亲和力之间没有相关性。这些化合物中，F8608 呈现了对 mu 阿片受体最好的结合选择性，这个新的化合物有可能用作为研究 mu 阿片受体的工具药。目前的研究确认了以前的观察，即羟甲芬太尼是一个新的选择性的 mu 阿片受体激动剂。

关键词 3-甲基芬太尼；强直性木僵；内啡肽受体；放射配体测定