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PII S0024-3205(99)00404-X

Life Sciences, Vol. 65, No. 15, pp. 1589-1595, 1999

0024-3205/99/$–see front matter

ANALGESIC ACTIVITY AND SELECTIVITY OF ISOTHIOCYANATE DERIVATIVES OF FENTANYL ANALOGS FOR OPIOID RECEPTORS

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(Received in final form June 17, 1999)

Summary

The analgesic activity and opioid receptor binding characteristics were studied for the isothiocyanate ohmefentanyl (OMFIT), and isothiocyanate carfentanil (CarFIT), isothiocyanate 4-methoxymethylfentanyl (MethoFIT), isothiocyanate 3-methyl-fentanyl (superFIT) and their amide analogs. Antinociceptive activity was evaluated using the mouse hot plate test; selectivity for opioid receptor was determined in bioassay and binding assay. SuperFIT, CarFIT, OMFIT and MethoFIT exhibited an analgesic ED_{50} lower than those of their parent compounds without isothiocyanate (SCN) group. Furthermore these compounds exhibited potent inhibitory actions on the electrically evoked contractions of mouse vas deferens, which could be antagonized by naloxone, but their actions were weaker than those of their parent compounds without SCN-group. The inhibitory actions of these compounds on binding of [^{3}H]OMF to mouse brain membrane was weaker than those of their parent compounds without SCN-group. CarFIT and MethoFIT showed weaker inhibitory actions on the binding of [^{3}H] DADLE than their parent compounds without SCN-group, but SuperFIT and OMFIT stronger than their parent compounds, 3-methylfentanyl and ohmefentanyl. The selectivity of these isothiocyanate derivatives for δ opioid receptors increased. In conclusion, introducing isothiocyanato-group into 1-position of phenyl ring of ohmefentanyl and other fentanyl analogs would enhance the selectivity of these compounds for δ-opioid receptors, but decrease their analgesic activity.

Key Words: isothiocyanate derivatives, fentanyl analogs, analgesia, opioid receptors, binding selectivity, bioassay

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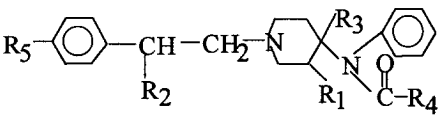
Probes with exclusive specificity for δ receptors have been heretofore unavailable, although several affinity reagents based on enkephaline (1) and naltrindole (2) have been prepared. Burke and colleagues had described the δ specific ligands isothiocyanato-fentanyl (FIT)(3) and isothiocyanato-methylfentanyl (SuperFIT)(4), derivatives of the potent opioid agonists fentanyl and 3-methylfentanyl. Ohmefentanyl (OMF) is a potent and highly selective agonist for μ opioid receptors, which was designed and synthesized in our laboratory (5). In this paper, we further investigate analgesic activities and opioid receptor binding characteristics of isothiocycanate (SCN) derivatives of ohmefentanyl and other fentanyl analogs, carfentanil, methomethylfentanyl, 3-methylfentanyl (6) and their amide analogs, since these isothiocyanate derivatives (7), like FIT and SuperFIT, may be changed to selective irreversibly ligands for δ opioid receptors.

Table 1

Structure of isothiocyanate derivatives of fentanyl analogs



Compound	Absolute configuration	R_1	R_2	R_3	R_4	R_5
3-methylfentanyl	3*R*,4*S*	CH_3	H	H	C_2H_5	H
Carfentanil		H	H	COOME	C_2H_5	H
Cis-±-Ohmefentanyl	3*R*,4*S*,2'*S*+3*R*,4*S*,2'*R*	CH_3	OH	H	C_2H_5	H
Methomethylfentanyl		H	H	CH_2OME	C_2H_5	H
SuperFIT	3*R*,4*S*	CH_3	H	H	C_2H_5	SCN
CarFIT		H	H	COOME	C_2H_5	SCN
Cis-±-OMFIT	3*R*,4*S*,2'*S*+3*R*,4*S*,2'*R*	CH_3	H	H	C_2H_5	SCN
MethoFIT		H	H	CH_2OME	C_2H_5	SCN
F9307		H	H	COOME		SCN
F9308		H	H	CH_2OME		SCN
F9317		H	H	COOME		SCN
F9318	3*R*,4*S*	CH_3	H	H		SCN
F9319		H	H	CH_2OME		SCN
F9320	3*R*,4*S*	CH_3	H	H		SCN
F9321	3*R*,4*S*	CH_3	H	H		SCN
F9323		H	H	CH_2OME		SCN

Materials and Methods

Cis-(±)-Ohmefentanyl (OMF), *cis*-3-methylfentanyl isothiocyanate (SuperFIT), *cis*-(±)-ohmefentanyl isothiocyanate (OMFIT), carfentanil isothiocycanate (CarFIT), methoxymethylfentanyl isothiocycanate (MethoFIT) and amide analogs of superFIT, CarFIT and MethoFIT, were synthesized by chemical group in our laboratory. These compounds were analyzed by chemical methods to identify their chemical structure and purity (>99%). Naloxone was obtained from Shanghai Medical University. [^{3}H]OMF ([^{3}H]ohmefentanyl, 54 C_i/mmol) was

labeled by Shanghai Institute of Nuclear Research, Chinese Academy of Sciences. [^{3}H]DADLE {[^{3}H](D-Ala^2, D-Leu^5)-enkaphalin, 33.5 μCi/μmol} was purchased from Dupont Co.

The Kunming strain mice (No. 9302) were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences.

Antinociceptive assay: Antinociceptive activities of these compounds were measured after subcutaneous injection in the mouse hot-plate assay as Eddy described (8). The number of animals in each dose group is 10. ED_{50} values were calculated using the method of Blis.

Bioassay: The mouse vas deferens (MVD) were prepared as described by Hughes et al. (9). The preparations were suspended in organ bath containing 4ml Krebs solution. The bath fluid was kept at 37°C±1°C and gassed continuously with 95% O_2 and 5% CO_2. The resting tension was maintained at 250 mg. The composition of Krebs solution contained (in mmol/L): NaCl 119, KCl 4.7, $CaCl_2$ 2.55, KH_2PO_4 1.6, $NaHCO_3$ 25, glucose 11. After equilibration for 30 min, longitudinal contractions were evoked by field stimulation through Pt-electrodes at the upper and lower ends of the bath. The stimulation trains consisted of 4 pulses at intervals of 200 ms (40 V, 1.0 ms duration, 15 s interval). The contractions were recorded by means of a force displacement transducer and recorder. The agonist potencies of these compounds were obtained from dose-response curves by calculating the concentration of the compound that reduced the height of the contractions by 50% (IC_{50}). The equilibrium dissociation constant, K_e values of antagonist were determined by the single dose method of Kosterlitz and Watt (10).

Binding assay: Mouse brain homogenates were prepared as described by Xu et al. (5). The μ and δ binding sites were determined with the selective μ ligand, [^{3}H]OMF and the selective δ ligand, [^{3}H]DADLE (0.5μmol/L DPDPE was added to prevent [^{3}H]DADLE binding to μ receptors), respectively. Nonspecific binding was measured by incubation in the presence of 0.5 μmol/L OMF or 30 μmol/L naloxone. The binding assays were conducted at 30°C in 0.05 mol/L Tris-HCl buffer (pH7.4), and each assay mixture (1 ml) contained 1.5 mg of crude synaptic plasma membranes of mouse brain. After a 30 min incubation, the samples were immediately cooled in an ice-bath and filtered through Whatman GF/B glass fiber filters on Millipore model 1225 sampling manifolds. The samples were washed with three 4 ml portions of ice-cold Tris-HCl buffer. The fiber filters were dried, and transferred to counting vials with scintillation cocktail. The radioactivity was determined by a model LS6500 Beckman multi-purpose scintillation counter. The IC_{50} values (the concentration which inhibited 50% binding of the [^{3}H]ligand by drug) were estimated by linear regression from logit method. The corresponding K_i (equilibrium dissociation constant of inhibitors) values were calculated according to the formula $K_i = IC_{50} / (1 + L/K_d)$ in which L is the concentration and K_d is dissociation constant of labeled ligand. The protein concentrations were determined by the method of Lowry (11).

Results

Antinociceptive activity: A summary of analgesic activities of superFIT, CarFIT, OMFIT, MethoFIT and their amide analogs is presented in Table 2. Antinociceptive activities of SuperFIT, CarFIT, OMFIT and MethoFIT were lower than those of their parent compounds without SCN-group, 3-methylfentanyl, carfentanil, ohmefentanyl and methomethylfentanyl, respectively. The analgesic activities of OMFIT and SuperFIT were 150 and 16 times lower than those of their parent compounds, OMF and 3-methylfentanyl, respectively. The amide analogs of SuperFIT,

CarFIT and MethoFIT were less potent. Except F9318 and F9320, the analgesic ED_{50} values of other amide analogs of SuperFIT, CarFIT and MethoFIT were more than 5 mg/kg.

Table 2

Analgesia ED_{50} (mouse hot plate, mg/kg, $n = 10$) of isothiocyanate derivatives of fentanyl analogs

Compound	ED_{50}	95% Confidence limits
3-methylfentanyl	0.021	0.019 - 0.023
Carfentanil	0.00041[a]	0.00029-0.00058[a]
Ohmefentanyl	0.0018	0.0019-0.0019
Methomethylfentanyl	0.00078[a]	0.00060 - 0.0010[a]
SuperFIT	0.33	0.21 - 0.51
CarFIT	0.46	0.34 - 0.63
OMFIT	0.53	0.43 - 0.65
MethoFIT	0.55	0.45 - 0.67
F9317	>5	
F9318	4	3.27 - 6.54
F9319	>5	
F9320	1.02	0.79 - 1.31
F9321	>5	
F9323	>5	

[a] Van Bever WFM et al. (12)

Table 3

The inhibitory action (IC_{50}, nmol/L) and naloxone K_e (nmol/L) values of some isothiocyanate derivatives of fentanyl analogs on electrically evoked contraction of mouse vas deferens (MVD), mean ± SD, $n = 4$

Compound	IC_{50}	K_e
3-methylfentanyl	2.19 ± 0.19	2.94 ± 0.72
Carfentanil	17.30 ± 8.00[a]	
Ohmefentanyl	0.89 ± 0.19	1.40 ± 0.30
Methomethylfentanyl	1200 ± 700[a]	
SuperFIT	22.29 ± 6.90	1.52 ± 1.12
CarFIT	21.28 ± 9.80	2.09 ± 1.07
OMFIT	28.29 ± 11.93	1.35 ± 0.51
MethoFIT	97.63 ± 27.03	1.99 ± 0.89
F9307	43.75 ± 22.41	1.32 ± 0.63
F9308	90.89 ± 8.00	1.08 ± 0.02
F9317	1234 ± 917	2.60 ± 2.27
F9318	>1000	
F9319	>1000	
F9320	32.49 ± 13.24	2.67 ± 0.42
F9321	92.30 ± 20.99	4.31 ± 0.53
F9323	195.9 ± 67.56	4.12 ± 0.07

[a]Maguire P. et al. (13)

Bioactivities in mouse vas deferens: The inhibitory actions of these compounds on electrically evoked contraction in MVD were shown in Table 3. Except F9318 and F9319, these compounds displayed inhibitory actions, and this action could be antagonized by naloxone. The activities of SuperFIT, OMFIT, CarFIT and MethoFIT were lower than those of the parent compounds without SCN-group, 3-methylfentanyl, ohmefentanyl, carfentanIl and methoxymethylfentanyl. Except F9320 and F9307, other amide analogs of SuperFIT, CarFIT and MethoFIT were less potency. The maximum inhibition of these compounds for the electrically evoked contractions in MVD was about 80 percent. In addition, the inhibitory actions of these compounds on MVD could not be easily washed out.

Binding assay: In binding studies using [^{3}H]DADLE and [^{3}H]OMF, some fentanyl isothiocyanate derivatives binding to mouse brain opioid receptors possessed good affinity (Table 4). The affinities of binding to μ opioid receptor for SuperFIT, CarFIT, OMFIT and MethoFIT were lower than those of their parent compounds, 3-methylfentanyl, carfentanil, ohmefentanyl and methomethylfentanyl respectively, but the affinities of binding to δ receptor for superFIT and OMFIT were higher than those of their parent compounds, 3-methylfentanyl and ohmefentanyl. Although the affinities of CarFIT and MethoFIT binding to δ opioid receptors were lower than those of their parent compounds, carfentanil and methomethylfentanyl, their ratios of μ/δ affinity were significantly higher than those of their parent compounds. Except F9308 and F9317, the affinities of furyl, thienyl and tetrahydrofuryl analogs of SuperFIT, CarFIT and MethoFIT to δ opioid receptors were lower than those of SuperFIT, CarFIT and MethoFIT, and the affinities of F9318 and F9320 to μ opioid receptors were more potent than affinities to δ opioid receptors.

Table 4

K_i values (nmol/L) and selectivity of some isothiocyanate derivatives of fentanyl analogs for μ and δ opioid receptors in mouse brain membrane , mean ± SD, n = 4

Compound	[^{3}H]-OMF(μ)	[^{3}H]-DADLE(δ)	$K_{i(\mu)}/K_{i(\delta)}$
3-methylfentanyl	0.49 ± 0.23	56.20 ± 11.34	0.0021
Carfentanil	0.024 ± 0.004	3.30 ± 0.04	0.0072[a]
Ohmefentanyl	0.18 ± 0.10	89.10 ± 0.80	0.0088
Methomethylfentanyl	0.09 ± 0.01	23.00 ± 3.00	0.0039[a]
SuperFIT	7.65 ± 1.76	3.66 ± 1.03	2.09
CarFIT	12.00 ± 6.28	15.40 ± 9.84	0.75
OMFIT	11.60 ± 2.09	18.90 ± 3.07	0.4
MethoFIT	24.10 ± 1.32	122.67 ± 65.86	0.19
F9307	25.63 ± 6.87	16.37 ± 5.64	1.57
F9308	22.10 ± 6.62	12.53 ± 0.92	2.35
F9317	53.70 ± 27.04	76.86 ± 31.20	0.69
F9318	8.76 ± 1.55	1048 ± 255	0.008
F9319	954 ± 322	1680 ± 573	0.57
F9320	23.47 ± 6.73	356 ± 73.7	0.07
F9321	123.3 ± 97.7	237.3 ± 58.26	0.52
F9323	141.5 ± 62.65	195.6 ± 78.4	0.72

[a]Maguire P. et al (13)

Discussion

It has been reported that many fentanyl analogs are μ–opioid receptor selective ligands (13). Our results revealed that the isothiocyanato-group introducing into 1-position of fentanyl analogs, could increase their selectivity for δ -opioid receptor, although decrease their analgesia. The compounds showed higher affinities for δ-opioid receptors and less for μ-opioid receptors than their parent compounds without SCN-group. These results were consisted with the results from Burke (3-4). In addition, Burke also reported that FIT and SuperFIT had irreversible binding to opioid receptor and might resist to be washed out (3-4). Our results displayed that the actions of SuperFIT, CarFIT, OMFIT, MethoFIT and their amide analogs on MVD could not be easily washed.

Brine reported the biological evaluation of 2- and 3-furancarboxamide, 2-and 3-thiophenecarboxamide derivatives of stereoisomers of ohmefentanyl and found some of such compounds were more selective for μ opioid receptor than the parent compound ohmefentanyl (14), but our results showed that the furyl, thienyl and tetrahydrofuryl group modifications of SuperFIT, CarFIT and MethoFIT were not beneficial to δ receptor binding affinity.

Sulfhydryl groups in sulfhydryl-containing amino acids (cysteine or lysine) in opioid receptors, which may control the conformation of opioid receptors, are known to interact irreversibly with the isothiocyanate moiety in opioid ligands to form a covalent bond. The likely explanation for observed selectivity of these isothiocyanate derivatives in receptor acylation is that the bound drug is orientated in the μ opioid receptor matrix with the electrophilic isothiocyanate moiety located too distant from a suitably reactive nucleophole for acylation to occur, in contrast to its interaction with δ opioid receptor where a nucleophile appears to have ready access to the electrophilic isothiocyanate moiety (15). The binding domain may be the segment from beginning of the first intracellular loop to the middle of the third transmembrane helix of the δ receptor (16).

Matthes and collegues (17) showed that mice lacking the μ-opioid receptor do not show any of the normal response to morphine and those mice did not become dependent on morphine after repeated treatment with the drug. So it seems that the key effects of morphine as both an analgesic and an additive euphoriant agent are completely lacking in the μ-opioid receptor knockout mice (18). The analgesic action and affinity for μ opioid recptors of OMFIT and SuperFIT are about 16 and 150, and 15 and 64 times less potent than those of their parent compounds, OMF and 3-methylfentanyl, respectively. It is indicated that the attenuation of the analgesic action for μ opioid recptor resulted in the reduction of affinity of OMFIT .

In conclusion, data in this paper and previous findings together with FIT and SuperFIT suggested that the further modification of FIT, SuperFIT and OMFIT structures will provide some approaches to develop δ specific agents.

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