ELSEVIER

European Journal of Pharmacology 278 (1995) 257–260

ejp

Short communication

CB-64D and CB-184: ligands with high σ_2 receptor affinity and subtype selectivity

Wayne D. Bowen *, Craig M. Bertha, Bertold J. Vilner, Kenner C. Rice

Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Received 4 January 1995; revised 15 March 1995; accepted 21 March 1995

Abstract

Four members of a novel class of sigma (σ) ligands were investigated for σ subtype selectivity. (−)-1*S*,5*S*- and (+)-1*R*,5*R*-(*E*)-8-Benzylidene-5-(3-hydroxyphenyl)-2-methylmorphan-7-one (CB-64L and CB-64D, respectively) exhibited σ_1 K_i = 10.5 nM and 3063 nM; σ_2 K_i = 154 nM and 16.5 nM, respectively. The corresponding 3,4-dichloro derivatives, (−)-1*S*,5*S*- and (+)-1*R*,5*R*-(*E*)-8-(3,4-dichlorobenzylidene)-5-(3-hydroxyphenyl)-2-methylmorphan-7-one (CB-182 and CB-184, respectively) were also examined. CB-182 ((−)-isomer) showed σ_1 and σ_2 K_i = 27.3 nM and 35.5 nM, respectively, whereas CB-184 ((+)-isomer) exhibited σ_1 and σ_2 K_i = 7436 nM and 13.4 nM, respectively. Thus, the two σ subtypes showed opposite enantioselectivity for these compounds, with (−) > (+) at σ_1 and (+) > (−) at σ_2. Importantly, CB-64D and CB-184 showed high σ_2 affinity and, respectively, 185-fold and 554-fold selectivity for σ_2 receptors over σ_1. While high σ_2 selectivity relative to σ_1 was achieved with these compounds, they both exhibited high affinity at mu (μ) opioid receptors (K_i = 37.6 nM and 4.5 nM, respectively). Despite this, CB-64D and CB-184 will be useful tools for further characterization of σ_2 receptors.

Keywords: σ Receptor; (Subtype selectivity); Phenylmorphan; Opioid

1. Introduction

Sigma (σ) receptors are unique binding sites located in the nervous system and peripheral organs and which exhibit affinity for a wide variety of structurally and pharmacologically diverse compounds (Walker et al., 1990; De Costa and He, 1994). These include typical neuroleptics, antidepressants, anticonvulsants, antitussives, and some opiate-related compounds. Although the physiological functions of σ sites are not yet completely known, they have been implicated in both the therapeutic effects of these compounds, as well as their side effects.

Recent evidence for σ site heterogeneity has further complicated the comprehension of σ receptor function. σ Receptors exist in at least two subtypes, termed σ_1 and σ_2 (Hellewell and Bowen, 1990; Quirion et al., 1992). σ Receptor subtypes are readily distinguishable pharmacologically by the high affinity of σ_1 sites for dextrorotatory benzomorphans such as (+)-pentazocine, and the low affinity of σ_2 sites for these compounds. These subtypes appear to mediate different effects. For example, σ_1 receptors have been implicated in negative modulation of agonist-stimulated phosphoinositide turnover, whereas σ_2 receptors have been implicated in the motor effects of σ ligands (Walker et al., 1990).

It is imperative that subtype-selective σ ligands be developed to further elucidate the functional roles of these subtypes. However, progress in delineating the differential functions of σ subtypes, particularly σ_2 sites, has been hampered since the vast majority of σ ligands available to date either fail to discriminate the two sites or exhibit selectivity for σ_1 sites. Furthermore, compared to the σ_1 receptor, σ_2 sites appear to have much more restrictive structural requirements for high affinity binding, making the development of σ_2-selective compounds even more difficult.

* Corresponding author. Unit on Receptor Biochemistry and Pharmacology, Laboratory of Medicinal Chemistry, NIDDK/NIH, Bldg. 8, Rm. B1-23, 8 Center Dr MSC 0815, Bethesda, MD 20892-0815, USA. Tel. (301) 402-3375, fax (301) 402-0589.

Elsevier Science B.V.
SSDI 0014-2999(95)00176-X

The 2-methyl-5-(3-hydroxyphenyl)morphans are a class of synthetic analgesic compounds developed by May and coworkers (May and Takeda, 1970). These compounds generally exhibit high affinity for mu (μ) opioid receptors, moderate affinity for kappa (κ) opioid receptors, and little or no affinity for delta (δ) opioid receptors (Froimowitz et al., 1992). Recently we reported that the incorporation of an (*E*)-8-benzylidene moiety into the 2-methyl-5-(3-hydroxyphenyl)morphan produces compounds with decreased opioid binding affinity and greatly increased σ receptor binding affinity (Bertha et al., 1994). Here we have investigated two enantiomeric (*E*)-8-benzylidene-2-methyl-5-phenylmorphan-7-ones for σ receptor subtype selectivity and report the most σ_2 subtype-selective compound currently known.

2. Materials and methods

2.1. Synthesis

Chemical synthesis, optical purity determination, and assignment of absolute configuration for (−)-1*S*,5*S*- and (+)-1*R*,5*R*-(*E*)-8-benzylidene-5-(3-hydroxyphenyl)-2-methylmorphan-7-one (CB-64L and CB-64D, respectively) and for (−)-1*S*,5*S*- and (+)-1*R*,5*R*-(*E*)-8-(3,4-dichlorobenzylidene)-5-(3-hydroxy-phenyl)-2-methylmorphan-7-one (CB-182 and CB-184, respectively) are as described previously (Bertha et al., 1994). The structures of these compounds are shown in Fig. 1.

2.2. Ligand binding assay (σ_1 receptors)

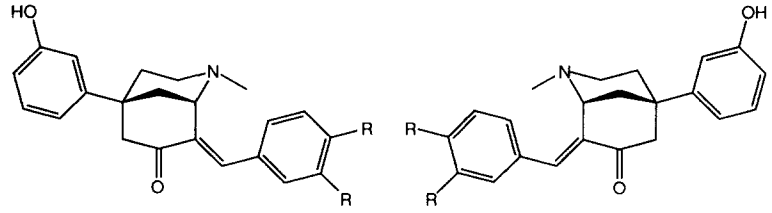
σ_1 Receptors were labeled as described previously, using the σ_1-selective probe [^{3}H](+)-pentazocine and guinea pig brain membranes (Bowen et al., 1993). Guinea pig brain membranes (350–500 μg of membrane protein) were incubated with 3 nM [^{3}H](+)-pentazocine in a total volume of 0.5 ml of 50 mM Tris-HCl, pH 8.0. Incubations were carried out for 120 min at 25° C. Non-specific binding was determined in the presence of 10 μM unlabeled haloperidol. Assays were terminated by dilution with 5 ml of ice-cold 10 mM Tris-HCl, pH 8.0 and vacuum filtration through glass fiber filters using a Brandel cell harvester (Gaithersburg, MD, USA). Filters were then washed twice with 5 ml of ice-cold 10 mM Tris-HCl, pH 8.0. Filters were soaked in 0.5% polyethyleneimine for at least 30 min at 25° C prior to use. Filters were counted in CytoScint cocktail (ICN, Costa Mesa, CA, USA) after an overnight extraction of counts. Membranes were prepared from frozen guinea pig brains (minus cerebella) as previously described (Bowen et al., 1993).

2.3. Ligand binding assay (σ_2 receptors)

σ_2 Receptors were labeled as previously described using rat liver membranes, a rich source of σ_2 sites, and [^{3}H]1,3-di-*o*-tolylguanidine ([^{3}H]DTG) in the presence of 1 μM dextrallorphan to mask σ_1 receptors (Hellewell et al., 1994). Assays were performed in 50 mM Tris-HCl, pH 8.0 for 120 min at 25° C in a volume of 0.5 ml with 160 μg membrane protein and 5 nM radioligand. Assays included 1 μM dextrallorphan to mask σ_1 binding. Non-specific binding was determined in the presence of 10 μM haloperidol. All other manipulations were as described above for the σ_1 receptor assay. Rat liver membranes were prepared from the livers of male Sprague-Dawley rats as previously described (Hellewell et al., 1994).

2.4. Chemicals

[^{3}H](+)-Pentazocine (51.7 Ci/mmol) was synthesized as described previously (Bowen et al., 1993). [^{3}H]DTG (39.1 Ci/mmol) was purchased from DuPont/New England Nuclear (Boston, MA, USA). Dextrallorphan was provided by Dr. F.I. Carroll (Research Triangle Institute, Research Triangle Park, NC, USA). Haloperidol, Tris-HCl, and polyethyleneimine



(-)-1*S*,5*S* (+)-1*R*,5*R*

CB-64L, R = H **CB-64D**, R = H
CB-182, R = Cl **CB-184**, R = Cl

Fig. 1. Structures of the novel (*E*)-8-benzylidene-5-(3-hydroxyphenyl)-2-methylmorphan-7-ones, CB-64L, CB-64D, CB-182, and CB-184.

were purchased from Sigma Chemicals (St. Louis, MO, USA).

3. Results

σ Receptor binding data are shown in Table 1. σ_1 and σ_2 receptors exhibited opposite enantioselectivity for CB-64L and CB-64D ((−)-1*S*,5*S*- and (+)-1*R*,5*R*-isomers, respectively). The σ_1 site was highly enantioselective for the (−)-isomer, whereas the σ_2 site preferred the (+)-isomer. Importantly, a comparison across subtypes reveals a 185-fold selectivity of CB-64D ((+)-isomer) for σ_2 sites over σ_1. In contrast, CB-64L ((−)-isomer) exhibited 15-fold selectivity for σ_1 sites over σ_2.

The 3,4-dichloro derivatives, CB-182 ((−)-1*S*,5*S*-isomer) and CB-184 ((+)-1*R*,5*R*-isomer), were examined. These compounds had similar enantioselectivity profiles as above, with (−) > (+) at σ_1 sites and (+) > (−) at σ_2 sites. Unlike CB-64L which showed selectivity for the σ_1 site, CB-182 had nearly equal affinity for σ_1 and σ_2 receptors, resulting from a concomitant loss in affinity at the σ_1 site and gain in affinity at the σ_2 site relative to CB-64L. A comparison of CB-64D and CB-184 shows that chlorine substitution of the (+)-isomer did not affect the σ_2 affinity, but decreased the σ_1 affinity by 2.5-fold. This resulted in a marked increase in the σ_2 selectivity to 554-fold as seen in CB-184. Relative to σ_1 receptors, CB-64D and CB-184 are among the most σ_2 subtype-selective compounds currently reported.

In a previous study, the binding affinities of these four compounds at μ-opioid receptors was determined (Bertha et al., 1994). This was undertaken since 2-methyl-5-(3-hydroxyphenyl)morphans are known to have high affinity for μ-opioid receptors (Froimowitz et al., 1992). These results are reproduced in the caption of Table 1. That study showed that all four (*E*)-8-benzylidene-5-(3-hydroxyphenyl)-2-methylmorphan-7-ones showed significant affinity for μ-opioid receptors. The 3,4-dichloro derivatives, CB-182 and CB-184, had 8-fold higher μ-opioid receptor affinity than the unsubstituted compounds. The (+)-isomers exhibited 10-fold higher affinity than the corresponding (−)-isomers, which is the same enantioselectivity pattern demonstrated at the σ_2 receptor. Thus, although CB-64D and CB-184 show high σ_2 receptor affinity and subtype selectivity, they are also potent at μ-opioid receptors.

4. Discussion

σ_1 and σ_2 receptors showed opposite enantioselectivity for enantiomeric (*E*)-8-benzylidene-2-methyl-5-phenylmorphan-7-ones, with (−) > (+) at σ_1 receptors and (+) > (−) at σ_2 receptors. Furthermore, marked subtype selectivity and high receptor affinity was exhibited. CB-64D and CB-184 showed 185-fold and 554-fold selectivity, respectively, for σ_2 receptors over σ_1. Conversely, CB-64L showed 15-fold selectivity for σ_1 sites over σ_2.

The affinity of (*E*)-8-benzylidene-2-methyl-5-phenylmorphan-7-ones at other receptors has been investigated previously (Bertha et al., 1994). With regard to other receptors, CB-64D and CB-184 had low or negligible affinity for kappa (κ) opioid, phencyclidine, and muscarinic receptors, with CB-184 having only moderate affinity for delta (δ) opioid receptors (K_i = 271 ± 35 nM) (Bertha et al., 1994). However, both σ_2-selective compounds exhibited significant affinity for μ-opioid receptors (see caption of Table 1), with CB-64D and CB-184 having K_i values at μ-opioid receptors of 37.6 nM and 4.5 nM, respectively (Bertha et al., 1994).

These results are particularly significant in light of

Table 1
Affinities of (*E*)-8-benzylidene-5-(3-hydroxyphenyl)-2-methylmorphan-7-ones at σ_1 and σ_2 receptors

Compound	Configuration	K_i (nM)		σ_1/σ_2
		σ_1	σ_2	K_i ratio
CB-64L	(−)-1*S*,5*S*	10.5 ± 1.6	154 + 3	0.07
CB-64D	(+)-1*R*,5*R*	3063 ± 78	16.5 + 2.7	185
CB-182	(−)-1*S*,5*S*	27.3 ± 2.8	35.5 + 8.8	0.77
CB-184	(+)-1*R*,5*R*	7436 ± 308	13.4 + 2.0	554

Assays were carried out under the conditions described in Materials and methods. Twelve concentrations of unlabeled test ligand ranging from 0.05 nM to 10000 nM or 0.5 nM to 100000 nM were incubated with guinea pig brain membranes and 3 nM [^{3}H](+)-pentazocine (σ_1 receptors) or with rat liver membranes and 5 nM [^{3}H]DTG in the presence of 1 μM dextrallorphan (σ_2 receptors). CB-64L and CB-64D were dissolved in dilute HCl. CB-182 and CB-184 were prepared by first dissolving the compound to a concentration of 20 mM in a solution of dimethyl sulfoxide/0.4 mM HCl. This was then diluted to 10 mM by adding an equal volume of Emulphor EL-620 (Rhone-Poulenc, Cranbury, NJ, USA), and then subsequently diluted to 1 mM in 1 mM HCl. Solvents had no effect on the assay. IC_{50} values were determined using the iterative curve-fitting program GraphPAD InPlot (San Diego, CA, USA). IC_{50} values were then converted to apparent K_i values using the Cheng-Prusoff equation and radioligand K_d values determined previously (Bowen et al., 1993; Hellewell et al., 1994). Values are the averages of 2–3 experiments, ± S.E.M. Each experiment was carried out in duplicate. The binding affinities of these compounds at μ-opioid receptors were determined previously in rat brain using [^{3}H]D-Ala^2,-*N*-methyl-Phe^4,Gly-ol^5 (Bertha et al., 1994). K_i values at μ-opioid receptors for CB-64L, CB-64D, CB-182, and CB-184 were 392 + 48 nM, 37.6 + 2.2 nM, 45.1 + 4.3 nM, and 4.5 + 1.3 nM, respectively.

the paucity of highly subtype-selective σ_2 ligands. There have been other reports of σ_2 subtype-selective compounds. However, these compounds have exhibited only moderate to low σ_2 affinity or have shown no greater than 10-fold selectivity for σ_2 receptors over σ_1 (Mewshaw et al., 1993; De Costa et al., 1994). The only other report of a highly σ_2 subtype-selective compound is that of the benzimidazolone, endo-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride (BIMU-1) (Bonhaus et al., 1993). BIMU-1, with a σ_2 $K_i = 32$ nM and σ_1 $K_i = 6300$ nM, is 200-fold selective for σ_2 sites over σ_1, but also has high affinity for 5-HT_3 and 5-HT_4 serotonin receptors. Thus, CB-184 with a σ_2 $K_i = 13.4$ nM and 554-fold selectivity is the most σ_2 subtype-selective ligand known to date.

In summary, a novel structural class has been discovered which exhibits selectivity for either σ_1 or σ_2 subtypes, depending on the enantiomer. The σ_2 selectivity of these compounds is particularly important. An extensive series of (*E*)-8-benzylidene-5-phenylmorphan-7-ones is currently under investigation in order to further determine the structural requirements for σ_2 subtype selectivity and to obtain compounds which lack affinity for μ-opioid sites and other receptors. Despite their high μ-opioid receptor affinity, CB-184 and CB-64D have both high affinity and high selectivity for σ_2 sites over σ_1 and will prove extremely useful in further delineating the functions and biochemical characteristics of σ_2 receptors.

References

Bertha, C.M., M.V. Mattson, J.L. Flippen-Anderson, R.B. Rothman, H. Xu, X.-Y. Cha, K. Becketts and K.C. Rice, 1994, A marked change of receptor affinity of the 2-methyl-5-(3-hydroxyphenyl)morphans upon attachment of an (*E*)-8-benzylidene moiety: synthesis and evaluation of a new class of σ receptor ligands, J. Med. Chem. 37, 3163.

Bonhaus, D.W., D.N. Loury, L.B. Jakeman, Z. To, A. DeSouza, R.M. Eglen and E.F. Wong, 1993, [^{3}H]BIMU-1, a 5-hydroxytryptamine$_3$ receptor ligand in NG-108 cells, selectively labels sigma-2 binding sites in guinea pig brain hippocampus, J. Pharmacol. Exp. Ther. 267, 961.

Bowen, W.D., B.R. De Costa, S.B. Hellewell, J.M. Walker and K.C. Rice, 1993, [^{3}H](+)-Pentazocine: a potent and highly selective benzomorphan-based probe for sigma-1 receptors, Mol. Neuropharmacol. 3, 117.

De Costa, B.R. and X.-S. He, 1994, Structure-activity relationships and evolution of sigma receptor ligands (1976–present), in: The Sigma Receptors, Neuroscience Perspectives Series, ed. Y. Itzhak (Academic Press, London) p. 45.

De Costa, B.R., X.-S. He, C. Dominguez, J. Cutts, W. Williams and W.D. Bowen, 1994, A new approach to the design of sigma-2 selective ligands: synthesis and evaluation of *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine-related polyamines at sigma-1 and sigma-2 receptor subtypes, J. Med. Chem. 37, 314.

Froimowitz, M., C.G. Pick and G.W. Pasternak, 1992, Phenylmorphans and analogues: opioid receptor subtype selectivity and effect of conformation on activity, J. Med. Chem. 35, 1521.

Hellewell, S.B. and W.D. Bowen, 1990, A sigma-like binding site in rat pheochromocytoma (PC12) cells: decreased affinity for (+)-benzomorphans and lower molecular weight suggest a different sigma receptor form from that of guinea pig brain, Brain Res. 527, 244.

Hellewell, S.B., A. Bruce, G. Feinstein, J. Orringer, W. Williams and W.D. Bowen, 1994, Rat liver and kidney contain high densities of σ_1 and σ_2 receptors: characterization by ligand binding and photoaffinity labeling, Eur. J. Pharmacol. Mol. Pharmacol. 268, 9.

May, E.L. and M. Takeda, 1970, Optical isomers of miscellaneous strong analgesics, J. Med. Chem. 13, 805.

Mewshaw, R.E., R.G. Sherrill, R.M. Mathew, C. Kaiser, M.A. Bailey and E.W. Karbon, 1993, Synthesis and in vitro evaluation of 5,6,7,8,9,10-hexahydro-7,10-iminocyclohept[*b*]indoles: high affinity ligands for the *N*,*N*′-di-*o*-tolylguanidine-labeled σ binding site, J. Med. Chem. 36, 343.

Quirion, R., W.D. Bowen, Y. Itzhak, J.L. Junien, J.M. Musacchio, R.B. Rothman, T.-P. Su, S.W. Tam and D.P. Taylor, 1992, A proposal for the classification of sigma binding sites, Trends Pharmacol. Sci. 13, 85.

Walker, J.M., W.D. Bowen, F.O. Walker, R.R. Matsumoto, B.R. De Costa and K.C. Rice, 1990, Sigma receptors: biology and function, Pharmacol. Rev. 42, 355.