ELSEVIER

European Journal of Pharmacology 442 (2002) 23–27

ejp

www.elsevier.com/locate/ejphar

Biochemical characterisation of newly developed β-etorphine and β-dihydroetorphine derivatives

Dauren Biyashev [a], Sándor Garadnay [b], János Marton [b], Sándor Makleit [b], Anna Borsodi [a], Sándor Benyhe [a,*]

[a] *Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O. Box 521, Temesvári krt. 62, Hungary*
[b] *Department of Organic Chemistry, University of Debrecen, H-4010 Debrecen, Egyetem tér 1, Hungary*

Received 14 August 2001; received in revised form 20 February 2002; accepted 1 March 2002

Abstract

The highly potent synthetic narcotic compound etorphine is known to cause strong analgesia, catatonia and blockade of conditioned reflexes in laboratory animals and is widely used for the immobilisation of game animals. In this study, a number of new structural analogues of etorphine, including C18-β-structures, 3-*O*-methylether derivatives and saturated C7–C8 dihydro-compounds, were synthesised and examined in in vitro ligand binding experiments. Opiate receptor-mediated activation of G-proteins by these derivatives was also investigated using the [^{35}S]GTPγS binding assay. The receptor binding affinity constant and G-protein stimulatory potency of the novel β-etorphins were compared with those of the corresponding C18-α-derivatives. In rat brain membrane preparations, all the compounds tested displayed high affinity (K_i's ranging 0.4–22 nM) using [^{3}H]naloxone in competition assays. The α-etorphines had somewhat higher affinity in comparison with the β-structures. Methylether derivatives were consistently weaker than the corresponding phenolic compounds. Dihydroetorphine and β-dihydroetorphine, which have a partially saturated ring structure, showed as good potency in the binding assays as did etorphine and β-etorphine with C7–C8 double bonds. The etorphine derivatives were potent but naloxone-reversible activators of G-proteins in the [^{35}S]GTPγS functional tests. It was also found that the C3 phenolic group is favourable for G-protein activation. On the basis of our experimental results, neither the configuration of C18 nor the saturation of the C7–C8 double bond appears to play a critical role in the biological activity of etorphines.

Keywords: Opioid receptor; Etorphine derivative; Radioligand binding; [^{35}S]GTPγS binding; Brain, rat

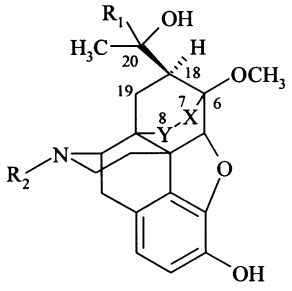
1. Introduction

Narcotic analgesics, of which morphine is the prototypic ligand, are the most powerful drugs for relieving pain (Pasternak, 1993; Benyhe, 1994). The primary targets of the analgesic action of these compounds are opioid receptors in the central nervous system. Opioid receptors belong to the G-protein-coupled receptor superfamily, the receptors of which are localised on the surface membrane of neurons and have seven helical hydrophobic transmembrane segments. Three main types of the opioid receptors, named μ-, δ- and κ-receptors, exist on the basis of their primary sequence (Kieffer, 1997). Agonist binding to the receptors results in conformational change in the binding domain permitting subsequent activation of heterotrimeric GTP-binding proteins (G-proteins). Activation and regulation of G-proteins are key elements in the transmembrane signalling triggered by opioid ligands and are believed to be involved in the cellular mechanisms of drug addiction (Childers et al., 1993; Standifer and Pasternak, 1997).

Oripavines, such as the pure agonist etorphine, the mixed agonist–antagonist buprenorphine and the narcotic antagonist diprenorphine are potent opioid drugs and are also important research tools. Opioid receptors themselves were discovered in 1973, with the demonstration of the specific binding of [^{3}H]etorphine to rat brain homogenates (Simon et al., 1973). The slowly dissociable oripavine radioligands [^{3}H]etorphine and [^{3}H]diprenorphine were successfully used for the solubilization and purification of opioid receptors (Simon et al., 1975) and for the determination of the molecular size of the native receptor–ligand complex in detergent extracts (Mollereau et al., 1988).

* Corresponding author. Tel.: +36-62-432-232x176; fax: +36-62-433-432.
E-mail address: benyhe@nucleus.szbk.u-szeged.hu (S. Benyhe).

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01492-9



1a: R_1= tBu, R_2= CPM, x-y = -CH_2-CH_2- (CPM= cyclopropylmethyl)
1b: R_1= nPr, R_2= CH_3, x-y = -CH=CH-
1c: R_1= nPr, R_2= CH_3, x-y = -CH_2-CH_2-
1d: R_1= CH_3 R_2= CPM, x-y = -CH_2-CH_2-

Fig. 1. Chemical structure of C18-β oripavines.

The first compound with the C18-β structure in the oripavine series, β-buprenorphine (Fig. 1, compound **1a**, 18*S*, 20*R*), was synthesised by Uff et al. (1985). Recently, we prepared β-buprenorphine using a different method, and we also succeeded in elaborating procedures for producing β-etorphine (**1c**, 18*S*, 20*S*) and β-dihydroetorphine (**1d**, 18*S*, 20*S*, Marton et al., 1998a). The synthesis of β-diprenorphine (**1b**, 18*S*) was described earlier (Marton et al., 1995).

A number of structure–activity relationship studies dealing with etorphine derivatives are available (Katsumata et al., 1995; Wang et al., 1995; Lee et al., 1999) but the biochemical and pharmacological properties of the β-substituted Bentley-type compounds (Bentley and Lewis, 1972) have not been reported. The aim of the present study is therefore to compare the receptor binding properties of the newly synthesised β-Bentley compounds in rat brain membrane preparations. The ligands are also investigated in guanosine-5′-*O*-(3-[γ-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding assays to uncover G-protein activation via opioid receptors by these compounds.

2. Materials and methods

2.1. Chemicals

The starting materials for the synthesis of **1a**–**1d**, namely 18β-thevinone (3,6-Dimethoxy-4,5α-epoxy-6,14β-ethano-7,8-didehydro-17-methyl-18β-acetylmorphinane) and 18β-dyhidro-thevinone, were prepared in our laboratory from the corresponding 18α compounds using either basic (Marton et al., 1998b) or acidic conditions (Garadnay et al., 2000). In the present work, four structurally 'identical' etorphine–β-etorphine pairs (18α–18β, **2a**–**2d**, **3a**–**3d**, Fig. 2) were characterised biochemically. Full analytical data for the tested compounds have been published by Marton et al. (1998a).

[^{3}H]Naloxone (35 Ci/mmol) was prepared as described previously (Tóth et al., 1982). Radiolabelled guanosine-5′-*O*-(3-[γ-[^{35}S]thio)triphosphate ([^{35}S]GTPγS; 37–42 Tbq/mmol) was purchased from the Isotope Institute (Budapest, Hungary). Guanosine diphosphate and unlabelled GTPγS were from Sigma-Aldrich (Hungary). All other chemicals were of analytical grade and obtained either from Sigma-Aldrich or Reanal Fine Chemicals (Budapest, Hungary).

2.2. Rat brain membrane preparations

A crude membrane fraction of Wistar rat brains was prepared for ligand binding experiments according to Benyhe et al. (1997). Briefly, the animals were decapitated, and their brains without cerebella were rapidly removed and homogenised in 30 volumes of ice-cold 50 mM Tris–HCl (pH 7.4) buffer with a teflon-glass homogeniser. After centrifugation at 40 000 × *g* for 25 min at 4 °C, the resulting pellets were suspended in 30 volumes of the same buffer and incubated for 30 min at 37 °C to remove endogenous opioids. Centrifugation was then repeated as described above. The final pellets were suspended in 5 volumes of 50 mM Tris–HCl (pH 7.4) buffer containing 0.32 M sucrose and stored at −70 °C. Membranes were thawed before use, diluted with buffer and centrifuged at 40 000 × *g* for 25 min at 4 °C to remove sucrose. The resulting pellets were taken up in appropriate fresh buffer and immediately used.

2.3. Radioligand binding studies

[^{3}H]Naloxone (specific activity 35 Ci/mmol, (Tóth et al., 1982)) binding assays were performed as described earlier (Simon et al., 1986). Briefly, reaction mixtures were composed of 50 mM Tris–HCl buffer (pH 7.4), 0.6–1 nM radioligand, 0.2–0.4 mg tissue protein and graded dilutions of the compounds tested in a final volume of 1 ml. Incubations (0 °C, 60 min) were terminated by rapid filtration under vacuum followed by washing with 3 × 6 ml ice-cold Tris–HCl (50 mM, pH 7.4) buffer through Whatman GF/B glass fiber filters, using a Brandel M24R cell harvester. Non-specific binding was defined as the radioactivity bound in the presence of 10 μM unlabelled naloxone. The radioactivity was measured in a toluene-based scintillation cocktail with a Wallac 1409 spectrophotometer. All assays were performed in duplicate and repeated several times. Equilibrium competition data were analysed with the GraFit computer program, using the 'four parameter logistic' fitting option for sigmoid displacement curves (Leatherbarrow, 1992). Data are expressed as arithmetic means ± standard error of at least three repeated assays.

2.4. [^{35}S]GTPγS binding assays

Rat brain membrane fractions (≈ 10 μg of protein/sample) were incubated at 30 °C for 60 min in Tris–EGTA buffer (50 mM Tris–HCl, 1 mM EGTA, 3 mM $MgCl_2$; pH 7.4) containing [^{35}S]GTPγS (0.05 nM) and increasing concentrations (10^{-9}–10^{-5} M) of the compounds tested in the presence of excess GDP (100 μM) in a final volume of 1 ml according to

Sim et al., 1995 and Traynor and Nahorsky, 1995, with slight modifications. Non-specific binding was determined with 10 μM GTPγS and subtracted. Bound and free [^{35}S]GTPγS were separated by vacuum filtration through Whatman GF/B filters with a Millipore manifold. Filters were washed with 3 × 5 ml ice-cold buffer, and the radioactivity on the dried filters was detected in a toluene-based scintillation cocktail with a Wallac 1409 scintillation counter (Turku, Finland).

3. Results

3.1. Competition assays

Four new analogues of etorphine with a C18-β configuration (**3a**–**3d** in Fig. 2) were prepared and were investigated by the means of in vitro opiate receptor binding assays. Corresponding α-etorphine derivatives (**2a**–**2d** in Fig. 2) were also tested for direct comparison. The potency of the compounds in inhibiting the specific binding of the general opioid antagonist [^{3}H]naloxone to rat brain membranes was measured in equilibrium heterologous competition experiments. These studies also included the determination of the so-called 'sodium shift'. Agonist binding has long been reported to be sensitive to the presence of 50–150 mM NaCl resulting in a substantial decrease in affinity, whereas antagonist ligands are not affected by the presence of NaCl (Pert et al., 1973). The results are summarised in Table 1. Etorphine derivatives effectively inhibited the equilibrium binding of [^{3}H]naloxone with relatively high affinity in rat brain membrane fractions. The rank order of potency was β-etorphine ≥ etorphine ≥dihydroetorphine ≥ β-dihydroetorphine > dihydroetorphine–methylether ≥ etorphine–methylether>β-dihydroetorphine–methylether>β-etorphine–methylether. The effect of 100 mM NaCl was characterised by a decrease in binding affinity. Significant increases in the K_i value in the presence of sodium ions suggested the agonist character of each compound tested (Table 1).

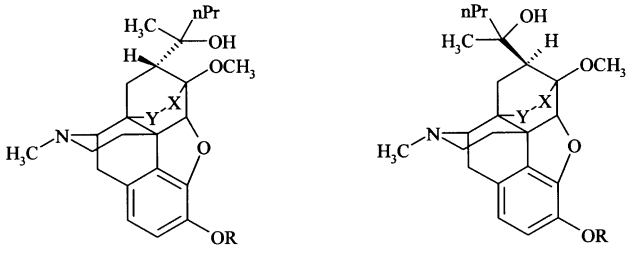
Table 1
Potency of various etorphine and β-etorphine derivatives in competing reversibly with [^{3}H]naloxone equilibrium binding to rat brain membranes

Compound		Equilibrium inhibition constant, K_i (nM)		Sodium index
		without Na^+ ions	100 mM Na^+ present	
Etorphine	**2b**	0.62 ± 0.08	4.26 ± 0.99	6.9
Etorphine–methylether	**2a**	4.54 ± 0.38	342 ± 183	75
Dihydroetorphine	**2d**	0.78 ± 0.39	13.1 ± 2.9	17
Dihydroetorphine–methylether	**2c**	3.58 ± 1.72	201 ± 88	56
β-Etorphine	**1c**	0.47 ± 0.09	11.5 ± 1.3	24
β-Etorphine–methylether	**1e**	22.7 ± 1.5	1390 ± 918	61
β-Dihydroetorphine	**1d**	1.61 ± 0.34	13.8 ± 1.8	8.6
β-Dihydroetorphine–methylether	**1f**	10.5 ± 1.9	409 ± 51	39

Heterologous displacement curves were analysed by using a non-linear regression algorithm (GraFit). IC_{50} values provided by the fitting procedure were then converted into equilibrium inhibitory constant (K_i) values, using the Cheng–Prusoff equation. Data represent the means (± S.E.M.) of three to five independent determinations, each done in duplicate. Na^+ index = $K_i(+Na^+)/K_i(-Na^+)$.

3.2. [^{35}S]GTPγS binding assays

In order to investigate the receptor-mediated activation of G-proteins by etorphines, [^{35}S]GTPγS binding assays, which are widely accepted as functional biochemical experiments for determining and characterising pharmacological agonism in vitro (Elliott and Traynor, 1994; Befort et al., 1996; Selley et al., 1998), were performed. Etorphine derivatives dose



2a: R = CH_3, x-y = -CH=-CH- (nPr = propyl)
2b: R = H, x-y = -CH=CH- [7, 8]
2c: R = CH_3, x-y = -CH_2-CH_2-
2d: R = H, x-y = -CH_2-CH_2- [9]

3a: R = CH_3, x-y = -CH=-CH-
3b: R = H, x-y = -CH=CH- (**1b**)
3c: R = CH_3, x-y = -CH_2-CH_2-
3d: R = H, x-y = -CH_2-CH_2- (**1c**)

Fig. 2. Structures for α- and β-etorphine derivatives used in this study.

Table 2
Stimulation of [^{35}S]GTPγS binding by etorphine compounds in rat brain membranes

Compound		ED_{50} (nM)	Maximal stimulation, %
Etorphine	**2b**	1.09 ± 0.47	210 ± 9
Etorphine–methylether	**2a**	405 ± 99	179 ± 8
Dihydroetorphine	**2d**	0.87 ± 0.11	200 ± 9
Dihydroetorphine–methylether	**2c**	113 ± 19.7	198 ± 11
β-Etorphine	**1c**	10.2 ± 6.7	189 ± 9
β-Etorphine–methylether	**1e**	675 ± 126	192 ± 7
β-Dihydroetorphine	**1d**	3.10 ± 1.02	171 ± 12
β-Dihydroetorphine–methylether	**1f**	205 ± 27	209 ± 5
No compounds added (basal level)		–	100

Agonist-stimulated [^{35}S]GTPγS binding was determined as described in Materials and methods. The parameters shown were obtained by non-linear regression analysis (Graph Pad Prism 2.01). ED_{50} values are defined as the concentration of the ligand producing 50% of the maximal response. Maximal stimulation represents the percent stimulation of [^{35}S]GTPγS binding over basal activity. Data are means ± S.E.M. of three experiments, each performed in triplicate. Basal activitiy measured in the absence of opioid ligands was 69 ± 5 fmol/mg protein in rat brain membranes.

dependently stimulated the binding of [^{35}S]GTPγS to rat brain membranes and this stimulation could be prevented by 10 μM naloxone, indicating opioid receptor-mediated processes (data not shown). The newly developed β-etorphines were also potent stimulators of G-proteins. Maximal stimulation, observed at 10 μM ligand concentration, was about 80–110% over the basal or non-stimulated level. Computer-assisted analysis of the sigmoidal dose–response curves revealed that compounds with phenolic C3 groups were again the better ligands. Molar concentrations necessary for half-maximal stimulation (ED_{50}) were lower for the phenolic compounds while methylether derivatives displayed substantially higher ED_{50} values. The gradual decrease in the potency of the C3-*O*-methyl derivatives was more pronounced in the functional assay than in the receptor binding assay (Table 2).

4. Discussion

From a structural point of view, the currently presented β-etorphines are completely novel compounds because only β-buprenorphine was constructed earlier (Uff et al., 1985; Marton et al., 1995). β-Etorphines have a similar high affinity for opioid binding sites as the corresponding C18-α structures. The agonist character of the compounds, determined in our experiments, is consistent with the results for α-etorphine reported by Kurowski et al. (1982), who described sodium-dependent inhibition of [^{3}H]etorphine but not [^{3}H]diprenorphine binding in rat brain. *O*-Methylation at the C3 position consistently reduced the affinity of the oripavine ligands whereas saturation of the C7–C8 double bond, resulting in dihydro derivatives, produced no substantial changes in the apparent affinity of the compounds. The C3-methylation-dependent decrease in affinity has long been recognised among the morphinane structures, where methylation of morphine results in a less effective analgesic compound, codeine. Methylated etorphine and β-etorphine derivatives were also less efficacious in functional studies (even though the maximal stimulation was approximately the same, the ED_{50} values were significantly higher for the methylated compounds), indicating that the affinity, which was decreased in ligand binding tests, is probably not high enough to activate signal transduction events through G-proteins. However, etorphine and dihydroetorphine derivatives behaved similarly in receptor binding assays and functional experiments, so the unique pharmacological features of dihydroetorphine, e.g., the low dependence potency (Tokuyama et al., 1993; Qin, 1996), cannot be explained by simple structural reasons. Significant differences in the ED_{50} value between the phenolic compounds and methylether derivatives could reflect a different efficacy of the compounds in activating heterotrimeric G-proteins in brain membranes. Equilibrium inhibitory constant K_i values correlated quite well with the ED_{50} values obtained in [^{35}S]GTPγS binding studies (correlation coefficient 0.88), suggesting the close connection of the receptor and transducer proteins in the membrane preparations. Overall, the pharmacological properties of drugs acting on the central nervous system receptors are dependent upon multiple factors, among others, metabolism, lipid/water solubility changes, altered transport properties across the blood–brain barrier and different ability of ligand-induced receptor internalisation (Keith et al., 1998). Similar factors may also be involved in the very high analgesic potency of etorphine-like compounds and in their reported capability of immobilising large animals (Osofsky, 1997).

In summary, the newly synthesised β-etorphine analogues have high affinity in [^{3}H]naloxone binding assays and potently stimulate [^{35}S]GTPγS incorporation in neuronal membranes. Both sodium ion regulation studies and receptor-mediated activation of the G-proteins strongly suggest the agonist nature of all the compounds tested. These biochemical findings are in agreement with the chemical structure of the ligands, and in several cases, are further supported by pharmacological observations (Tokuyama et al., 1996; Aceto et al., 1997). Use of the etorphine and β-etorphine series of ligands in binding and functional studies shows that opioid receptors are relatively insensitive in detecting diastereoisomery at the C18 atom in the oripavine structure. The outstanding affinity and efficacy of etorphine and dihydroetorphine in ligand binding assays (Katsumata et al., 1995; Rosenbaum et al., 1984) and their high potency in pharmacological investigations render these compounds and the new β-structures to be valuable experimental tools in opioid research and probably in medicine.

Acknowledgements

This paper is dedicated to Prof. Dr. W. Fleischhacker on the occasion of his 70th birthday. Supports were provided by OTKA T035211 (S.B.), OTKA T025047 (S.M.), OTKA T032907 and NKFP 1/027 (A.B.) grants from the Hungarian Scientific Research Fund and from the Ministry of Education, Budapest, Hungary.

References

Aceto, M.D., Harris, L.S., Bowman, E.R., 1997. Etorphines: μ-opioid receptor-selective antinociception and low physical dependence capacity. Eur. J. Pharmacol. 338, 215–223.

Befort, K., Tabbara, L., Kieffer, B.L., 1996. [^{35}S]GTPγS binding: a tool to evaluate functional activity of a cloned opioid receptor transiently expressed in COS cells. Neurochem. Res. 21, 1301–1307.

Bentley, K.W., Lewis, J.W., 1972. The relationship between structure and activity in the 6,14-endoethenotetrahydrothebaine series of analgesics. In: Kosterlitz, H.W., Collier, H.O.J., Villarreal, J.E. (Eds.), Agonist and Antagonist Actions of Narcotic Analgesic Drugs. Univ. Park Press, Baltimore, pp. 8–16.

Benyhe, S., 1994. Morphine: new aspects in the study of an ancient compound. Life Sci. 55, 969–979.

Benyhe, S., Farkas, J., Tóth, G., Wollemann, M., 1997. Met^{5}-enkephalin-Arg^{6}-Phe^{7}, an endogenous neuropeptide, binds to multiple opioid and nonopioid sites in rat brain. J. Neurosci. Res. 48, 249–258.

Childers, S.R., Fleming, L.M., Selley, D.E., McNutt, R.W., Chang, K.J., 1993. BW373U86: a nonpeptidic delta-opioid agonist with novel receptor-G protein-mediated actions in rat brain membranes and neuroblastoma cells. Mol. Pharmacol. 44, 827–834.

Elliott, J., Traynor, J.R., 1994. [^{35}S]GTPγS binding in SH-SY5Y human neuroblastoma cells as a model for the study of opioid tolerance. Regul. Pept. 54, 91–92.

Garadnay, S., Marton, J., Makleit, S., 2000. Acid-catalyzed isomerization of α-thevinone and α-dihydrothevinone into β-thevinone and β-dihydrothevinone. ACH-Models Chem. 137, 793–796.

Katsumata, S., Minami, M., Nakagawa, T., Iwamura, T., Satoh, M., 1995. Pharmacological study of dihydroetorphine in cloned mu-, delta- and kappa-opioid receptors. Eur. J. Pharmacol. 291, 367–373.

Keith, D.E., Anton, B., Murray, S.R., Zaki, P.A., Chu, P.C., Lissin, D.V., Monteillet-Agius, G., Stewart, P.L., Evans, C.J., Von Zastrow, M., 1998. μ-Opioid receptor internalization: opiate drugs have differential effects on a conserved endocytic mechanism in vitro and in the mammalian brain. Mol. Pharmacol. 53, 377–384.

Kieffer, B.L., 1997. Molecular aspect of opioid receptors. In: Dickenson, A., Besson, J.-M. (Eds.), Handbook of Experimental Pharmacology, The Pharmacology of Pain, vol. 130. Springer-Verlag, Berlin, pp. 281–303.

Kurowski, M., Rosenbaum, J.S., Perry, D.C., Sadée, W., 1982. [^{3}H]etorphine and [^{3}H]diprenorphine receptor binding in vitro and in vivo: differential effect of Na+ and guanylyl imidodiphosphate. Brain Res. 249, 345–352.

Leatherbarrow, R.J., 1992. GraFit Version 3.0. Erithacus Software, Staines, UK.

Lee, K.O., Akil, H., Woods, J.H., Traynor, J.R., 1999. Differential binding properties of oripavines at cloned mu- and delta-opioid receptors. Eur. J. Pharmacol. 378, 323–330.

Marton, J., Miklós, Sz., Hosztafi, S., Makleit, S., 1995. Synthesis of N-substituted 7β-diprenorphine derivatives. Synth. Commun. 25, 829–848.

Marton, J., Szabó, Z., Garadnay, S., Miklós, Sz., Makleit, S., 1998a. Studies on the synthesis of β-thevinone derivatives. Tetrahedron 54, 9143–9152.

Marton, J., Garadnay, S., Szabó, Z., Hosztafi, S., Makleit, S., 1998b. Isomerization reactions of 7-substituted 6,14-bridged thebaine derivatives (Bentley compounds). Acta Chem. Scand. 52, 1234–1238.

Mollereau, C., Pascaud, A., Baillat, G., Mazarguil, H., Puget, A., Meunier, J.-C., 1988. 5′-Guanylylimidodiphosphate decreases affinity for agonists and apparent molecular size of a frog brain opioid receptor in digitonin solution. J. Biol. Chem. 263, 18003–18008.

Osofsky, S.A., 1997. A practical anesthesia monitoring protocol for free-ranging adult African elephant (*Loxodonta africana*). J. Wildl. Dis. 33, 72–77.

Pasternak, G.W., 1993. Pharmacological mechanisms of opioid analgesics. Clin. Neuropharmacol. 16, 1–18.

Pert, C.B., Pasternak, G.W., Snyder, S.H., 1973. Opiate agonists and antagonists discriminated by receptor binding in brain. Science 182, 1359–1361.

Qin, B.Y., 1996. Advances in dihydroetorphine: from analgesia to detoxification. Drug Dev. Res. 39, 131–134.

Rosenbaum, J.S., Holford, N.H.G., Sadée, W., 1984. Opiate receptor binding-effect relationship: sufentanil and etorphine produce analgesia at the mu-site with low fractional receptor occupancy. Brain Res. 291, 317–324.

Selley, D.E., Liu, Q., Childers, S.R., 1998. Signal transduction correlates of mu opioid agonist intrinsic efficacy: receptor-stimulated [^{35}S]GTPγS binding in μMOR-CHO cells and rat thalamus. J. Pharmacol. Exp. Ther. 285, 496–505.

Sim, L.J., Shelley, D.E., Childers, S.R., 1995. In vitro autoradiography of receptor-activated G-proteins in rat brain by agonist-stimulated guanylyl 5′(γ-[^{35}S]thio)triphosphate binding. Proc. Natl. Acad. Sci. U.S.A. 92, 7242–7246.

Simon, E.J., Hiller, J.M., Edelman, I., 1973. Stereospecific binding of the potent narcotic analgesics [^{3}H]etorphine to rat brain homogenate. Proc. Natl. Acad. Sci. U.S.A. 70, 1947–1949.

Simon, E.J., Hiller, J.M., Edelman, I., 1975. Solubilization of a stereospecific opiate-macromolecular complex from rat brain. Science 190, 389–390.

Simon, J., Benyhe, S., Abutidze, K., Borsodi, A., Szűcs, M., Wollemann, M., 1986. Kinetics and physical parameters of rat brain opioid receptors solubilized by digitonin and CHAPS. J. Neurochem. 46, 695–701.

Standifer, K.M., Pasternak, G.W., 1997. G proteins and opioid receptor-mediated signalling. Cell Signalling 9, 237–248.

Tokuyama, S., Takahashi, M., Kaneto, H., 1993. Antinociceptive effect of dihydroetorphine and its tolerance/dependence liability in mice. Biol. Pharm. Bull. 16, 774–777.

Tokuyama, S., Nakamura, F., Takahashi, M., Kaneto, H., 1996. Antinociceptive effect of dihydroetorphine following various routes of administration: a comparative study with morphine. Biol. Pharm. Bull. 19, 477–479.

Tóth, G., Krámer, M., Sirokmán, F., Borsodi, A., Rónai, A., 1982. Preparation of (7,8,19,20-^{3}H)naloxone of high specific activity. J. Labelled Compd. Radiopharm. 19, 1021–1027.

Traynor, J.R., Nahorsky, S.R., 1995. Modulation by μ-opioid agonists of guanosine-5′-*O*-(3-[γ-[^{35}S]thio)triphosphate binding to membranes from human neuroblastoma SH-SY5Y cells. Mol. Pharmacol. 47, 848–854.

Uff, B.C., Mallard, A.S., Davis, J.A., Henson, R., 1985. NMR spectra and stereochemistry of some 7-substituted^{+} 6,14-bridged thebaine derivatives. Magn. Reson. Chem., 454–459.

Wang, D.X., Lu, X.Q., Qin, B.Y., 1995. Dihydroethorphine is a μ-receptor-selective ligand. J. Pharm. Pharmacol. 47, 669–673.