ELSEVIER

European Journal of Pharmacology 319 (1997) 109–114

ejp

Determination of δ-opioid receptors in NG108-15 cells

Begonia Y. Ho *, Blythe B. Holmes, Jinchuan Zhao, James Fujimoto

Department of Pharmacology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA

Received 29 July 1996; revised 10 October 1996; accepted 15 October 1996

Abstract

The δ-opioid receptors in mouse neuroblastoma × rat glioma hybridoma NG108-15 cells were characterized by receptor binding and cAMP assays. Saturation binding assays using [^{3}H]naltrindole, [^{3}H][D-Pen^2,D-Pen^5]enkephalin (DPDPE) or [^{3}H][D-Ser^2,Leu^5,Thr^6]enkephalin (DSLET) gave similar binding capacities (B_{max}). Competition binding assays showed that DPDPE and DSLET have similar affinity for the [^{3}H]DPDPE or [^{3}H]DSLET binding sites. The rank order of potency of competition with [^{3}H]DPDPE and [^{3}H]DSLET was similar: naltriben ~ DSLET ≥ DPDPE > 7-benzylidenenaltrexone (BNTX). Both DPDPE and DSLET were found to decrease cAMP formation. The action of DSLET was antagonized by naltriben but not BNTX, while the action of DPDPE was reversed by both antagonists. Therefore, the δ-opioid receptor in NG108-15 cells has similar affinity for the agonists DPDPE and DSLET, and a higher affinity for the antagonist naltriben than BNTX.

Keywords: δ-Opioid receptor; NG108-15 cell; DPDPE ([D-Pen^2,D-Pen^5]enkephalin); DSLET ([D-Ser^2,Leu^5,Thr^6]enkephalin); BNTX (7-benzylidenenaltrexone); Naltriben

1. Introduction

The δ-opioid receptor is one of the three major types of opioid receptors (designated as μ-, δ- and κ-opioid receptors) classified by pharmacological studies. It has high affinity for enkephalins and has been proposed to be the receptor for these endogenous opioid peptides. The δ-opioid receptors are involved in a wide spectrum of pharmacological actions, including analgesia, gastroparesis and antitussis (Loh and Smith, 1990). Their major cellular actions include adenylyl cyclase inhibition, calcium current inhibition and potassium channel opening (Di Chiara and North, 1992). According to analgesic studies in vivo, the δ-opioid receptor can be further classified into δ_1 and δ_2 which can be differentiated by their selectivity to [D-Pen^2,D-Pen^5]enkephalin (DPDPE) and 7-benzylidenenaltrexone (BNTX) (δ_1-opioid receptor agonist and antagonist) and to [D-Ser^2,Leu^5,Thr^6]enkephalin (DSLET) and naltriben (δ_2-opioid receptor agonist and antagonist) (Sofuoglu et al., 1991a,b,1993; Portoghese et al., 1992; Takemori et al., 1992).

The mouse neuroblastoma × rat glioma hybridoma NG108-15 cell has been used as a model for studying cellular actions of the δ-opioid receptor as well as the mechanism of desensitization because they express endogenous δ-opioid receptors, while the μ- and κ-opioid receptors are undetectable (Wahlstrom et al., 1977; Knapp and Yamamura, 1990). However, the types of endogenous δ-opioid receptors expressed in NG108-15 cell are still unclear. It has been shown that both δ_1- and δ_2-opioid receptor agonists such as [D-Ala^2,D-Leu^5]enkephalin (DADLE), DPDPE and DSLET can elicit various pharmacological actions in NG108-15 cells (Bergsbaken et al., 1993; Jin et al., 1994). Molecular cloning using NG108-15 cell cDNA revealed the mouse form of the δ-opioid receptor (Evans et al., 1992; Kieffer et al., 1992). After expression of this δ-opioid receptor in monkey kidney COS or Chinese hamster ovary CHO cells, it was found to exhibit a higher affinity for the δ_2-opioid receptor antagonist naltriben than the δ_1-opioid receptor antagonist BNTX. Therefore, this clone was proposed to encode the δ_2-opioid receptor (Law et al., 1994; Raynor et al., 1994). The purpose of the present study is to characterize the endogenous δ-opioid receptor subtypes in NG108-15 cells by both receptor binding and functional cAMP assays. The conventional δ_1- and δ_2-opioid receptor agonists (DPDPE and DSLET) and antagonists (BNTX and naltriben) were used to test whether the cellular actions can be correlated with one of the known δ-opioid receptor subtypes.

* Corresponding author. Tel.: (1-414) 456-8625; Fax: (1-414) 266-8456.

PII S0014-2999(96)00827-8

2. Materials and methods

2.1. Cell culture

NG108-15 cells were grown in Dulbecco's modified Eagle medium containing 5% fetal bovine serum, 0.1 μM hypoxanthine, 10 μM aminopterin and 17 μM thymidine in a humidified environment of 95% air and 5% CO_2 at 37°C.

2.2. Preparation of membranes

Confluent cells were harvested, lysed by sonication in buffer A (Tris pH 7.4 50 mM, $MgCl_2$ 5 mM, EDTA 1 mM, aprotinin 1 μg/ml, phenylmethylsulfonyl fluoride 0.1 mM). Membranes were centrifuged at $1000 \times g$ to remove cell debris. The supernatant was then centrifuged at $30\,000 \times g$. The pellet was resuspended in buffer A. Membranes were used either immediately for binding or frozen at −80°C and used within 2 weeks.

2.3. Binding assays and analysis

Radioligand binding assays were performed in buffer A in an incubation volume of 500 μl in duplicates (27°C for 45 min). Saturation binding assays were performed with 12 concentrations of [^{3}H]naltrindole, [^{3}H]DPDPE or [^{3}H]DSLET. In competition binding assays, different concentrations of competitive ligands were used to compete with 1 nM [^{3}H]DPDPE or [^{3}H]DSLET. Nonspecific binding was determined with 10 μM naloxone for both saturation and competition binding. Incubation was terminated by rapid filtration with a cell harvester (Brandel) onto glass fiber GF/C membranes, followed by three washes of 3 ml buffer A. Radioactivity was determined by liquid scintillation counting. Protein concentrations were determined by the Bradford assay using bovine serum albumin as the standard (Bradford, 1976). Binding results were analyzed with the software Prism to obtain B_{max}, K_d and K_i values. The B_{max} and K_d values are expressed as mean ± standard error of mean (S.E.M.). The K_i values usually follow a log-normal distribution, therefore, they are expressed as the geometric mean (95% confidence interval) (Hancock et al., 1988). The K_i values are considered to be significantly different when the geometric means are outside each other's 95% confidence interval.

2.4. cAMP assays

Adenylyl cyclase activity was assayed by cAMP formation from whole cells using radioimmunoassay kits according to the manufacturer's instruction (BTI Technologies, Stoughton, MA, USA). Cells (0.5×10^5/tube) were preincubated with Krebs-Ringer-Hepes (KRH) buffer (NaCl 110 mM, KCl 5 mM, $MgCl_2$ 1 mM, $CaCl_2$ 1.8 mM, Hepes pH 7.4 10 mM, sucrose 5.5 mM, glucose 25 mM) and 100 μM isobutylmethylxanthine (IBMX) as a phosphodiesterase inhibitor for 20 min at 37°C. The reaction was started by the addition of 1 μM prostaglandin E_1 (adenylyl cyclase stimulator) and different concentrations of δ-opioid receptor agonist/antagonist prepared in KRH buffer containing 100 μM IBMX. After a 5 min incubation (37°C), the reaction was terminated with trichloroacetic acid (final concentration 5%). Basal cAMP level was determined in the absence of prostaglandin E_1. The supernatant was extracted 3 times with water-saturated diethyl ether. Aliquots of 50 μl were used for cAMP radioimmunoassay. Data are represented as percent prostaglandin E_1-stimulated cAMP level. Results from different treatments were compared using unpaired Student's *t*-test.

2.5. Drugs

DPDPE and DSLET were purchased from Sigma (St. Louis, MO, USA) and Peninsula Laboratories (Belmont, CA, USA), respectively. BNTX and naltriben were gifts from Dr. Portoghese (University of Minnesota, MN, USA). [^{3}H]Naltrindole, [^{3}H]DPDPE and [^{3}H]DSLET were purchased from DuPont NEN (Boston, MA, USA). Tissue culture reagents were purchased from Gibco (Grand Island, NY, USA). Other chemicals were purchased form Sigma (St. Louis, MO, USA) or Fisher Scientific (Itasca, IL, USA).

3. Results

3.1. Binding characteristics of the δ-opioid receptor in NG108-15 cell membranes

Preliminary time-course experiments showed that the binding of 1 nM [^{3}H]DPDPE and 1 nM [^{3}H]DSLET reached saturation after incubation at 27°C between 45–60 min. Therefore, binding assays were performed at 27°C for 45 min throughout. Longer incubation times were not used to avoid potential breakdown of the ^{3}H-labeled peptides as suggested by the manufacturer (DuPont NEN). Saturation binding with the nonselective δ-opioid receptor antagonist [^{3}H]naltrindole, the putative δ_1-opioid receptor agonist [^{3}H]DPDPE and the putative δ_2-opioid receptor agonist [^{3}H]DSLET all gave best fits to a single site model with Hill coefficients of approximately 1 (Table 1 and Fig. 1). The B_{max} of [^{3}H]naltrindole binding sites was found to be

Table 1
Binding characteristics of [^{3}H]naltrindole, [^{3}H]DPDPE and [^{3}H]DSLET in NG108-15 cell membranes

	B_{max} ± S.E.M. (pmol/mg protein)	K_d ± S.E.M. (nM)	Hill coefficient
[^{3}H]Naltrindole	0.98 ± 0.15 (3)	0.12 ± 0.04	0.94 ± 0.01
[^{3}H]DPDPE	0.76 ± 0.13 (3)	0.97 ± 0.18	0.98 ± 0.02
[^{3}H]DSLET	0.77 ± 0.29 (3)	0.40 ± 0.03	0.94 ± 0.07

Saturation binding assays were performed as described for Fig. 1. Data are expressed as mean ± S.E.M. (number of experiments).

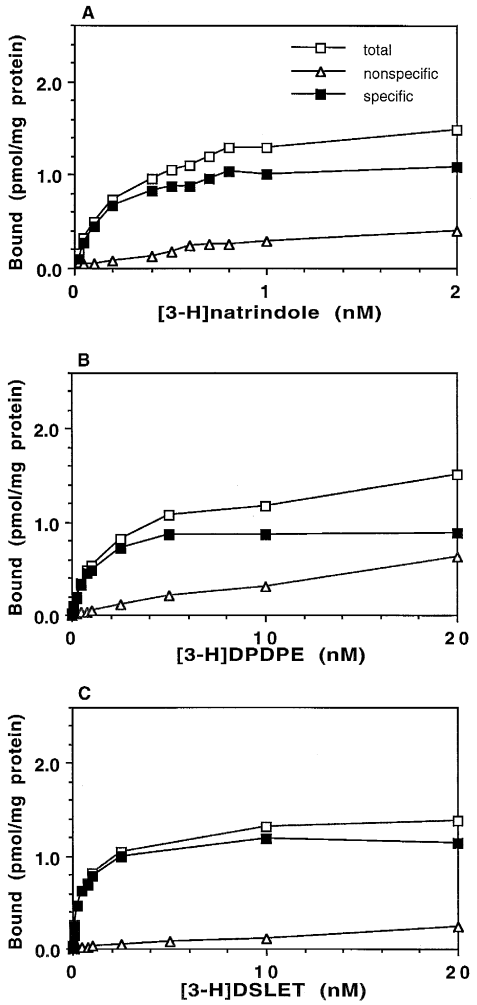

Fig. 1. Saturation binding of [^{3}H]naltrindole (A), [^{3}H]DPDPE (B) and [^{3}H]DSLET (C) in NG108-15 cell membranes. Binding was performed with 12 concentrations of [^{3}H]naltrindole (0.01–2 nM), [^{3}H]DPDPE (0.01–20 nM) or [^{3}H]DSLET (0.01–20 nM). Nonspecific binding was determined in the presence of 10 μM naloxone. Each panel shows results from a representative binding experiment. Similar results were obtained in two other independent experiments.

0.98 ± 0.15 pmol/mg protein, which is not significantly different from that of [^{3}H]DPDPE (0.76 ± 0.13 pmol/mg protein) or [^{3}H]DSLET (0.77 ± 0.29 pmol/mg protein).

Table 2
Competition of DPDPE, DSLET, BNTX and naltriben for [^{3}H]DPDPE and [^{3}H]DSLET binding sites in NG108-15 cell membranes

Competitive ligand	K_i (95% confidence interval) (nM)	
	[^{3}H]DPDPE	[^{3}H]DSLET
DPDPE	1.01 (0.92–1.11) (3)	0.86 (0.64–1.15) (4)
DSLET	0.76 (0.55–1.06) (3)	0.54 (0.36–0.82) (4)
BNTX	4.54 (3.69–5.59) (4)	1.49 (1.03–2.14) (4) [a]
Naltriben	0.76 (0.50–1.17) (4) [b]	0.46 (0.36–0.57) (3) [a,b]

Competition binding assays were performed as described for Fig. 2. Data are expressed as geometric mean (95% confidence interval) (number of experiments). [a] The K_i values are significantly different between [^{3}H]DPDPE and [^{3}H]DSLET. [b] The K_i values are significantly different between BNTX and naltriben.

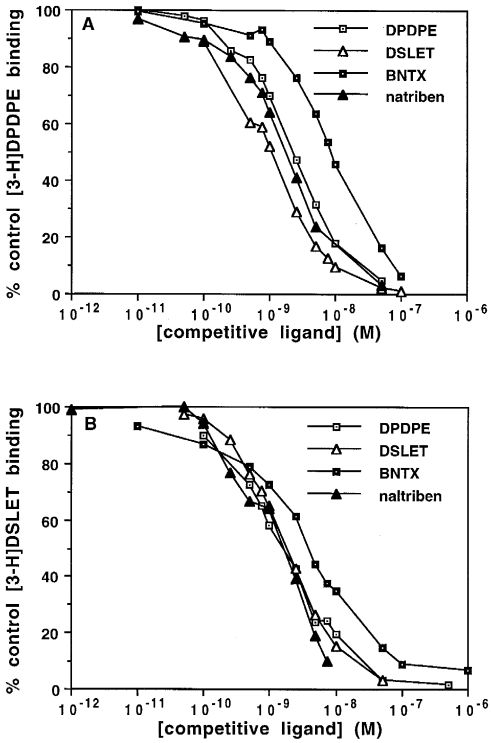

Fig. 2. Competition of specific binding of [^{3}H]DPDPE and [^{3}H]DSLET by DPDPE, DSLET, BNTX and naltriben in NG108-15 cell membranes. Eleven to twelve concentrations of the competitive ligand were incubated with 1 nM of [^{3}H]DPDPE (A) or [^{3}H]DSLET (B) as described in Section 2. Nonspecific binding was determined with 10 μM naloxone. Control binding represents specific binding of the ^{3}H-labeled ligand in the absence of competitive ligands. Each figure shows representative inhibition curves of the competitive ligands. Similar results were obtained in two to three other independent experiments.

Therefore, it is possible that the binding sites of [^{3}H]DPDPE and [^{3}H]DSLET overlap in NG108-15 cells. To test this hypothesis, the ability of δ_1- (DPDPE and BNTX) and δ_2- (DSLET and naltriben) opioid receptor

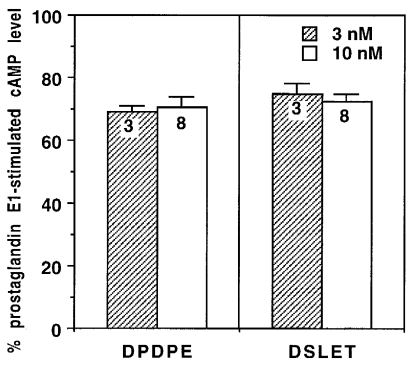

Fig. 3. Inhibition of prostaglandin E_1-stimulated cAMP formation by DPDPE and DSLET in NG108-15 cells. Cells were incubated with 1 μM prostaglandin E_1 (to stimulate cAMP formation) in the absence or presence of DPDPE or DSLET. Results are expressed as percent prostaglandin E_1-stimulated cAMP formation and represented as mean ± S.E.M. Numbers inside columns represent the number of experiments. Basal and prostaglandin E_1-stimulated cAMP levels in NG108-15 cells were 2.19 ± 0.56 and 18.96 ± 2.76 pmol/1×10^5 cells, respectively.

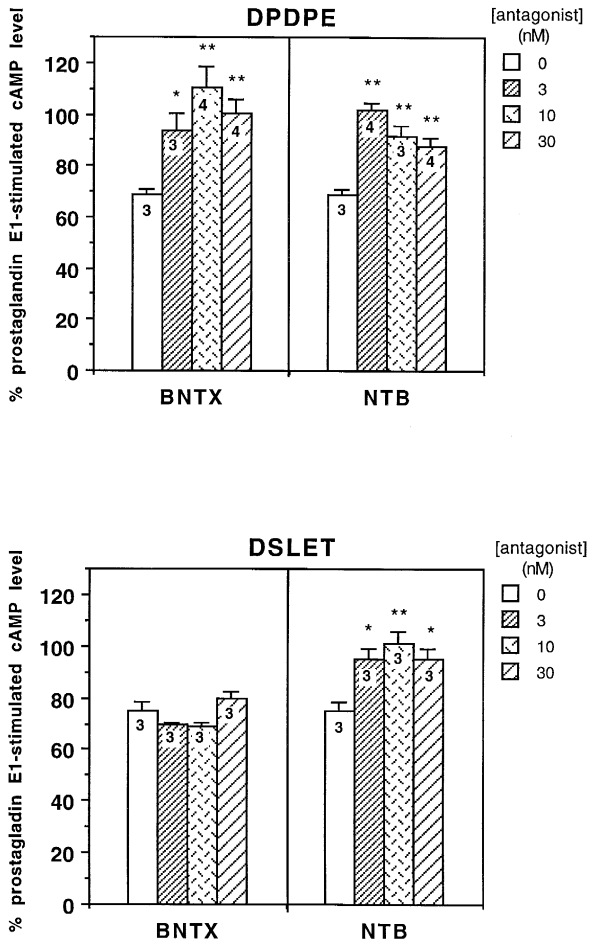

Fig. 4. Effects of BNTX and naltriben on DPDPE- or DSLET-induced inhibition of cAMP formation. Cells were incubated with 1 μM prostaglandin E_1, the agonist (3 nM DPDPE or DSLET) and the indicated concentration of the antagonist (naltriben or BNTX). Data are expressed as percent prostaglandin E_1-stimulated cAMP level and represented as mean ± S.E.M. Numbers inside columns represent the number of experiments. The same concentrations of naltriben and BNTX alone did not cause any significant change in prostaglandin E_1-stimulated cAMP level. * $P < 0.05$, ** $P < 0.01$, significantly different from the result obtained in the absence of antagonist.

ligands to compete with [^{3}H]DPDPE and [^{3}H]DSLET was studied in competition binding assays.

The K_i value of DPDPE (1.01 nM) in the competition assay was found to be very similar to the K_d value of [^{3}H]DPDPE (0.97 nM) in the saturation assay (Fig. 2 and Table 2). The K_i (0.54 nM) and K_d (0.40 nM) values of DSLET were also found to be similar. Comparison of the K_i values of DPDPE and DSLET showed that they have similar potency in competing with [^{3}H]DPDPE and [^{3}H]DSLET, suggesting that their binding sites probably overlap. Both antagonists BNTX and naltriben were found to be more potent in competing with [^{3}H]DSLET than [^{3}H]DPDPE: BNTX was about 3 times more potent in competing with [^{3}H]DSLET than [^{3}H]DPDPE and naltriben 2 times more potent. The rank order of potency of the unlabeled ligands in competing with [^{3}H]DPDPE and [^{3}H]DSLET was found to be similar: naltriben ~ DSLET ≥ DPDPE > BNTX.

3.2. Ability of δ-opioid receptor antagonists to reverse the inhibition of cAMP formation induced by DPDPE and DSLET

To characterize the receptors functionally, BNTX and naltriben were tested for their antagonism of DPDPE- or DSLET-induced inhibition of cAMP formation. Both DPDPE and DSLET were able to decrease prostaglandin E_1-stimulated cAMP formation (Fig. 3). A complete concentration-response curve was not performed because the small percentage of inhibition renders it difficult to establish such a curve. DPDPE-induced inhibition was reversed by both naltriben and BNTX, whereas DSLET-induced inhibition was antagonized by naltriben but not BNTX (Fig. 4).

4. Discussion

The present finding that DPDPE and DSLET could compete with [^{3}H]DPDPE and [^{3}H]DSLET with similar potency suggests that [^{3}H]DPDPE and [^{3}H]DSLET probably interact with the same population of δ-opioid receptors in NG108-15 cells. This is consistent with the finding that [^{3}H]DPDPE, [^{3}H]DSLET and [^{3}H]naltrindole had similar B_{max} values. The K_d values from saturation binding studies showed that even though the binding sites probably overlap, these ligands interact with the receptor with different affinities, with a rank order of potency of [^{3}H]naltrindole > [^{3}H]DSLET ≥ [^{3}H]DPDPE. The high affinity of this δ-opioid receptor to both DPDPE and DSLET explains the finding that nM concentrations of DPDPE and DSLET were able to inhibit adenylyl cyclase in NG108-15 cells (Bergsbaken et al., 1993; Fig. 3). The K_i values from the competition binding assays show that naltriben has a higher affinity than BNTX for both the [^{3}H]DPDPE and [^{3}H]DSLET binding sites. These binding characteristics are similar to those of the mouse δ-opioid receptor cloned from NG108-15 cells and expressed in COS or CHO cells (Evans et al., 1992; Kieffer et al., 1992; Yasuda et al., 1993; Law et al., 1994; Raynor et al., 1994). Naltriben was demonstrated to have a higher affinity than BNTX in competing with [^{3}H]naltrindole or [^{3}H]diprenorphine. In these studies, however, naltriben showed a much higher affinity (30–50-fold) than BNTX for the expressed receptor but we only observed a 3–6-fold difference in affinity for the endogenous receptor. The disparity may be due to the use of different ^{3}H-labeled ligands in these studies. The binding characteristics of the agonists DPDPE and DSLET for the expressed receptor are less well defined. DSLET was found to have a 3–8-fold higher affinity than DPDPE for the expressed receptor in some

studies (Evans et al., 1992; Yasuda et al., 1993; Raynor et al., 1994) but have a lower affinity than DPDPE in another expression study reported by Law et al. (1994). Therefore, both the endogenous and expressed δ-opioid receptor appear to have a higher affinity for naltriben than BNTX but a difference in the affinity for the agonists DSLET and DPDPE was not as consistently demonstrated.

The δ-opioid receptor in NG108-15 cells also did not function exactly like the δ_1- or δ_2-opioid receptor in the cAMP assay. Both DSLET and DPDPE resulted in the inhibition of prostaglandin E_1-induced cAMP formation. DSLET-induced adenylyl cyclase inhibition was reversed by naltriben but not by the same concentrations of BNTX. These findings are consistent with results from the competition binding assays in which naltriben showed a 3-fold higher affinity than BNTX for [^{3}H]DSLET. However, DPDPE-induced adenylyl cyclase inhibition was antagonized by both BNTX and naltriben. It is possible that although DPDPE and DSLET are binding to the similar population of δ-opioid receptors, there may be subtle differences in the agonist-antagonist interaction within the binding domain of the receptor. This difference is revealed by the K_i values of naltriben and BNTX for [^{3}H]DPDPE and [^{3}H]DSLET. Furthermore, even though BNTX is significantly less potent than naltriben in competing with [^{3}H]DPDPE, the affinity of BNTX is still relatively high ($K_i = 4.54$ nM) (Table 2).

The classification of δ_1- and δ_2-opioid receptors is best defined by analgesic assays in mice. The analgesic action of DPDPE was antagonized by BNTX and this subset of δ-opioid receptors is designated as the δ_1-opioid receptor (Portoghese et al., 1992; Sofuoglu et al., 1993). The analgesic action of DSLET was antagonized by naltriben and this subset is designated as the δ_2-opioid receptor (Sofuoglu et al., 1991a; Takemori et al., 1992). In addition to the differential antagonism, there is also a lack of cross-tolerance between the δ_1- and δ_2-opioid receptors (Sofuoglu et al., 1991a,b). However, the classification of δ-opioid receptors is not as definitive in other preparations. Studies in vivo and in vitro have demonstrated a heterogeneity of δ-opioid receptors, which exists among different assays, different species and sometimes among different tissues of the same species. For example, BNTX was shown to have a 100-fold higher affinity for [^{3}H]DPDPE binding sites than those of [^{3}H]DSLET in guinea pig cerebellum, and was 8-fold more potent in antagonizing DPDPE- than DSLET-induced analgesia in the mouse tail-flick assay, but it showed similar IC_{50} values for DPDPE- and DSLET-induced inhibition of mouse vas deferens contractions (Portoghese et al., 1992). Naltriben showed almost identical ability to antagonize DPDPE (δ_1-opioid receptor agonist) and [D-Ala^2,Glu^4]deltorphin (δ_2-opioid receptor agonist) in the mouse vas deferens preparation (Wild et al., 1993) and rat analgesic assays (Stewart et al., 1994). So far, only one δ-opioid receptor clone has been identified from mouse brain (Yasuda et al., 1993) and NG108-15 cells (Evans et al., 1992; Kieffer et al., 1992). It remains to be demonstrated whether the receptor heterogeneity found in various pharmacological assays is the result of different genes or different post-translational modifications of the same gene. Taken together, the endogenous δ-opioid receptor in NG108-15 cells appears to be different from the typical δ_1- or δ_2-opioid receptors identified in mouse analgesic assays with respect to the selectivity to the agonists DPDPE and DSLET and the ability of BNTX and naltriben in antagonizing their respective agonist. It has similar affinity for the δ-opioid receptor agonists DPDPE and DSLET and a higher affinity for the δ-opioid receptor antagonist naltriben than BNTX.

Acknowledgements

This investigation was supported by the Pharmaceutical Research and Manufacturers of America Foundation, American Heart Association, Medical College of Wisconsin Research Grant (B.Y.H.) and U.S. Public Health Service Grants from the National Institute on Drug Abuse (J.F.). The authors thank Dr. James G. Drewett for reading the manuscript critically.

References

Bergsbaken, C.L., S.L. Sommers and P.Y. Law, 1993, Effect of forskolin and isobutylmethylxanthine on delta-opioid receptor activity in neuroblastoma×glioma NG108-15 cells, J. Pharmacol. Exp. Ther. 264, 1474.

Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248.

Di Chiara, G. and R.A. North, 1992, Neurobiology of opiate abuse, Trends Pharmacol. Sci. 13, 185.

Evans, C.J., D.E. Keith Jr., H. Morrison, K. Magendzo and R.H. Edwards, 1992, Cloning of a delta opioid receptor by functional expression, Science 258, 1952.

Hancock, A.A., E.N. Bush, D. Stanisic, J.J. Kyncl and C.T. Lin, 1988, Data normalization before statistical analysis: keeping the horse before the cart, Trends Pharmacol. Sci. 9, 29.

Jin, W., N.M. Lee, H.H. Loh and S.A. Thayer, 1994, Opioids mobilize calcium from inositol 1,4,5-triphosphate-sensitive stores in NG108-15 cells, J. Neurosci. 14, 1920.

Kieffer, B.L., K. Befort, C. Gaveriaux-Ruff and C.G. Hirth, 1992, The δ-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization, Proc. Natl. Acad. Sci. USA 89, 12048.

Knapp, R.J. and H.I. Yamamura, 1990, [^{3}H][D-Pen^2,D-Pen^5]Enkephalin binding to delta opioid receptors on intact neuroblastoma–glioma (NG 108-15) hybrid cells, Life Sci. 46, 1457.

Law, P.Y., T.M. McGinn, M.J. Wick, L.J. Erikson, C. Evans and H.H. Loh, 1994, Analysis of delta-opioid receptor activities stably expressed in CHO cell lines: function of receptor density?, J. Pharmacol. Exp. Ther. 271, 1686.

Loh, H.H. and A.P. Smith, 1990, Molecular characterization of opioid receptors, Annu. Rev. Pharmacol. Toxicol. 30, 123.

Portoghese, P.S., M. Sultana, H. Nagase and A.E. Takemori, 1992, A highly selective δ_1-opioid receptor antagonist: 7-benzylidenenaltrexone, Eur. J. Pharmacol. 218, 195.

Raynor, K., H. Kong, Y. Chen, K. Yasuda, L. Yu, G.I. Bell and T. Reisine, 1994, Pharmacological characterization of the cloned κ-, δ-, and μ-opioid receptors, Mol. Pharmacol. 45, 330.

Sofuoglu, M., P.S. Portoghese and A.E. Takemori, 1991a, Differential antagonism of delta opioid agonists by naltrindole and its benzofuran analog (NTB) in mice: evidence for delta opioid receptor subtypes, J. Pharmacol. Exp. Ther. 257, 676.

Sofuoglu, M., P.S. Portoghese and A.E. Takemori, 1991b, Cross-tolerance studies in the spinal cord of β-FNA-treated mice provides further evidence for delta receptor subtypes, Life Sci. 49, PL153.

Sofuoglu M., P.S. Portoghese and A.E. Takemori, 1993, 7-Benzylidenenaltrexone (BNTX): a selective δ_1 opioid receptor antagonist in the mouse spinal cord, Life Sci. 52, 769.

Stewart, P.E., E.M. Holper and D.L. Hammond, 1994, δ Antagonist and κ agonist activity of naltriben: evidence for differential κ interaction with the δ_1 and δ_2 opioid receptor subtypes, Life Sci. 55, PL79.

Takemori, A.E., M. Sultana, H. Nagase and P.S. Portoghese, 1992, Agonist and antagonist activities of ligands derived from naltrexone and oxymorphone, Life Sci. 50, 1491.

Wahlstrom, A., M. Brandt, L. Moroder, E. Wunsch, G. Lindeberg, U. Ragnarsson, L. Terenius and B. Hamprecht, 1977, Peptides related to β-lipotropin with opioid activity: effects on level of adenosine 3′:5′-cyclic monophosphate in neuroblastoma × glioma hybrid cells, FEBS Lett. 77, 28.

Wild, K.D., V.J. Carlisi, H.I. Mosberg, W.D. Bowen, P.S. Portoghese, M. Sultana, A.E. Takemori, V.J. Hruby and F. Porreca, 1993, Evidence for a single functional opioid delta receptor subtype in the mouse isolated vas deferens, J. Pharmacol. Exp. Ther. 264, 831.

Yasuda, K., K. Raynor, H. Kong, C.D. Breder, J. Takeda, T. Reisine and G.I. Bell, 1993, Cloning and functional comparison of κ and δ opioid receptors from mouse brain, Proc. Natl. Acad. Sci. USA 90, 6736.