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Characterization of the δ-opioid receptor found in SH-SY5Y neuroblastoma cells

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Abstract

The δ-opioid receptor found in SH-SY5Y cells was characterized in terms of binding profile and ability to mediate the inhibition of forskolin-stimulated cAMP accumulation. Both DPDPE ([D-Pen^2,D-Pen^5]enkephalin) and deltorphin II, compounds reported to be selective for the δ_1- and δ_2-opioid receptor respectively, were potent agonists in these cells. Binding studies indicated that naltrindole benzofuran (NTB) had significantly higher affinity than 7-benzylidenenaltrexone (BNTX); however, both compounds have high affinity for the δ-opioid receptor found in SH-SY5Y cells. Naltrindole benzofuran was found to be a potent antagonist, with an IC_{50} of less than 1 nM, while 7-benzylidene naltrexone was found to be a relatively weak antagonist, requiring greater than 100 nM to inhibit 50% of agonist activity. Binding to intact SH-SY5Y cells was compared to binding to cell membranes and guinea-pig brain membranes. In each case, binding affinities were very similar. These studies suggest that the receptor found in SH-SY5Y cells could probably be classified as a δ_2-opioid receptor. However, the very similar binding characteristics of SH-SY5Y cells and guinea-pig brain membranes call into question the ability to label δ_1-opioid receptors. © Elsevier Science B.V. All rights reserved.

Keywords: Opiate receptor; Neuroblastoma; δ-Opioid receptor, subtype; SH-SY5Y

1. Introduction

It has been known for some time that opioid compounds produce their diverse effects through more than a single receptor type. Based upon years of binding studies, in vitro and in vivo pharmacology, autoradiography, and now receptor cloning, there is hard evidence (binding plus function plus a clone) for only three receptors, now called μ-, δ- and κ-opioid receptors. Nevertheless, considerable evidence has been accumulated for subtypes of each of the three main classes of opioid receptor. Utilizing the non-equilibrium antagonist naloxonazine, Pasternak and colleagues have presented a great deal of evidence for a site that they named the μ_1-opioid receptor site (Wolozin and Pasternak, 1981). This site has high affinity for most opiate receptor ligands, and may mediate supraspinal analgesia. In addition, several groups have presented binding and some pharmacological studies that indicate the presence of from one to three additional κ-opioid receptors (Zukin et al., 1988; Clark et al., 1989; Rothman et al., 1990; Webster et al., 1993).

More recently, considerable evidence has been presented that suggests the existence of δ-opioid receptor subtypes. The initial observations were differences in the actions of the δ-opioid receptor-selective antagonist naltrindole and its analog naltriben (NTB) (Sofuoglu et al., 1991), differences in the actions of the non-equilibrium antagonists naltrindole isothiocyanate and DALCE ([D-Ala^2,Leu^5,Cys^6]enkephalin) (Jiang et al., 1991), and a lack of analgesic cross-tolerance between two very selective δ-opioid receptor ligands, DPDPE ([D-Pen^2,D-Pen^5]enkephalin) and deltorphin II (Mattia et al., 1991). Another δ-opioid receptor antagonist, 7-benzylidenenaltrexone (BNTX) (Portoghese et al., 1992; Sofuoglu et al., 1993), has been used in conjunction with naltriben as a tool for the identification of δ-opioid receptor subtypes. Although the majority of the evidence for δ-opioid receptor subtypes comes from in vivo experiments, there is a small amount of in vitro data suggesting the presence of δ-opioid receptor subtypes. In particular, Cox and colleagues have shown DPDPE to be inhibited more readily by 7-benzylidenenaltrexone, while deltorphin II was more sensitive to naltriben in both caudate-putamen and nucleus accumbens (Buzas et al., 1994). In addition, they have found DPDPE to be, at best, a poor agonist for the stimulation of an

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increase in free intracellular Ca^{2+} in a neuroblastoma-dorsal root ganglion hybrid cell line, while deltorphin II is quite active in this system (Tang et al., 1994).

Deriving binding parameters to match the functional data has been difficult. A few studies have shown low Hill coefficients, suggesting the presence of multiple binding sites or states (Negri et al., 1991; Xu et al., 1991), or some differences in affinity using apparently δ-opioid receptor-selective binding conditions (Sofuoglu et al., 1992). However, binding to brain membranes or striatum using δ_1-opioid receptor-selective [^{3}H]DPDPE or δ_2-opioid receptor-selective [^{3}H]deltorphin II seems to give very similar values for the affinities of both naltriben and 7-benzylidenenaltrexone (Buzas et al., 1994). Furthermore, it has not been possible to identify δ-opioid receptor subtypes by in vitro bioassay, as the mouse vas deferens apparently has only δ_2-opioid receptors (Wild et al., 1993a).

The cloning of the δ-opioid receptor (Evans et al., 1992; Kieffer et al., 1992) has not aided in the characterization of δ-opioid receptor subtypes. So far, only a single type of δ-opioid receptor has been identified. Based upon the affinities of the selective antagonists naltriben and 7-benzylidenenaltrexone, this has been characterized as a δ_2-opioid receptor (Raynor et al., 1994). Although the initial receptor cloned was the mouse δ-opioid receptor from NG108-15 cells, the rat and human receptors have been cloned and represent homologs of the same gene (Fukuda et al., 1993; Knapp et al., 1994).

In order to characterize the δ-opioid receptor in vitro, and to determine whether δ-opioid receptor subtypes can be identified, the human neuroblastoma cell line SH-SY5Y was used. This cell line has been shown to contain both μ- and δ-opioid receptors, both of which are functional in terms of inhibition of cAMP accumulation (Yu et al., 1986; Kazmi and Mishra, 1987; Yu and Sadeé, 1988). SH-SY5Y cells have been widely used for the study of the activity of μ-opioid receptors (Toll, 1992, 1995), tolerance development and receptor desensitization (Yu and Sadeé, 1988). In order to characterize the receptor(s) present we have utilized selective ligands and compared binding affinities with affinities determined in guinea-pig brain. Our results suggest the presence of a single δ-opioid receptor type in SH-SY5Y cells. Furthermore, the affinity of a variety of ligands for this site matches closely with binding affinities determined in brain membranes.

2. Materials and methods

2.1. Cell culture

SH-SY5Y cells were obtained from Dr. Wolfgang Sadeé. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum and 10% calf serum as described previously (Toll, 1992).

2.2. Receptor binding

For binding to δ-opioid receptors on intact cells, cells were plated on 24-well tissue culture plates, and used at confluence. Plates were washed once with modified Krebs buffer containing: 130 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM glucose, 1 mg/ml bovine serum albumin and 25 mM Hepes, pH 7.4. Cells were then incubated for 1 h at room temperature, in 0.5 ml of the same buffer containing [^{3}H]Cl-DPDPE (approximately 1.5 nM), 1 mg/ml bovine serum albumin and the appropriate concentration of the unlabeled opioid compound. Binding equilibrium was reached within 1 h when binding to intact cells (data not shown). After 1 h, the radioligand was removed, and the wells washed 3 times with 0.5 ml of ice-cold buffer. After the third wash, 0.5 ml of 0.5 M NaOH was added to each well to digest the protein. After at least 2 h, 0.4 ml was added to scintillation vials for counting, the remainder was used for protein determination in each well.

For binding to cell membranes, the cells were plated on 100 mm culture dishes and used at confluence. The cells were washed off the plates with 50 mM Tris buffer, pH 7.5, and homogenized using a Polytron homogenizer. The homogenate was then centrifuged at $40\,000 \times g$ for 10 min, resuspended in Tris and centrifuged once more. The pellet was resuspended in Tris buffer and used for binding studies. Binding was conducted for 2 h at 25°C, using approximately 1.0 nM [^{3}H]Cl-DPDPE, in a total volume of 1.0 ml of Tris buffer in the absence of other salts. After the incubation, the samples were filtered over glass fiber filters using a cell harvester (Brandel), prior to counting.

Guinea-pig brains were rapidly removed from Hartley guinea pigs immediately after decapitation. Membrane preparation and binding to guinea-pig brain membranes was conducted as described for binding to cell membranes, except a 2 ml incubation volume was used for all experiments. At least seven concentrations of the competing drug were used in all experiments, usually ranging from 0.1 nM to 1.0 μM. For compounds with particularly high or low affinity, the concentrations were modified so that in each experiment inhibition of [^{3}H]Cl-DPDPE ranged from 0 to 100% of specific binding.

2.3. cAMP determination

The effect of opiates on cAMP accumulation was determined basically as described previously (Toll, 1995). Briefly, cells were incubated in the modified Krebs buffer used for the intact cell binding experiments, either in 6-well or 24-well plates. Cells were preincubated for 15 min in the presence or absence of antagonist, as desired, this medium was removed, and replaced with fresh medium containing the appropriate agonist, antagonist and forskolin (10 μM). In the experiments to measure the activity of antagonists, agonist concentration was set at 100 nM. The second incubation lasted 10 min, at which time the buffer

was removed, and replaced with 1 ml (6-well plates) or 0.4 ml (24-well plates) of 0.5 M formic acid. The formic acid was left on the plates for at least 1 h, at which time the formic acid containing cAMP was removed and lyophilized. The residue was suspended in 100 mM sodium acetate buffer pH 4.0, and used for the determination of cAMP. cAMP was assayed by the method of Gilman (1970).

2.4. Materials

DMEM was from Sigma, and horse and fetal calf serum were from Hyclone. Naltrindole, naltriben and 7-benzylidenenaltrexone were from Research Biochemicals International, deltorphin II and Cl-DPDPE were from Peninsula Labs, [^{3}H]Cl-DPDPE was from Dupont/New England Nuclear. The remainder of the drugs were obtained from the NIDA drug supply program.

3. Results

It has been shown previously that SH-SY5Y cells contain δ-opioid receptors (Yu et al., 1986; Kazmi and Mishra, 1987). These can be labeled, either in intact cells or cell membranes using the δ-opioid receptor-selective ligand [^{3}H]Cl-DPDPE (Fig. 1). In both cases, non-linear analysis of saturation binding and linear Scatchard plots suggest the presence of a single binding site. The affinities of Cl-DPDPE determined by the two binding methods were also similar. As expected, the B_{max} values were not similar in intact cells and cell membranes. The B_{max} value in the cell membranes was higher per mg protein probably because much of the cytosolic protein is discarded when making the membrane preparation.

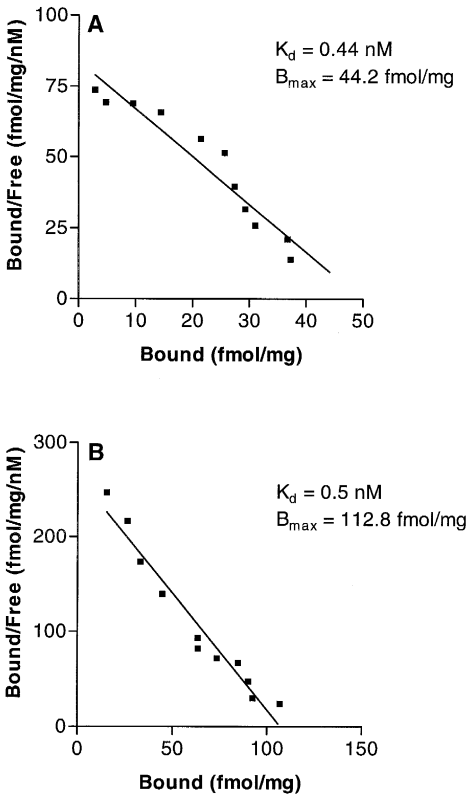

Fig. 1. Scatchard analysis of [^{3}H]Cl-DPDPE binding to SH-SY5Y (A) intact cells and (B) cell membranes. Data shown are from single experiments conducted in triplicate, as described in Section 2. Saturation data shown were analyzed by non-linear analysis, using the curve-fitting program PRISM. Scatchard plots were derived from these data. Each experiment was repeated three additional times with similar results.

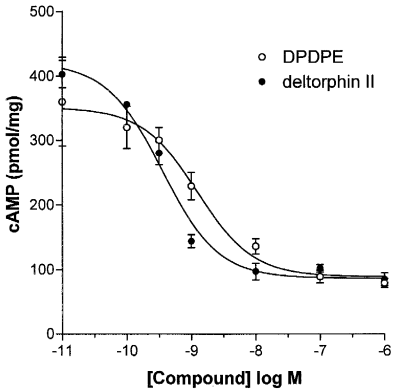

Fig. 2. Inhibition of forskolin-stimulated cAMP accumulation in SH-SY5Y cells by δ-selective agonists. cAMP accumulation was inhibited by DPDPE (○) and deltorphin II (●), during a 10 min incubation in the presence 10 μM forskolin, as described in Section 2. Data shown are from individual experiments, that were repeated two additional times. In the three separate experiments, maximal inhibition of cAMP accumulation varied between 45% and 75%; however, IC_{50} values for the agonists did not change significantly.

Although DPDPE and deltorphin II are considered to be selective for δ_1- and δ_2-opioid receptors respectively, they are both very potent as inhibitors of forskolin stimulation of cAMP accumulation in SH-SY5Y cells. cAMP is inhibited to the same extent with each compound, and at slightly lower concentrations by the δ_2-opioid receptor-selective agonist deltorphin II (Fig. 2). The potencies of both ligands are in the nanomolar range, as expected from the binding affinities, and other in vitro evaluations of these compounds.

Table 1
Inhibition of the activity of δ-agonists by selective δ-antagonists

Agonist	IC_{50} (nM)	
	7-Benzylidene naltrexone	Naltrindole benzofuran
DPDPE	163 ± 32	0.88 ± 0.13
Deltorphin II	580 ± 104	0.80 ± 0.18

Values shown represent IC_{50} ± S.D. for the inhibition of the opiate-induced decrease of forskolin stimulation of cAMP accumulation in intact SH-SY5Y cells. The experiments were carried out as described in Section 2, and represent three or four individual experiments in each case. The concentrations of DPDPE and deltorphin II were 100 nM, while the concentrations of the antagonists varied between 10 pM and 10 μM.

Table 2
Affinities of opioid ligands at δ-opioid receptors in SH-SY5Y cells and guinea-pig brain membranes

Compound	K_i (nM)		
	SH-SY5Y intact cells	SH-SY5Y cell membranes	Guinea-pig brain membranes
DPDPE	9.6 ± 0.85	6.8 ± 4.2	2.8 ± 0.3
Cl-DPDPE	0.90 ± 0.15	0.59 ± 0.09	0.3 ± 0.05
Deltorphin II	0.96 ± 0.22	1.28 ± 0.12	0.7 ± 0.1
DSLET	1.46 ± 0.6	2.2 ± 0.6	0.5 ± 0.1
DADLE	7.2 ± 5.3	1.1 ± 0.9	2.7 ± 1.7
Naltrindole	0.09 ± 0.04	0.06 ± 0.016	0.09 ± 0.01
NTB	0.07 ± 0.025	0.09 ± 0.023	0.06 ± 0.01
BNTX	1.56 ± 0.42	1.63 ± 0.42	3.1 ± 1.1
Etorphine	5.6 ± 2.6	1.12 ± 0.05	0.70 ± 0.02
Morphine	765 ± 181	103 ± 11.7	50 ± 6.5
DAMGO	5272 ± 124	433 ± 47	180 ± 16

Binding was conducted as described in Section 2. Values shown are K_i ± S.D. from at least two experiments conducted in triplicate. K_i values were determined from the equation $K_i = IC_{50}/(1+[L]/K_d)$. K_d for [^{3}H]Cl-DPDPE was determined using the curve fitting program LIGAND (Munson and Rodbard, 1980). None of the compounds had Hill coefficients significantly less than 1.0, indicating that we could only identify a single binding site, and allowing for the use of the above equation. DADLE is [D-Ala^2,D-Leu^5]enkephalin.

We next tested the ability of the δ-opioid receptor-selective antagonists naltriben and 7-benzylidenenaltrexone to inhibit the activities of DPDPE and deltorphin II. In SH-SY5Y cells, both antagonists are able to block the inhibition of forskolin-stimulated cAMP accumulation induced by either selective agonist. However, as seen in Table 1, in SH-SY5Y cells, naltriben is considerably more potent. Furthermore, although naltriben is equally potent against either agonist, 7-benzylidenenaltrexone seems to be slightly more potent against DPDPE.

In order to better compare the receptor found in SH-SY5Y cells with the receptor that is routinely labeled in brain, additional binding studies were conducted with [^{3}H]Cl-DPDPE. In these experiments, binding to intact cells was compared with binding to both cell membranes and guinea-pig brain membranes. Binding to intact cells was conducted in a Krebs buffer, while binding to both cell and brain membranes was conducted in a Tris buffer, in the absence of salts and guanine nucleotides. As seen in Table 2, the binding was very similar in each case, in spite of the fact that we are comparing guinea-pig receptor from brain with the human receptor in SH-SY5Y cells. In general, binding affinities were as expected. The δ-opioid receptor agonists were all found to have high affinity, while the μ-opioid receptor-selective compounds morphine and DAMGO ([D-Ala^2,(Me)Phe^4,Glyol^5]enkephalin) both have very low affinity. Each of the δ-opioid receptor antagonists were found to have high affinity, although the affinities of naltrindole and its analog naltriben were significantly higher than 7-benzylidenenaltrexone. It is important to note that the affinity we derived for 7-benzylidenenaltrexone in both tissues is 10-fold lower than Portoghese et al. (1992) reported in the initial publication with this compound. Interestingly, the affinities determined for the δ-opioid receptor agonists were the same in intact cells as in cell or brain membranes. This was not the case for the μ-opioid receptor agonists morphine and DAMGO, which shifted considerably to lower affinity in the salt-containing Krebs buffer used on the intact cells.

4. Discussion

The in vivo evidence for δ-opioid receptor subtypes is compelling. This evidence includes: actions of selective agonists and antagonists on analgesia (Sofuoglu et al., 1991; Jiang et al., 1991), lack of cross-tolerance of selective δ-opioid receptor ligands (Mattia et al., 1991), differences in the actions of δ_1- and δ_2-opioid receptor ligands on the development of physical dependence on morphine in mice (Miyamoto et al., 1993), differences in the actions of selective δ-opioid receptor ligands in weaned and non-weaned rats (Crook et al., 1992), electrophysiological studies in spinal cord neurons (Glaum et al., 1994) and the administration of receptor antisense oligonucleotides (Cha et al., 1995). This project was initiated by asking the simple question, whether the δ-opioid receptor found in SH-SY5Y cells was a δ_1- or δ_2-opioid receptor type, or perhaps both receptors could be found.

Initial studies clearly showed both δ_1- and δ_2-opioid receptor-'selective' ligands DPDPE and deltorphin II were quite active in SH-SY5Y cells (Fig. 2). These results indicate that either two receptors are present, and both agonists are active at each receptor, or a single receptor is present, with both compounds acting as potent agonists. Our results are totally in accord with the results of Wild et al. (1993a), who showed both selective ligands to be active in mouse vas deferens, and Henderson and colleagues (Keir et al., 1995) who found no differences for selective δ-opioid receptor agonists and antagonists when measuring opioid-stimulated increases in intracellular Ca^{2+}. Based upon the activity of the non-equilibrium antagonist DALCE, Wild et al. (1993a) concluded that the only δ-opioid receptor found in the mouse vas deferens was δ_2-opioid receptor. Experiments using DALCE on SH-SY5Y cells were not able to reveal a difference between DPDPE and deltorphin II, but were difficult to interpret because of acute agonist activity of DALCE (data not shown). Acute agonist activity had been shown previously for the peptide DALCE (Bowen et al., 1987).

We next used the selective antagonists to help determine which site is found in SH-SY5Y cells. As found by Raynor et al. (1994) in transfected Chinese hamster ovary (COS) cells, the δ_2-opioid receptor ligand naltriben has considerably higher affinity (15–20-fold) for the receptor in SH-SY5Y cells than the δ_1-opioid receptor ligand 7-benzylidenenaltrexone. Raynor et al. (1994) used this crite-

rion to call the cloned receptor a δ_2-opioid receptor. However, 7-benzylidenenaltrexone still has very high affinity (1–2 nM). The affinity that we found for 7-benzylidenenaltrexone in SH-SY5Y cells is 10-fold lower than reported by Portoghese et al. (1992) for inhibiting [^{3}H]DPDPE binding to guinea-pig brain. However, in our hands, the apparent binding affinities for all of the compounds tested are practically identical for the receptor in SH-SY5Y cells and for the receptor labeled in guinea-pig brain membranes (see Table 2). Furthermore, Hill coefficients are routinely close to 1.0 using [^{3}H]Cl-DPDPE, and computer analysis of the saturation experiments suggested a single binding site. From these data, we cannot identify two binding sites, and it seems clear that the binding site found in SH-SY5Y cells is quite similar to the site found in brain membranes. If we used the criterion of relative affinities of naltriben and 7-benzylidenenaltrexone, we would have to conclude that the receptor labeled by [^{3}H]Cl-DPDPE in SH-SY5Y cells and brain is the δ_2-opioid receptor.

The antagonist activity of naltriben and 7-benzylidenenaltrexone might provide additional clues as to the nature of the receptor in SH-SY5Y cells. In these cells, 7-benzylidenenaltrexone is a very weak antagonist (Table 1). Although a Schild analysis was not conducted, and the potency of the antagonist depends upon the concentration of agonist, the difference between binding K_i and IC_{50} for inhibition of agonist activity was 100–400-fold for 7-benzylidenenaltrexone and approximately 10-fold for naltriben. Furthermore, naltriben was at least 200-fold more potent than 7-benzylidenenaltrexone in SH-SY5Y cells. When given intracerebroventricularly (i.c.v.) as inhibitors of analgesia, 7-benzylidenenaltrexone and naltriben seem to require approximately equal doses (10–20 pmol per mouse) to produce significant antagonism of the appropriate agonist (DPDPE and DSLET ([D-Ala^2,Leu^5,Thr^6]enkephalin), respectively) (Sofuoglu et al., 1991; Portoghese et al., 1992). Although it is hard to understand how 7-benzylidenenaltrexone can bind with such high affinity but be such a weak antagonist, these are additional indications that the receptor found in SH-SY5Y cells could be classified as a δ_2-opioid receptor.

Some of the results in vitro seem to conflict with the in vivo analgesia data. Although, under the appropriate conditions, there seems to be no cross-reactivity between DPDPE and deltorphin II in vivo, they seem to act quite similarly in SH-SY5Y cells and mouse vas deferens (Wild et al., 1993a). One simple explanation for this might be that dose-response curves are very steep when measuring analgesia, but much shallower when measuring in vitro activities. This being the case, one would expect to get a greater separation of activities in vivo, and perhaps find concentrations where only one of the compounds has agonist activity. Another explanation might be that DPDPE has lower efficacy at the δ_2-opioid receptor. If this were the case, DPDPE might not have agonist activity in many δ_2-opioid receptor-mediated functions, but still could act as an agonist in systems with high receptor reserve, potentially mouse vas deferens and SH-SY5Y cells.

An explanation for the difficulty in seeing δ-opioid receptor subtypes in binding experiments might be that one of the receptors represents a small proportion of binding sites. If δ_1-opioid receptors, for instance, represented only 10% of the total δ-opioid receptors, but [^{3}H]Cl-DPDPE bound with reasonably high affinity to both sites, it would be very difficult to characterize both sites by receptor binding.

It is interesting that the binding affinities found in intact cells match so closely with values determined in either cell membranes or guinea-pig brain membranes. This result is quite different than affinities determined for [^{3}H]CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2) binding to μ-opioid receptors on intact SH-SY5Y cells when compared to either cell membranes or guinea-pig brain membranes (Toll, 1992). The most likely explanation for this difference is that [^{3}H]Cl-DPDPE is an agonist, so that it binds with high affinity only to the agonist conformation of the receptor, and that only this conformation is labeled whether binding to intact cells or cell membranes. This would occur if the dissociation rate of [^{3}H]Cl-DPDPE from the lower affinity states were too fast to capture using our binding protocols. If this were the case, each of the δ-opioid receptor agonists might be expected to have the same apparent affinity in the three different preparations. In contrast, [^{3}H]CTOP is a μ-opioid receptor-selective antagonist, so that in intact cells it would bind to the low affinity agonist conformation that would be present in large amounts in intact cells. For this reason, most of the μ-opioid receptor agonists had low apparent affinity when binding to the μ-opioid receptor in intact SH-SY5Y cells (Toll, 1992).

On the other hand, there are clear differences between intact cell binding to μ- and δ-opioid receptors. In intact cells, we could observe no significant [^{3}H]DAMGO binding, and only a single apparent binding site for a variety of ligands in competition with [^{3}H]CTOP, suggesting that there was very little, if any, high affinity μ-opioid receptor binding found in the intact cells. This is clearly not the case for δ-opioid receptors. Furthermore, some agonists, such as etorphine, maintained high affinity even in intact cells. This led us to the conclusion that a single μ-opioid receptor conformation was present in intact cells, and it was ligand-dependent as to whether they had high or low affinity (Toll, 1992). This is consistent with the unusual finding that the affinities of morphine and DAMGO (but not the δ-opioid receptor agonists) also decrease significantly when binding to the δ-opioid receptor on intact cells (see Table 2). It is possible that the δ-opioid receptor agonists do maintain high affinity in intact cells despite the presence of salts in the buffer and GTP inside the cells, and that morphine and DAMGO have lower affinity under those conditions regardless whether they are binding to μ-

or δ-opioid receptors. Many, but not all, δ-opioid receptor-selective ligands show a large sodium effect when binding to δ-opioid receptors in brain membranes (Wild et al., 1993b), suggesting that high and low affinity conformations should be present in intact cells. However, intact cells, with their full complement of ions and proteins, plus a membrane potential, may be different than any binding conditions that can be developed for brain membrane homogenates.

In conclusion, there is apparently a single δ-opioid receptor type in SH-SY5Y cells. Based upon the comparison of the affinity and activity of 7-benzylidenenaltrexone and naltriben, we would conclude that this is a δ_2-opioid receptor. In addition, this receptor has binding characteristics that suggest that it is the same receptor that was cloned from NG108-15 cells, and is the same receptor that accounts for the majority of the binding in guinea-pig brain, even when using [^{3}H]Cl-DPDPE, a supposed δ_1-opioid receptor-selective ligand (Sofuoglu et al., 1992). This makes several things unclear. First, the δ_1-opioid receptor is more difficult to characterize, and there apparently is no appropriate binding assay for this site. Consequently, we feel that the affinities of all ligands are unknown at this site. Furthermore, it is unclear why DPDPE and deltorphin II do not act as agonists at both sites in vivo. Although we do not know the affinity of deltorphin II at the δ_1-opioid receptor, we know that DPDPE has high affinity at the δ_2-opioid receptor, and acts as an agonist in at least two in vitro assays. The remaining possibility is that apparent δ-opioid receptor subtypes are a result of pharmacokinetics, second messenger coupling, or some other phenomenon that is difficult to assess in vitro. This would explain why only a single δ-opioid receptor subtype has been cloned in spite of intensive investigations via molecular biological techniques. The final answers will require either another cloned δ-opioid receptor subtype, or more selective ligands for the in vitro identification of another δ-opioid receptor binding site.

Acknowledgements

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