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Agonist-Induced State of the δ-Opioid Receptor That Discriminates between Opioid Peptides and Opiate Alkaloids

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SUMMARY

We report a novel agonist-induced change in δ-opioid receptor binding properties in NG108–15 cells. Pretreatment of these cells with opioid agonists substantially diminishes the binding of peptide agonists and a peptide antagonist to opioid receptors in intact cells or membrane preparations. However, similar agonist-induced changes in the binding of opiate alkaloid agonists and antagonists were not detected. The change in opioid peptide binding occurs rapidly at 37° ($t_{½} \approx 10$ min) but is not induced by agonist treatment at 4°. Because of its lability at 37°, the binding change is only detected when equilibrium binding assays are performed at 4°. Both alkaloid and peptide agonists induce the binding change in a dose-dependent manner, with an ED_{50} for etorphine of approximately 10 nM. The induction of the binding change is completely blocked by the opiate antagonist naloxone. Stimulation of muscarinic receptors (which, like opioid receptors, inhibit adenylate cyclase in these cells) does not induce or block the binding change. These data reveal the operation of a homologous regulation mechanism that rapidly diminishes the interaction of δ-subtype opioid receptors with peptide ligands but does not detectably change the interaction of receptors with alkaloid ligands.

Molecular cloning has established that many receptors for neurotransmitters and neuromodulators are structurally similar transmembrane proteins that modulate intracellular effectors via coupling to specific G proteins (1). Functional studies suggest that δ-opioid receptors are members of the G protein-coupled receptor family, because they are coupled via pertussis toxin-sensitive G proteins to adenylate cyclase and ion channels and because they exhibit GTP-sensitive binding of agonists (2–7). Recent molecular cloning data provide structural confirmation that the δ-opioid receptor is a member of the G protein-coupled receptor family, with a predicted topology containing seven putative membrane-spanning hydrophobic domains (8).

The neuroblastoma-glioma hybrid cell line NG108–15 is a useful model system for studying the function and regulation of δ-opioid receptors. These cells can be readily grown and manipulated in culture and express large numbers of δ-opioid receptors; these receptors are functionally coupled to adenylate cyclase and calcium channels via pertussis toxin-sensitive G proteins (6, 7). δ-Opioid receptors in NG108–15 cells display agonist-induced desensitization that occurs within minutes and down-regulation that occurs more slowly over several hours (9). The internalization of receptor-agonist complexes can be detected with kinetics similar to those of down-regulation (10, 11). In addition, small agonist-induced changes in agonist binding affinity, without changes in antagonist affinity, have been reported (12, 13). However, there is no evidence that binding selectivity between peptide and alkaloid ligands can be regulated.

Here we describe an agonist-induced state of δ-opioid receptors, detected in NG108–15 cells or in membranes prepared from cell lysates, that is characterized by markedly reduced binding of opioid peptides without a detectable change in the binding of alkaloid agonists or antagonists. In addition to its peptide selectivity, this binding effect differs from previously reported regulatory phenomena in magnitude and speed. These findings challenge the widely held belief that opiate alkaloids and endogenous opioid peptides are equivalent in their interaction with opioid receptors (i.e., opioid receptors cannot distinguish a peptide from an alkaloid agonist) and may be relevant to understanding more completely the interaction of alkaloid drugs with the endogenous opioid system.

Materials and Methods

Etorphine HCl, naloxone, and diprenorphine were gifts from the National Institute on Drug Abuse. Morphine sulfate was obtained from Stanford Pharmacy with a National Institute on Drug Abuse research

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ABBREVIATIONS: G protein, guanine nucleotide-binding protein; DADLE, 2-D-Ala,5-D-Leu-enkephalin; TIPP, Tyr-tetrahydroisoquinoline-3-carboxylic acid-Phe-Phe; PBS, phosphate-buffered saline; KRHB, Krebs-Ringer-HEPES buffer; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

permit. Opioid peptides were either purchased from Peninsula Laboratories (San Carlos, CA) or synthesized using standard solid-phase procedures (14). Tritiated diprenorphine (30–50 Ci/mmol) was purchased from Amersham, and tritiated DADLE (approximately 35 Ci/mmol) was obtained from New England Nuclear. Carbachol and general biochemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Cell culture reagents were obtained from the University of California, San Francisco, Cell Culture Facility and GIBCO.

Cell culture. NG108–15 cells were cultured in Dulbecco's modified Eagle's medium containing 7% (v/v) fetal bovine serum and hypoxanthine/aminopterin/thymidine (9) and were maintained at 37° in an atmosphere containing 10% CO_2. Cells were grown to a confluent monolayer in 100-mm plastic dishes (Corning), after which a medium change was performed 16–24 hr before drug or sham addition in 1/20 volume of warmed medium. After experimental incubations but before harvesting, culture dishes were chilled for 15 min on ice, and all subsequent manipulations were performed at 4°. After removal of the culture medium, cells were harvested by incubating monolayers for 15 min with ice-cold PBS, pH 7.3, containing 1 mM EDTA, followed by gentle scraping using a rubber policeman. Cell suspensions were pelleted in a refrigerated clinical centrifuge at 200 × *g* for 3 min, the supernatant was decanted, and pellets were washed four times in PBS. After the final wash, cells were pelleted and resuspended in 10 ml of KRHB (9) for binding assays. Membrane fractions for binding assays were prepared from washed cells as described (12).

Binding determinations. Binding assays of intact cells were performed essentially as described by Law *et al.* (9), with the important difference that all incubations and manipulations were performed at 4°. [^{3}H]Diprenorphine (30–50 Ci/mmol; Amersham) was used to probe opiate binding sites. A probe concentration of 2.5 nM (approximately the K_d under these conditions; see Fig. 2b) was used unless specified otherwise. Equilibrium binding assay incubations were performed in KRHB, in a final volume of 1 ml (100 μl of tracer, 100 μl of drug solution, and 800 μl of cell suspension described above). After a 1-hr incubation in 12- × 75-mm borosilicate glass tubes, cells were washed with 20 ml of cold PBS by filtration on Whatman GF/B paper, using a Brandel harvester. For direct examination of opioid peptide binding, [^{3}H]DADLE was used. Binding conditions used were as described above, except that incubations were carried out in a total volume of 100 μl in 96-well plastic microtiter plates. Binding assays on membrane fractions were conducted at 4°, as described above, in a buffer containing 25 mM HEPES and 10 mM $MgCl_2$, pH 7.4. CytoScint cocktail (ICN) was added to the filters, and samples were counted in a Beckman LS3000 counter operating at approximately 66% efficiency. Nonspecific binding was determined by assay in the presence of 400 nM unlabeled diprenorphine. Competition binding results are expressed as the percentage of specific binding measured in the absence of added competitor. Saturation binding data are expressed as dpm of specific binding (using binding in the presence of 400 nM unlabeled diprenorphine to define nonspecific binding) normalized to the amount of cell protein added to the assay, as determined by the method of Bradford (15) using Bio-Rad dye reagent. All data points represent the mean of three separate experimental determinations unless noted otherwise.

Results

Opioid agonists markedly reduce the binding of the opioid peptide DADLE to NG108–15 cells but do not affect the binding of agonist or antagonist alkaloids. We examined opiate binding to intact NG108–15 cells using equilibrium competition at 4°, with a high affinity δ-opiate antagonist ([^{3}H]diprenorphine) as probe. We used low temperature in an attempt to stabilize modifications that may occur during drug treatments and to prevent the possible loss of labile modifications when the cells are withdrawn from opioid drugs and incubated with antagonist for assay. A secondary motivation for the use of low temperature was to diminish the possibility of proteolytic artifact. Competition binding assays, using either peptide or alkaloid competitor, yielded identical results when incubation times ranging from 45 min to >2 hr were used, indicating that equilibrium binding conditions were achieved. Subsequently, 1-hr incubation was chosen for routine assay.

Treatment of cells for up to 2 hr with 25 nM etorphine had no detectable effect on the binding of this alkaloid agonist (Fig. 1a). We also found no change in the binding of the partial agonist morphine or the alkaloid antagonists diprenorphine and naltrindole (Fig. 2; see also Table 2). After treatment with etorphine for several hours, we began to detect a loss of opiate binding sites, consistent with the established time course (9) of receptor down-regulation (data not shown). In contrast to the lack of effect on opiate alkaloid binding, etorphine treatment caused a dramatic change in the binding properties of the opioid peptide agonist DADLE. DADLE competition was so impaired by this treatment that typically 70–90% of specifically bound [^{3}H]diprenorphine could not be displaced by this opioid peptide (Fig. 1b). Treatment of cells with 10 nM DADLE induced a change in peptide, but not alkaloid, binding comparable to that produced by etorphine (Table 1). Thus, the change in peptide-binding properties was induced by peptide as well as alkaloid agonists. This binding change was blocked by the opiate antagonists naloxone (Table 1) and diprenorphine (data not shown).

One explanation for the large change in opioid peptide binding could be that, despite extensive washing, residual agonist might dissociate so sluggishly from cells at 4° that receptor sites could be "blocked" in competition binding assay. Several observations suggest that such blockade does not occur; 1) the amount of diprenorphine tracer bound in the absence of competitor was unchanged by etorphine treatment, 2) only competition by opioid peptides (not that by alkaloids) was affected, and 3) the binding change was not observed after treatment of cells with etorphine for several hours at 4° (see below). However, to test this possibility further, we performed saturation binding analysis with diprenorphine. Untreated and etorphine-treated cells displayed essentially identical saturation binding properties (Fig. 2b). Therefore, the binding data truly indicate a selective binding change and do not result artifactually from residual bound etorphine.

The agonist-induced binding change results from a loss of peptide binding and is observed with a variety of opioid peptides. To test whether the binding change results from a direct loss of opioid peptide binding or whether it results indirectly from a diminished ability of peptide to compete for alkaloid binding sites, we examined peptide binding using [^{3}H]DADLE saturation. Labeled opioid peptide bound to untreated cells in a dose-dependent manner but was substantially reduced in agonist-treated cells (Fig. 3). These data suggest that the agonist-induced binding change results directly from a selective loss of opioid peptide binding sites.

Saturation binding detected high affinity peptide binding sites that comprised approximately 7% of receptor binding detected by diprenorphine (compare Figs. 2b and 3). This observation is consistent with the multiple affinity states of δ-opioid receptor described extensively in a previous study, where it was determined that most receptors are in a state of low agonist affinity (16). Furthermore, these data indicate that agonist treatment of cells markedly reduces both high affinity (measured by saturation; Fig. 3) and low affinity (measured by competition; Fig. 1b) peptide binding. Therefore, the binding

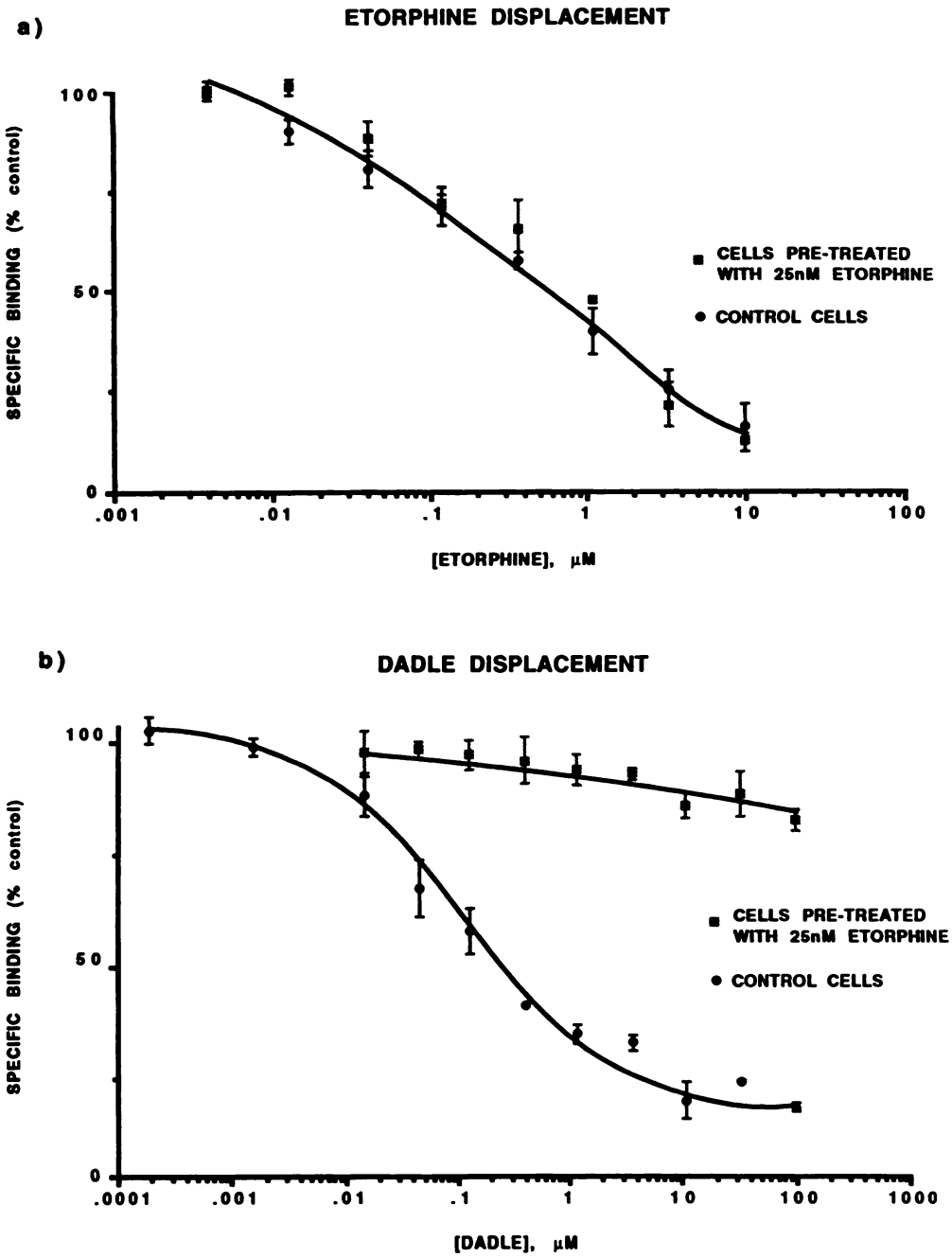


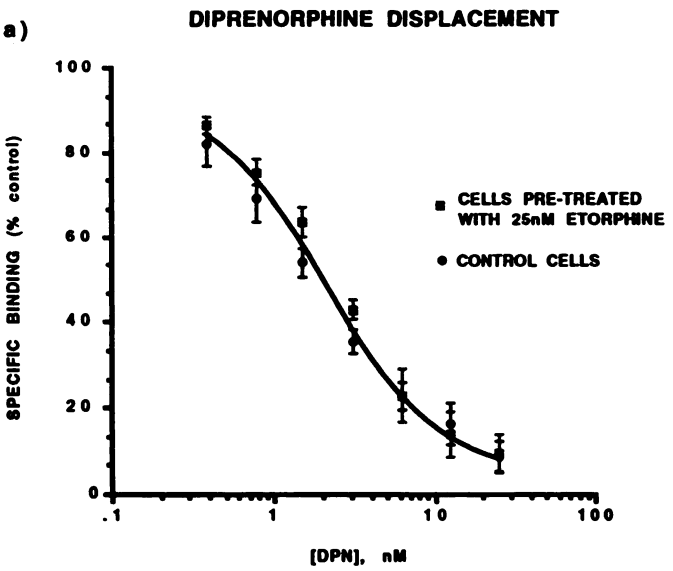
Fig. 1. Effect of etorphine pretreatment of NG108–15 cells on the displacement of 2.5 nM [^{3}H]diprenorphine by etorphine and DADLE. Confluent cell monolayers were treated in 100-cm dishes for 2 hr at 37° with either 25 nM etorphine delivered in 1/20 volume of warmed medium (etorphine-treated cells) or 1/20 volume of warmed medium containing no drugs (control cells). Monolayers were chilled, harvested, and washed as described in Materials and Methods. Binding assay was conducted at 4° for 1 hr, using 2.5 nM [^{3}H] diprenorphine as tracer and either etorphine (a) or DADLE (b) as competitor, as described in Materials and Methods. *Data points*, mean specific binding of quadruplicate determinations; *error bars*, standard deviations.

change can be detected using either method; we routinely assayed high and low affinity states in aggregate by competition of diprenorphine binding.

This selective change in opioid peptide binding was not unique to the synthetic enkephalin analog DADLE. Agonist-induced loss of peptide binding was also observed in competition binding experiments using a number of naturally occurring opioid peptides as well as deltorphan and the peptide antagonist TIPP (Table 2). Taken together, the data obtained using four alkaloids and 10 peptides indicate that the agonist-induced binding change detected in our assay distinguishes opioid peptides from opiate alkaloids, regardless of agonist or antagonist properties. Therefore, we refer to this receptor regulation as a peptide-selective binding change.

The peptide-selective binding change is dose dependent and is not altered or induced by stimulation of another G_i-coupled receptor system. The peptide-selective binding change was detected after treatment of cells with relatively low doses of agonist (Fig. 4a). Etorphine induced a readily detectable change in peptide binding at a concentration of only 2.5 nM, which is considerably below its K_i for competition binding measured in intact cells (in the presence of sodium) at either 4° (Fig. 1a) or 37° (5, 13). Nearly maximal effect was obtained with 25 nM etorphine, which is still below the measured K_i at 4°. Only slightly more change in peptide binding was induced by 250 nM etorphine. Even at this high concentration, no evidence of receptor blockade by residual etorphine was observed, inasmuch as the total binding of diprenorphine remained unchanged (Fig. 4a).

NG108–15 cells express muscarinic acetylcholine receptors,



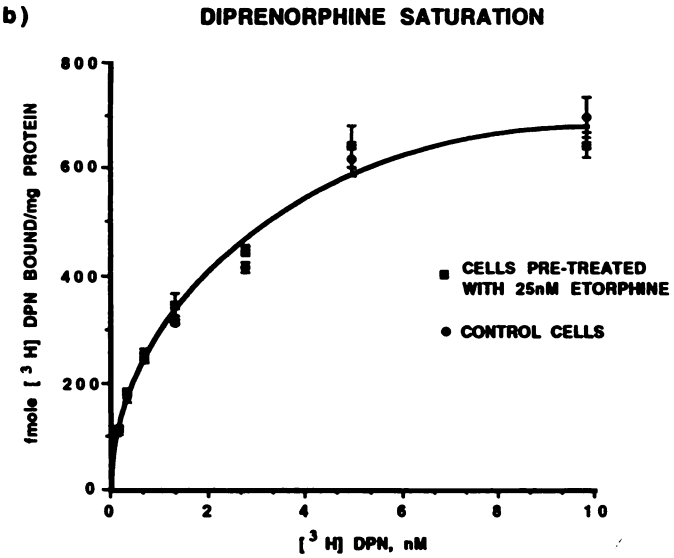


Fig. 2. Effect of etorphine pretreatment of NG108–15 cells on displacement and saturation binding curves for diprenorphine (*DPN*). Cells were treated exactly as described for Fig. 1. Displacement of binding (a) was measured at 4° using competition of 2.5 nM [^{3}H]diprenorphine with unlabeled diprenorphine, as described in Materials and Methods. *Data points*, mean specific binding from triplicate determinations; *error bars*, standard deviations. Saturation binding (b) was measured at 4° using various concentrations of [^{3}H]diprenorphine, as described in Materials and Methods. *Data points*, mean of triplicate determinations of specific binding, expressed as fmol bound/mg of cell protein present in the assay; *error bars*, standard deviation. We verified that the peptide-selective binding change occurred in the etorphine-treated cells by checking displacement of the binding of [^{3}H]diprenorphine by DADLE.

which, like δ-subtype opiate receptors, are coupled to adenylate cyclase via pertussis toxin-sensitive G proteins (17). To determine whether the change in opiate peptide binding is homologous (9, 18) or whether the effect can be induced via another G protein-coupled signal transduction system, we examined peptide binding in cells treated with the muscarinic agonist carbachol. Carbachol (100 μM) stimulates and causes desensitization and down-regulation of muscarinic receptors in NG108–15 cells (19), but this agent did not block or induce the peptide-selective binding change (Table 1).

TABLE 1

Effect of various drug treatments on the induction of the peptide-selective binding change

The peptide-selective binding effect was measured by determination of specific [^{3}H]diprenorphine binding remaining in the presence of 30 μM DADLE (as described in the legend to Fig. 5). NG108-15 cell monolayers were treated for 1 hr at 37° with various drugs before binding assay at 4°. Data represent the mean of duplicate determinations of specific [^{3}H]diprenorphine binding, as described in Materials and Methods.

Treatment	Specific diprenorphine binding remaining in the presence of 30 μM DADLE
	%
Control (untreated)	18
25 nM Etorphine	59
25 nM Etorphine + 100 μM naloxone	15
25 nM DADLE	65
25 nM DADLE + 100 μM naloxone	17
100 μM Carbachol	18
100 μM Carbachol + 25 nM etorphine	61

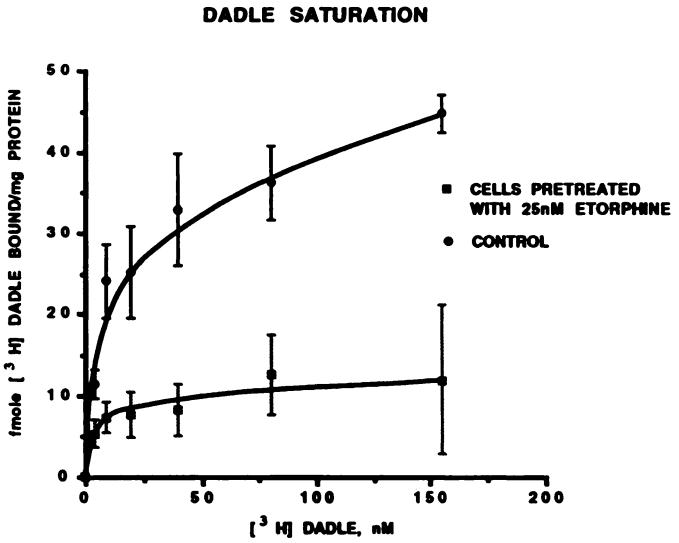


Fig. 3. Effect of etorphine pretreatment of NG108–15 cells on binding of [^{3}H]DADLE. Cells were treated and harvested as in Fig. 1 and were incubated at 4° with various concentrations of [^{3}H]DADLE in KRHB before determination of bound ligand by filtration, as described in Materials and Methods. *Data points*, mean values of triplicate determinations; *error bars*, standard error.

The time course and temperature dependence of the peptide-selective affinity change. Treatment of NG108–15 cells with 25 nM etorphine at 37° resulted in rapid and substantial loss of peptide binding, but we observed no change in peptide binding after incubation of cells with etorphine for 2 hr at 4° (Fig. 4b). Therefore, we used the strict temperature dependence of the binding change to dissect its kinetics from the association kinetics of etorphine. Cells were incubated with etorphine for 2 hr at 4° to allow ample time for agonist binding, quickly warmed to 37° for various time periods to allow the binding change to occur, and then chilled to 4° on ice for subsequent washing and assay. These experiments indicated that the development of the etorphine-induced binding change is quite rapid at 37° (Fig. 4b). No binding change was observed after treatment of cells for 2 hr with etorphine at 4°, a small effect was detected after 1 min at 37°, and half-maximal binding change occurred in <10 min.

The peptide-selective binding change is retained in

TABLE 2

Effect of etorphine pretreatment of NG108-15 cells on the displacement of 2.5 nM [^{3}H]diprenorphine by a variety of opioid peptides and opiate alkaloids

Cells were treated and harvested as in Fig. 1 and were incubated with 2.5 nM [^{3}H] diprenorphine and various competitor opioid receptor ligands as indicated. Data are presented as the percentage of specific [^{3}H]diprenorphine binding remaining in the presence of the indicated concentration of each competing ligand.

Competing ligand	Specific diprenorphine binding remaining in the presence of competing ligand	
	Control cells	Etorphine-treated cells
	%	
Etorpine (1 μM)	21	23
Naltrindole (10 μM)	10	8
Morphine (2.5 μM)	62	53
TIPP (10 μM)	14	66
TIPP (100 nM)	29	96
Tyr-Gly-Gly-Phe (100 μM)	12	62
DADLE (30 μM)	22	66
Deltorphan (10 μM)	28	73
β-Endorphin (30 μM)	23	63
Dynorphin-1–9 (30 μM)	28	61
Met-Enkephalin (30 μM)	14	79
γ-Endorphin (30 μM)	24	77
Met-Enkephalin-RGL (30 μM)	33	64
Metorphamide (30 μM)	28	50

membranes prepared from agonist-treated cells. To elucidate the molecular mechanisms responsible for the peptide-selective binding change, it will be useful to reconstitute this effect in a cell-free system. As an initial step in this direction, we examined receptor binding in membrane fractions prepared at 4° from cell lysates. Membranes prepared from untreated cells exhibited specific binding of diprenorphine that was readily competed for by both alkaloid (diprenorphine) and peptide (DADLE) ligands. In membranes prepared from etorphine-treated cells, however, competition of diprenorphine binding by peptide was markedly diminished, without an apparent change in alkaloid competition binding (Fig. 5). Therefore, the peptide-selective binding change can be retained in membrane fractions prepared from agonist-treated cells.

Discussion

In this paper we describe a state of δ-opioid receptors that is induced by either alkaloid or peptide agonists and does not affect the binding of alkaloid agonists or antagonists but results in a substantial loss of binding of a variety of opioid peptides, including a synthetic antagonist peptide. This effect has a rapid onset and does not occur at 4°. The binding change is induced by low concentrations of etorphine that are comparable to the K_i for cyclase inhibition (13) and is blocked by opiate antagonists. However, inhibition of adenylate cyclase is not itself sufficient to induce the binding change, because carbachol, which also inhibits adenylate cyclase via G protein coupling, does not induce the opioid binding change.

Because the binding change distinguishes all 10 opioid peptides tested from four agonist or antagonist alkaloids tested, we refer to this agonist-induced regulation as a peptide-selective binding change. This type of binding change has not been reported previously, but we note that earlier studies have generally performed washing and binding assays at room temperature or at 37°. Due to the rapid kinetics and temperature dependence of the peptide-selective binding change, it may be

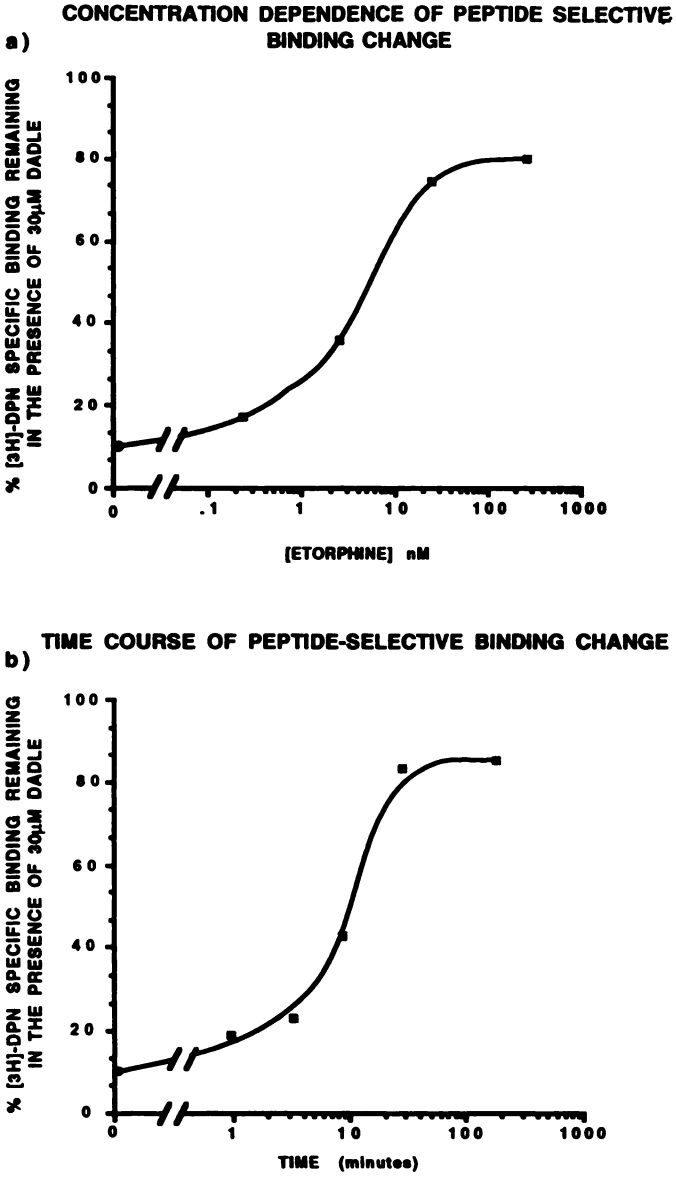


Fig. 4. Dose dependence and time course for the onset of the etorphine-induced binding change. Essentially all specifically bound [^{3}H]diprenorphine (*DPN*) is displaced from control NG108–15 cells by 30 μM DADLE, but substantially less is displaced from etorphine-pretreated cells (see Fig. 1). Therefore, we measured the binding change by assaying the specific binding of [^{3}H]diprenorphine remaining in the presence of 30 μM DADLE, as described in Materials and Methods. Concentration dependence (a) of the binding effect was measured in this way, after 2-hr treatment of NG108–15 monolayers with the indicated concentrations of etorphine. The time course for the onset of the binding change (b) was measured by preincubating cells at 4° for 2 hr with 25 nM etorphine (to allow ample time for agonist binding at reduced temperature, which does not allow the binding change to occur), followed by warming of cells to 37° for the indicated time periods, followed by chilling on ice for subsequent harvest and binding assay. *Data points*, mean of duplicate determinations.

obscured after washing (opiate withdrawal) and assay (incubation with opiate antagonist) at elevated temperature. Consistent with this, we have noted only small binding changes after rapid cell washing and binding assay (30 min) at 37°, and we have been unable to detect the change under more prolonged binding conditions at 37°. In studies currently in progress, we find that the reversal of the peptide-selective binding change,

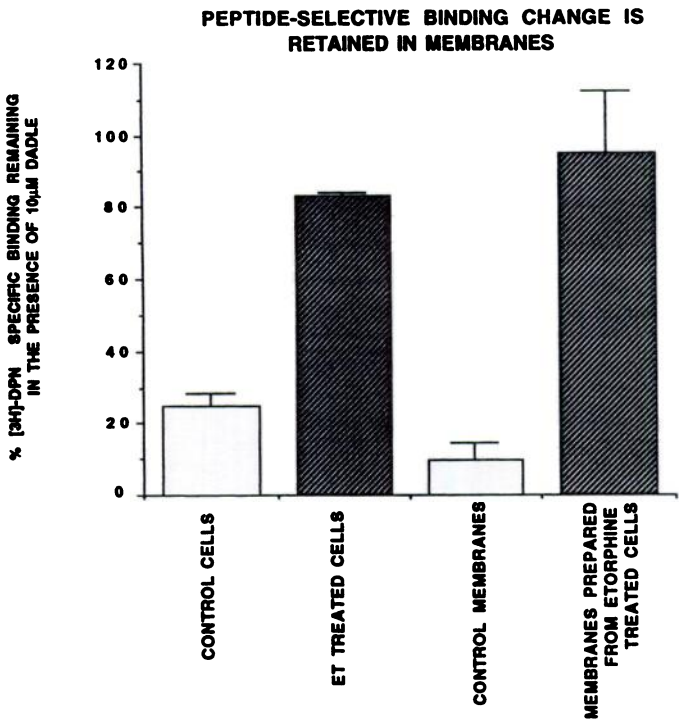


Fig. 5. Detection of the peptide-selective binding change in membranes prepared from etorphine (*ET*)-treated cells. Control and etorphine-treated cells were assayed for the peptide-selective binding change as in Fig. 1. Membrane fractions were prepared from a portion of the cells, and the peptide-selective binding change was assayed in these membranes, as described in Materials and Methods. Data are presented as percentage of specific diprenorphine (*DPN*) binding remaining in the presence of 10 μM DADLE and are expressed as the mean of triplicate determinations.

like its onset, is rapid and temperature dependent (data not shown).

What mechanisms could account for the peptide-selective binding change? The repertoire of regulatory mechanisms described for various G protein-coupled receptors includes agonist-induced functional uncoupling of receptor-modulated effector systems, receptor internalization, and regulatory phosphorylation of specific cytoplasmic receptor domains (18, 20–22). It will be important to determine whether the peptide-selective binding change described here is mediated by these mechanisms or whether the binding change represents the operation of a novel mechanism.

Modulation of functional coupling of opioid receptors to G proteins with GTP analogs and sodium selectively changes agonist binding properties (peptide or alkaloid) without affecting antagonist binding (3–5). It is doubtful that the peptide-selective binding change results solely from functional uncoupling, because this binding change affects peptide agonists and one peptide antagonist but does not affect alkaloid agonists. Furthermore, we have been unable to block or induce the peptide-selective binding change with pertussis toxin, which causes functional uncoupling of opioid receptors (data not shown). However, it is interesting to note that the etorphine concentration dependence for desensitization of cyclase is almost superimposable on that for induction of the affinity change (9).

It is possible that the peptide-selective binding change results from receptor internalization, so that internalized receptors are inaccessible to relatively hydrophilic peptides but are freely accessible to more hydrophobic alkaloids. If this were the case, one might not expect the binding discrepancy between peptides and alkaloids to be retained as it is in membranes prepared from lysed cells. However, the integrity of membrane vesicles containing internalized β-adrenergic receptors can be maintained after cell lysis, and internalized receptors in isolated membrane preparations can remain inaccessible to a hydrophilic ligand (23). Two other points suggest more strongly that the peptide-selective binding change observed in our assay does not result from receptor internalization. First, receptor internalization measured by ligand uptake in NG108–15 cells is much slower ($t_{1/2} \approx 2$ hr) (11) than the loss of peptide binding measured in our assay ($t_{1/2} \approx 10$ min). Second, the magnitude of the peptide-selective binding change is large (70–90% loss of peptide binding within 20 min) relative to the loss of surface receptors typically measured for G protein-coupled receptors that undergo rapid internalization, such as β-adrenergic receptors (20–40% within 20 min) (18, 24, 25). However, these points are only suggestive, and we cannot presently rule out the possibility that receptor internalization may contribute to the peptide-selective binding change. This question is quite important, both because the role that internalization plays in the physiological regulation of G protein-coupled receptors is not clear and because of the potential clinical significance of regulated peptide/alkaloid selectivity. Further biochemical examination of receptors in this regulated binding state will be required to fully evaluate the possible role of receptor internalization in the peptide-selective binding change. These experiments will be facilitated by the ability, demonstrated in this study, to retain the peptide-selective binding change in a membrane fraction prepared from lysed cells.

As discussed above, we doubt that the peptide-selective binding change results from functional uncoupling or receptor internalization. Rather, we currently favor the hypothesis that the binding change is mediated by a regulated conformational change in the δ-opioid receptor, causing the selective rearrangement of structural determinants required for peptide but not alkaloid binding. The existence of distinct structural determinants involved in peptide versus nonpeptide binding to neurokinin/substance P receptors and cholecystokinin-B/gastrin receptors has been demonstrated recently (26–29). We anticipate that opioid receptors also contain distinct structural features that participate selectively in peptide versus alkaloid ligand binding. Beyond the identification of these features, a most interesting question is how a conformational change leading to the rearrangement of peptide-selective domains within the receptor molecule could be regulated by agonist. One candidate mechanism responsible for the peptide-selective binding change is agonist-regulated phosphorylation, and this posttranslational modification has been implicated previously in the desensitization of opioid receptors (30). δ-Opioid receptors possess multiple serine and threonine residues in the intracellular domains (8) which are potential sites for regulation by phosphorylation (18, 21–25). The availability of a cDNA encoding δ-opioid receptors should facilitate studies investigating this hypothesis by allowing the site-directed mutagenesis of candidate phosphorylation sites.

Beyond questions of molecular mechanism, it will be important to determine the physiological function of the peptide-selective binding change. The binding change is induced at physiological temperature and by an opioid peptide similar to

the endogenous agonists of the δ-opioid receptor. However, the detection of the binding effect requires a distinctly nonphysiological condition (4°). Therefore, we must question to what extent the binding change exists *in vivo* and whether it plays a role in the regulation of the endogenous opioid system. For example, it will be interesting to determine whether opioid receptors in this state of selectively diminished peptide binding can still activate G protein-coupled effectors when the receptors are activated by alkaloid agonists.

Why might opioid receptors discriminate between alkaloid and peptide agonists? It is reasonable to assume that receptor adaptations have evolved to regulate the interaction of receptors with endogenous ligands. In this light, it is not surprising that alkaloid drugs, which differ markedly in structure from the endogenous opioid peptides, may not be subject to all receptor regulatory processes. The peptide-selective binding change described here calls into question the notion that opiate drugs function entirely by mimicking endogenous opioid peptides. The possibility that opiate drugs may elude certain physiological mechanisms that regulate endogenous peptidergic signal transduction could have important implications for understanding the biology of opiate tolerance and addiction.

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