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Selective and Interactive Down-regulation of μ- and δ-Opioid Receptors in Human Neuroblastoma SK-N-SH Cells

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SUMMARY

Human neuroblastoma SK-N-SH cells, which contain both μ- and δ-opioid receptors, were grown under conditions that provided a μ:δ ratio of 1.5:1. Both receptors were down-regulated after 72 hr of exposure to 100 nM etorphine. Selective down-regulation was demonstrated using selective opioid agonists; the μ agonist Tyr-D-Ala^2-Gly-(Me)Phe^4-Gly-ol down-regulated μ- but not δ-opioid receptors, whereas prolonged exposure to the selective δ agonist D-Pen^2,D-Pen^5-enkephalin resulted in δ- but not μ-opioid receptor down-regulation. Morphine, which binds μ- as well as δ-opioid receptors, down-regulated both receptor subtypes. NG108–15 cells, which contain δ receptors exclusively, were also tested. NG108–15 cells did not exhibit δ-opioid receptor down-regulation when exposed to morphine. The discrepancy between the effect of chronic morphine treatment on δ receptors in SK-N-SH cells and in NG108–15 cells raised the question of whether the coexistence of μ receptors in the former allowed morphine to down-regulate δ receptors. The role of μ-opioid receptors in morphine-induced δ receptor down-regulation was studied by using the irreversible μ antagonist β-funaltrexamine. Pretreatment of SK-N-SH cells with β-funaltrexamine prevented down-regulation of δ receptors in response to chronic exposure to morphine but did not affect down-regulation of δ receptors in response to D-Pen^2,D-Pen^5-enkephalin. The experimental data indicate that morphine-induced δ-opioid receptor down-regulation is dependent on the presence of functional μ receptors in the same cell.

Tolerance to opiates, which follows a prolonged exposure to opioid agonists, is accompanied by one or several molecular events that attenuate the effect of the agonist. Among these are down-regulation of opioid receptors (1–3), reduction in affinity for opioid agonists (1, 2, 4), modulation of G protein subunits (5, 6), and modification of the intracellular responding element (e.g., adenylate cyclase activity) (7, 8). The complexity of these processes led to the study of tolerance in simple preparations, which enabled a detailed molecular investigation. Thus, brain slices and cultured cells that contain one subtype of opioid receptors solely or predominantly were used. These studies revealed that different preparations that contained one of the various opioid receptors (μ, δ, or κ) responded differently to chronic exposure to opioid agonists. For example, δ-opioid receptors in NG108–15 cells and μ receptors in 7315c cells were down-regulated by 60–70% upon chronic agonist treatment (1, 2), whereas κ receptors in spinal cord-dorsal root ganglion cocultures exhibited only slight down-regulation (9). On the other hand, desensitization of the κ response in spinal cord-dorsal root ganglion cocultures was associated with reduction of α_{i1} subunits of G proteins (5), whereas no alteration in G proteins was found after chronic exposure to δ agonists in NG108–15 cells (10, 11).

It is not clear, however, whether the differences described above are receptor specific (namely, each receptor is subject to a different mode of regulation) or tissue specific. For example, morphine was previously shown to down-regulate μ receptors in 7315c cells (2) but not δ receptors in NG108–15 (1, 12, 13), M-8 (14), or N4TG1 (15) cells. It is not known whether morphine can differentiate between μ and δ receptors when both are expressed in the same cell. This question is relevant for the *in vivo* situation, because different subtypes of opioid receptors may exist in the same neuron (16–18). This issue can be studied in cultured cells that include a heterogeneous population of opioid receptors.

The human neuroblastoma SK-N-SH cell line and its subclone SH-SY5Y were reported to contain both μ- and δ-opioid receptors in a ratio of 5:1 (19, 20) or 2:1 (21). We have successfully grown an SK-N-SH cell line with a μ:δ ratio of 1.5:1. This ratio between the two subtypes of opioid receptors enables the study of each receptor separately. The present study shows that μ- and δ-opioid receptors that are expressed in the same cell can be down-regulated differentially upon exposure to selective agonists. Morphine, on the other hand, down-regulates both μ-

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ABBREVIATIONS: G protein, guanine nucleotide-binding protein; DAMGO, Tyr-D-Ala^2-Gly-(Me)Phe^4-Gly-ol; DPDPE, D-Pen^2,D-Pen^5-enkephalin; U50,488, (*trans*)-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamidemethanesulfonate; β-FNA, β-funaltrexamine.

and δ-opioid receptors; however, the δ receptor down-regulation is μ receptor dependent.

Experimental Procedures

Materials. DAMGO, DPDPE, and U50,488 were purchased from Sigma. Naloxone hydrochloride was a gift of DuPont. Morphine hydrochloride was purchased from Asia-Maabadot (Petah-Tikva, Israel). Etorphine was from Reckitt (UK) and β-FNA from Research Biochemicals, Inc., MA. [^{3}H]Diprenorphine (37 Ci/mmol), [^{3}H]DAMGO (60 Ci/mmol), and [^{3}H]DPDPE (48 Ci/mmol) were obtained from Amersham. EDTA (as a dipotassium salt) was from Fluka. Dulbecco's modified Eagle's medium containing D-glucose and L-glutamine and RPMI 1640 medium containing L-glutamine, fetal calf serum, hypoxanthine/aminopterin/thymidine, penicillin, and streptomycin were purchased from Beit-Haemek (Israel). Tissue culture flasks (75-cm^2) were purchased from Costar.

Cell cultures. SK-N-SH cells were originally obtained from the American Type Culture Collection (Rockville, MD) and were grown in RPMI 1640 medium, supplemented with 10% fetal calf serum, 40 units/ml penicillin, and 40 units/ml streptomycin, at 37° in a humidified atmosphere of 5% CO_2/95% air. Cells were seeded at a density of about 4×10^6 cells/flask and medium was replaced once, 5–7 days after seeding. Cells were harvested after 7–10 days in culture by mechanical agitation in phosphate-buffered saline (138 mM NaCl, 15.6 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, 0.5 mM $MgCl_2$, 1 mM EDTA).

NG108–15 cells (a gift of M. Nirenberg, National Institutes of Health) were grown in Dulbecco's modified Eagle's medium containing D-glucose and L-glutamine, supplemented with 0.1 mM hypoxanthine, 1 μM aminopterin, 16 μM thymidine, 5% fetal calf serum, 40 units/ml penicillin, and 40 units/ml streptomycin, at 37° in a humidified atmosphere of 10% CO_2/90% air. Medium was replaced once, 2–4 days after seeding. Cultured cells were harvested and seeded at a density of about 1×10^6 cells/flask every 5–7 days.

Membrane preparation. Membranes were prepared according to the method of Keren *et al.* (22). SK-N-SH cultures at confluency (about 3×10^7 cells/flask) were harvested and centrifuged at $1000 \times g$ for 10 min. The cells were resuspended in ice-cold 50 mM Tris·HCl buffer, pH 7.4, containing 1 mM EDTA, and were homogenized by using a Polytron. The homogenate was centrifuged at $40,000 \times g$ for 20 min at 4°. The resulting pellet was suspended in 0.32 M sucrose and stored at −70°. Before binding experiments, membranes were diluted 8-fold with the EDTA-Tris buffer.

NG108–15 cultures at confluency (about 5×10^6 cells/flask), were harvested and centrifuged at $1000 \times g$ for 10 min. The resulting pellet was suspended in 0.32 M sucrose and stored at −70°. Before binding experiments, cells were suspended in ice-cold 50 mM Tris·HCl buffer, pH 7.4, containing 1 mM EDTA, and were homogenized by using a Polytron. The homogenate was centrifuged at $20,000 \times g$ for 20 min at 4°. The resulting pellet was resuspended in EDTA-Tris buffer.

In some experiments, freshly prepared membranes of SK-N-SH and NG108–15 cells were used for binding assays. No difference was observed between stored and fresh membranes.

Prolonged exposure of cultures to opioid ligands. Cultures were exposed to various opioid ligands at concentrations of 10 nM to 100 μM, for 5–96 hr. Control cells of the same cultures were grown in the absence of opioids.

To eliminate the effect of residual agonist in treated cells, two procedures were carried out. 1) Control cells were exposed to an identical concentration of the same ligand, at 37°, 5 min before harvesting. Both control and treated cells were then harvested and centrifuged at $1000 \times g$ for 10 min. The resulting pellets were homogenized and incubated for 1 hr at room temperature in 50 mM Tris·HCl buffer containing 100 mM NaCl. The membranes were then centrifuged and used for binding experiments. 2) Control cells were exposed to an identical concentration of the same ligand for 1 min, and then both control and treated cells were washed three times in phosphate-buffered saline before harvesting. No difference was observed between the two procedures.

Receptor binding experiments. Binding experiments were performed in a total volume of 0.6 ml, consisting of 0.5 ml of membranes (0.1–0.2 mg of protein) in EDTA-Tris buffer, 0.05 ml of labeled ligand, and 0.05 ml of EDTA-Tris buffer (to determine total binding) or, alternatively, 0.05 ml of naloxone at a final concentration of 0.2 mM (to determine nonspecific binding) or 0.05 ml of opioid ligands at various concentrations (for competition experiments). Incubation was carried out at 30° for 30 min, followed by 15 min in ice-cold water. Incubation was terminated by the addition of 2 ml of 50 mM Tris·HCl buffer, pH 7.4, containing 1 mM EDTA, and rapid vacuum filtration through Whatman GF/B filters, which were then counted in scintillation fluid.

Statistics. Student's paired *t* test (one tail) was used to determine significant differences between treated and control cells.

Results

Opioid receptors in SK-N-SH cells. The presence of opioid receptors in SK-N-SH cells was detected using the nonselective opioid antagonist diprenorphine. The specific binding of increasing concentrations of [^{3}H]diprenorphine was found to be saturable. Saturation isotherm and Scatchard analyses of the data revealed a K_d value of 0.5–0.6 nM and a B_{max} of about 270 fmol/mg of protein (Fig. 1A).

Characterization of opioid receptor subtypes was carried out using two different procedures, 1) competition experiments in which [^{3}H]diprenorphine was displaced by selective agonists

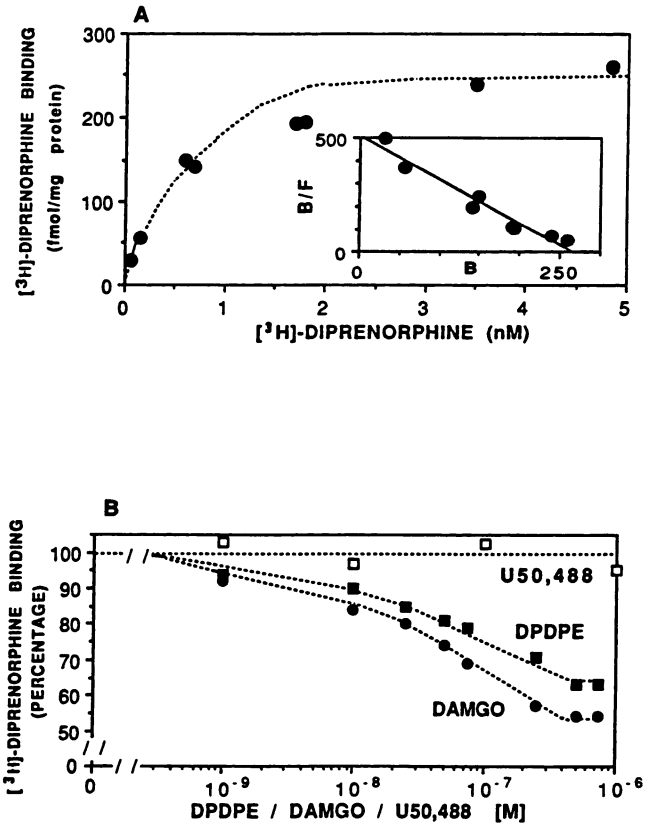

Fig. 1. A, Binding of [^{3}H]diprenorphine to SK-N-SH cell membranes. The graph illustrates a saturation isotherm and Scatchard plot (*inset*) of a representative experiment. B, Displacement of [^{3}H]diprenorphine by DAMGO (●), DPDPE (■), and U50,488 (□). The graph illustrates the binding of [^{3}H]diprenorphine in the presence of each ligand as a percentage of binding in the absence of displacer.

for μ- (DAMGO) or δ-opioid (DPDPE) receptors and 2) binding of labeled selective ligands ([^{3}H]DAMGO and [^{3}H]DPDPE). In competition experiments, increasing concentrations of either DAMGO or DPDPE reduced [^{3}H]diprenorphine specific binding until a plateau was reached at concentations of 400–500 nM (Fig. 1B). The plateau indicated that the selective ligand blocked the desired receptors but did not cross-bind to the other subtype of opioid receptors. DAMGO, at concentrations of 400–500 nM, displaced 60% of [^{3}H]diprenorphine specific binding, whereas DPDPE, at the same concentrations, displaced 40% of the specific binding. The selective κ agonist U50,488 did not inhibit [^{3}H]diprenorphine binding at concentrations up to 1000 nM (Fig. 1B).

The specific binding of increasing concentrations (0.5–9 nM) of each of the selective ligands was found to be saturable. Binding of [^{3}H]DAMGO resulted in B_{max} and K_d values of 199 ± 33 fmol/mg of protein and 3.2 ± 1.7 nM (12 experiments), respectively. [^{3}H]DPDPE binding to the same membranes revealed a B_{max} value of 130 ± 23 fmol/mg of protein and a K_d value of 2.2 ± 1.6 nM (12 experiments). It should be noted that the maximal binding varied appreciably among different stocks of cells and different passage numbers. Therefore, the ratio between μ and δ receptors was calculated from experiments carried out with cells from the same stock and passage number. Both methods yielded similar μ:δ ratios, of 1.5:1, in SK-N-SH cells.

Down-regulation of μ- and δ-opioid receptors. To test whether both μ- and δ-opioid receptors in SK-N-SH cells are prone to down-regulation, cultures were exposed to the nonselective agonist etorphine and tested for the binding of [^{3}H]diprenorphine, [^{3}H]DAMGO, and [^{3}H]DPDPE. Exposure of SK-N-SH cells to 100 nM etorphine for 72 hr reduced the maximal binding of the nonselective antagonist [^{3}H]diprenorphine by 50–80%. A similar reduction in the maximal binding of [^{3}H]DAMGO and [^{3}H]DPDPE was observed after exposure to etorphine (Fig. 2). No significant change in the K_d values of either the antagonist or the two agonists was detected in these experiments. The present data show that both μ and δ receptors in SK-N-SH cells can be down-regulated upon chronic exposure to an opioid agonist.

Selective down-regulation of μ- and δ-opioid receptors. One goal of this study was to find out whether opioid receptors that are expressed in the same cell can be down-regulated separately. For this purpose cells were exposed for 72 hr to 100 nM concentrations of either the selective μ (DAMGO) or the selective δ (DPDPE) agonist.

Both DAMGO and DPDPE down-regulated opioid receptors in SK-N-SH cells, as was evident from the reduction in the maximal binding of [^{3}H]diprenorphine by 30–40%. The subtype of opioid receptors undergoing down-regulation was studied by using the binding of [^{3}H]DAMGO and [^{3}H]DPDPE. In five experiments, exposure of cells to DAMGO reduced the maximal binding of [^{3}H]DAMGO by 40–70% ($p = 0.015$) (Fig. 3A). No significant change in the binding of [^{3}H]DPDPE after exposure to DAMGO was observed (Fig. 3B). Chronic exposure of SK-N-SH to the selective δ agonist DPDPE reduced the maximal binding of [^{3}H]DPDPE by 50–90% ($p = 0.017$, eight experiments), with no significant change in the B_{max} value for [^{3}H]DAMGO (Fig. 3, C and D). These results indicate that μ- and δ-opioid receptors that exist in the same cell can be down-regulated separately.

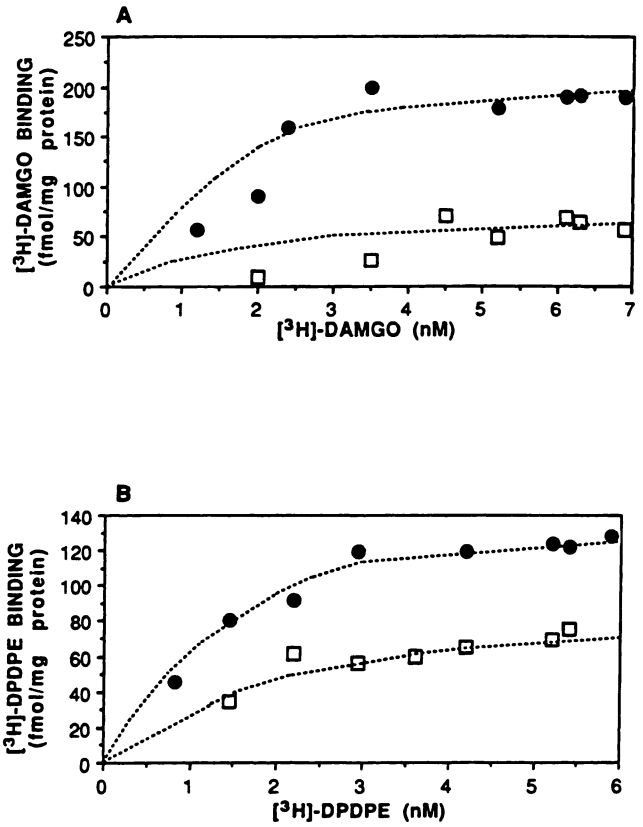

Fig. 2. Effect of chronic exposure of SK-N-SH cells to etorphine on the binding of [^{3}H]DAMGO (A) and [^{3}H]DPDPE (B). Membranes were prepared from cells that had been exposed to 100 nM etorphine for 72 hr (□) and from control cells that had been grown in the absence of the opiate agonist (●). The graphs illustrate representative experiments.

Chronic exposure to morphine. Another opioid agonist that might induce a selective down-regulation of μ- but not δ-opioid receptors is morphine. Although morphine binds to both μ and δ receptors, it failed to induce δ receptor down-regulation in preparations that contained δ receptors solely or predominantly (1, 12–15). On the other hand, morphine down-regulated μ receptors in 7315c cells (2) and SH-SY5Y cells (Ref. 23; see, however, Ref. 24). We confirmed the absence of down-regulation of δ receptors in response to morphine in our experiments with NG108–15 cells. Exposure of this culture to 10 μM morphine for 48 hr did not alter the specific binding of [^{3}H]diprenorphine by >10%. However, the δ-opioid receptors in these cells underwent down-regulation of 63 ± 10% (seven experiments) when cultures were exposed to DPDPE.

Exposure of SK-N-SH cells to 10 μM morphine for 72 hr reduced the specific binding of the nonselective antagonist [^{3}H]diprenorphine by 40–55% ($p = 0.005$), compared with control cells, in five experiments. Displacment of [^{3}H]diprenorphine specific binding by increasing concentrations of either DAMGO or DPDPE showed that the μ:δ ratio was similar to that in control cultures, indicating that both μ and δ receptors were down-regulated. The effect of chronic morphine exposure on each of the opioid receptors was further tested in four experiments using the selective labeled agonists; [^{3}H]DAMGO maximal binding was reduced by 35–65% ($p = 0.01$) and [^{3}H]DPDPE binding was decreased by 45–80% ($p = 0.01$). Similar results were obtained after the exposure of cultures to 10 μM morphine for 24 or 96 hr.

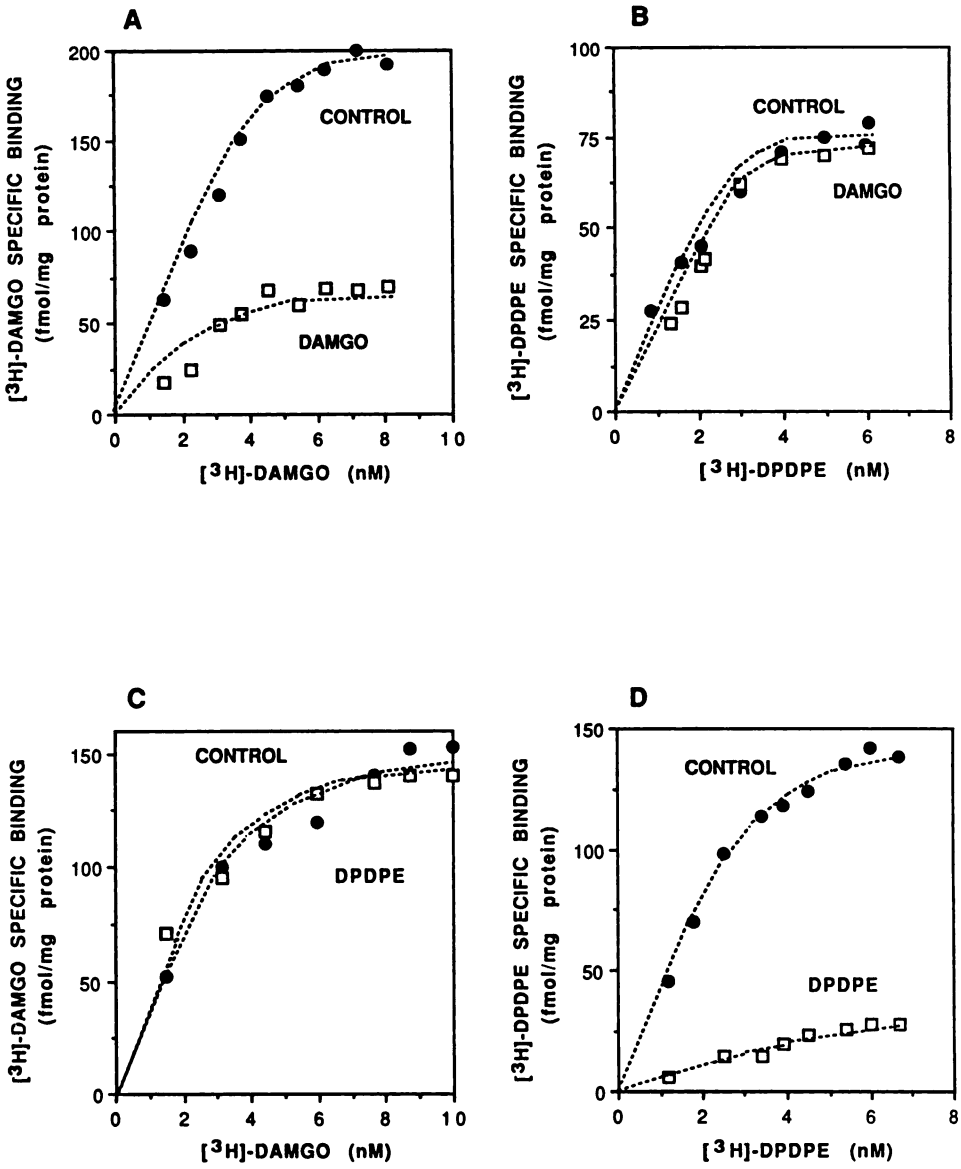

Fig. 3. Selective down-regulation of opioid receptor subtypes. SK-N-SH cells were grown in the presence (□) or absence (●) of either 100 nM DAMGO (A and B) or 100 nM DPDPE (C and D) for 72 hr. Membranes were prepared as described in Experimental Procedures and the binding of either [^{3}H]DAMGO (A and C) or [^{3}H]DPDPE (B and D) was measured. The graphs illustrate representative experiments with five to 10 repetitions under each condition.

To maximize the chances of morphine inducing a selective down-regulation of μ- but not δ-opioid receptors, more moderate exposure protocols were applied. SK-N-SH cells were exposed to morphine either at lower concentrations or for shorter durations. Parallel down-regulation of μ and δ receptors by morphine was observed under all exposure conditions. Thus, exposure of the culture to 100 nM morphine for 72 hr reduced only slightly and insignificantly the density of both μ and δ receptors in nine experiments (24 ± 5%, $p = 0.16$, and 20 ± 3%, $p = 0.15$, respectively). A short (5-hr) exposure to a high concentration (10 μM) of morphine did not attenuate the maximal binding of [^{3}H]DAMGO or [^{3}H]DPDPE.

β-FNA treatment. The finding that chronic exposure of SK-N-SH cells to morphine induces down-regulation of δ-opioid receptors (in addition to the expected μ receptor down-regulation) is in apparent contradiction to the failure of morphine to down-regulate δ receptors in NG108–15 cells. One difference between the two cultures is the coexistence of μ-opioid receptors in the former. To test the hypothesis that the presence of μ receptors is a prerequisite for down-regulation of δ receptors by morphine, SK-N-SH cells were exposed to morphine after μ receptors had been blocked by the irreversible μ-selective antagonist β-FNA (25). Control experiments showed that treatment of SK-N-SH cells with 10 nM β-FNA for 72 hr reduced the binding of [^{3}H]DAMGO by >90%, whereas no change in [^{3}H]DPDPE binding was found.

SK-N-SH cells were grown in the presence of 10 nM β-FNA and, 24 hr later, 10 μM morphine was added for the next 72 hr. Morphine did not significantly alter the maximal binding of the opiate antagonist [^{3}H]diprenorphine in six experiments, suggesting that no down-regulation of the remaining (δ) receptors took place (Fig. 4A). On the other hand, the maximal binding of the opioid peptide agonist [^{3}H]DPDPE was significantly reduced, by 32% (Fig. 4B) (see Discussion).

The failure of morphine to reduce the binding of [^{3}H]diprenorphine to the remaining δ receptors in β-FNA-treated SK-N-SH cells raised the question of whether β-FNA had induced a type of δ receptor modification that abolished the ability of the receptors to undergo down-regulation. To answer this question we tested the effect of β-FNA on DPDPE-induced down-regu-

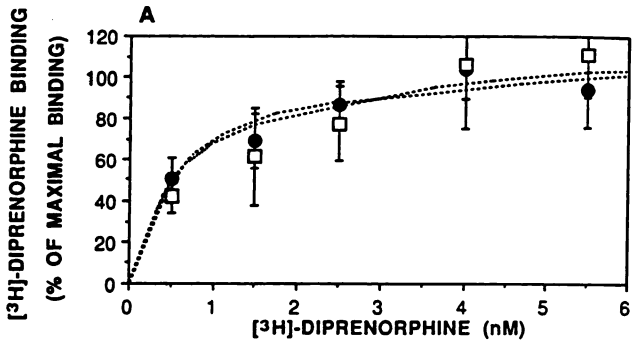

Fig. 4. Effect of chronic exposure to morphine on β-FNA-treated SK-N-SH cells. β-FNA-treated cells were grown in the presence (□) or absence (●) of 10 μM morphine, as described in the text. The binding of [^{3}H]diprenorphine (A) or [^{3}H]DPDPE (B) to membranes prepared from these cells was measured. The graphs illustrate the results of four (A) or nine (B) normalized experiments in which the maximal binding in control cells was considered to be 100%.

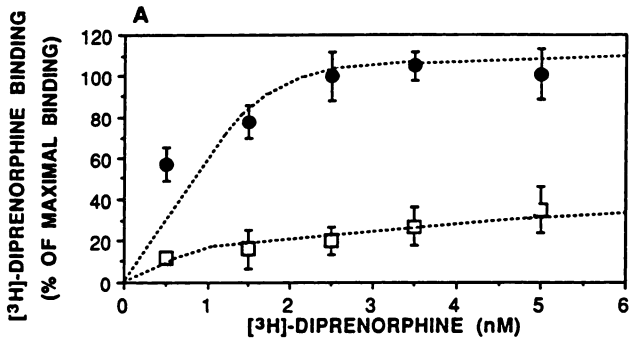

Fig. 5. Effect of chronic exposure to DPDPE on β-FNA-treated SK-N-SH cells. β-FNA-treated cells were grown in the presence (□) or absence (●) of 100 nM DPDPE, as described in the text. The binding of [^{3}H]diprenorphine (A) or [^{3}H]DPDPE (B) to membranes prepared from these cells was measured. The graphs illustrate the results of two (A) or three (B) normalized experiments in which the maximal binding in control cells was considered to be 100%.

lation of δ receptors. SK-N-SH cells were pretreated with 10 nM β-FNA for 24 hr, followed by 72 hr of exposure to 100 nM DPDPE in the presence of β-FNA. Under these conditions, chronic DPDPE reduced the binding of both [^{3}H]diprenorphine and [^{3}H]DPDPE, by 65% and 74%, respectively (Fig. 5). Thus, we conclude that in β-FNA-treated SK-N-SH cells the remaining δ receptors can undergo down-regulation when exposed to δ agonist but not when exposed to morphine.

Discussion

Down-regulation is one of various molecular events that may contribute to desensitization. Several studies indicated that opioid receptors in the brain were down-regulated after chronic treatment with opiates (3), whereas other studies found no change (26) or even up-regulation of opioid binding (27). The relevance of down-regulation to the phenomenon of tolerance is not clear. It is assumed that different events take place in various brain regions and under different conditions. The complexity of the *in vivo* situation has led to the study of neuronal cells grown in culture. Chronic exposure of cultures to opiates showed that different processes occur at the cellular level. For example, δ receptors were down-regulated upon chronic agonist exposure (1, 12, 13), whereas desensitization to κ agonists was accompanied by a reduction in G proteins, with no change in opiate receptors (5, 9). This discrepancy raises the question of whether the molecular events underlying desensitization are specific for the receptor subtypes or represent specificity for the examined tissue. This issue can be studied in cultures that express heterogeneous populations of opioid receptors. For that purpose, in the present study we used the human neuroblastoma SK-N-SH cells.

The SK-N-SH cell line and its subclone SH-SY5Y were reported to contain both μ- and δ-opioid receptors (19, 20). Electrophysiological experiments showed that both receptors exist in the same cell (28). Binding studies revealed a μ:δ ratio of 5:1 for the two cell lines (19, 20). Another study suggested a μ:δ ratio of 2:1 in SH-SY5Y cells (21). The diversity of results may represent phenotypic heterogeneity due to differences in culture conditions (29). Indeed, we found that the proportion of δ receptors was elevated if cultures were left to grow for a longer time.[1] Under the specified conditions of the present study, SK-N-SH cells expressed μ- and δ-opioid receptors in a ratio of 1.5:1. This proportion enabled us to investigate each receptor separately.

The down-regulation of opioid receptors demonstrated here is in agreement with the results of Zadina *et al.* (23) in SH-SY5Y cells but at variance with the results of Yu *et al.* (24) in the same cells. The discrepancy between our findings and those of Yu *et al.* is not due to differences in experimental methodology, because the same down-regulation was observed when cells were treated precisely according to the protocol of Yu *et*

[1] Baumhaker and Sarne, unpublished observations.

al. This difference may reflect the fact that SH-SY5Y is a subclone of SK-N-SH neuroblastoma and may therefore carry a mutation that prevents down-regulation. Mutations that modulated the process of down-regulation were reported for the β_2-adrenoceptor (30) and for the M_1 muscarinic receptor (31).

Differential regulation of the two opioid receptor subtypes in SK-N-SH cells was observed in the present study when cells were exposed to selective agonists. Chronic treatment with the δ agonist DPDPE resulted in δ but not μ receptor down-regulation, whereas DAMGO down-regulated μ but not δ receptors. Thus, the two opioid receptors can be regulated independently. In this context, the effect of morphine was of special interest. It was shown that prolonged exposure to morphine resulted in down-regulation of μ-opioid receptors in 7315c (2) and SH-SY5Y cells (23) but did not induce down-regulation of δ receptors, which were expressed solely or predominantly in NG108–15 (1, 12, 13), M-8 (14), or N4TG1 cells (15). Similarly, morphine induced down-regulation of μ but not δ receptors in the guinea pig brain (32). The present study shows that morphine down-regulated μ- and δ-opioid receptors that coexisted in the same cell. Furthermore, the effect of exposure to morphine on δ receptors in SK-N-SH cells was dependent on the presence of functional μ receptors in the same cells; when μ receptors were blocked by β-FNA, morphine failed to induce down-regulation of the remaining δ receptors. These findings indicate that μ- and δ-opioid receptors interact with each other in the process of down-regulation. Previous studies demonstrated interactions between μ and δ receptors in both binding experiments (33) and pharmacological tests (34). These interactions were later explained by the existence of a μ-δ opioid complex (35, 36). Biochemical studies indicated, however, that opioid receptors in SK-N-SH cells were not complexed (22, 37). Furthermore, the μ-δ complex was shown to undergo up-regulation upon chronic treatment with morphine (36), whereas in the present study both receptors were down-regulated after prolonged exposure to morphine. Thus, we conclude that the dependency of δ down-regulation on the presence of μ receptors does not represent a situation where the two binding sites reside in the same complex. It is reasonable to assume that the interaction between μ and δ receptors may occur only after the binding of morphine. It was previously shown that the binding of morphine led to aggregation of opioid receptors into clusters (38). This aggregation may bring the two receptor subtypes into close proximity; only under these conditions can the μ receptor affect the δ receptor (both being bound to morphine) and induce its comigration into the cytoplasm. This may explain the dependency of δ receptor down-regulation by morphine on the presence of functional μ receptors in the same cell.

The lack of δ receptor down-regulation in the absence of functional μ receptors (i.e., after β-FNA treatment) was indicated only when [^{3}H]diprenorphine and not when [^{3}H]DPDPE was used to detect the remaining δ receptors. The reduction in the binding of the agonist [^{3}H]DPDPE but not of the antagonist [^{3}H]diprenorphine could result from receptor-G protein uncoupling and a dramatic reduction in affinity for agonists (39). Thus, when only δ receptors are present in the cell, morphine reduces the agonist high affinity site with no alteration in total receptor number. In this respect, SK-N-SH cells pretreated with β-FNA behave like NG108–15 cells (1). However, in no case in our study did exposure to morphine (or to any other agonist) significantly affect the affinity of the opioid receptors. This is in agreement with a previous study in which no reduction in the affinity of SH-SY5Y cells for agonists after exposure to morphine was found (24). An alternative explanation is that morphine modified the remaining δ receptors to a state that did not bind the hydrophilic peptide [^{3}H]DPDPE but still bound the hydrophobic opiate [^{3}H]diprenorphine. A state of an opioid receptor that is "blind" to peptides but not to alkaloids was suggested previously (40). A critical review of the literature reveals that this situation is not an exception; exposure of NG108–15 cells to morphine attenuated the maximal binding of [^{3}H]methionine-enkephalin by up to 30% (13), whereas the maximal binding of [^{3}H]diprenorphine was reduced by only 7% (1). We suggest that morphine induces a type of internalization that leaves δ receptors available for the hydrophobic opiates (such as diprenorphine) but not for the hydrophilic peptides (such as DPDPE). Only when both μ and δ receptors exist in the same cell does morphine induce a real down-regulation of δ-opioid receptors.

In summary, SK-N-SH cells may serve as a relatively simple model that enables the study of the more complex situation of coexistence of more than one opioid receptor subtype in the same cell. This model demonstrates that each receptor subtype can be regulated separately, yet interactions between μ and δ receptors take place under certain conditions of chronic exposure to opioid drugs.

Acknowledgments

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