Dissociation of Opioid Receptor Upregulation and Functional Supersensitivity

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CHANG, S. C. J. L., K. LUTFY, V. SIERRA AND B. C. YOBURN. *Dissociation of opioid receptor upregulation and functional supersensitivity.* PHARMACOL BIOCHEM BEHAV **38**(4) 853–859, 1991.—Alterations in brain opioid binding and opioid pharmacodynamics following chronic (8-day) naltrexone (NTX) treatment were determined in pertussis toxin (PTX)-treated mice. Intrathecal (IT) and intracerebroventricular (ICV) PTX produced a time-dependent, long-lasting inhibition of morphine (SC) analgesia without modifying basal nociception. Inhibition was maximal 16 days following PTX treatment, and was still observed at 40 days. Relative to placebo controls, NTX treatment produced supersensitivity to morphine analgesia in all control mice and in mice pretreated with PTX 1 day before NTX. Supersensitivity was not observed in 7-day PTX-pretreated mice. [^{3}H][D-Ala^2-D-Leu^5]enkephalin ([^{3}H]DADLE) and [^{3}H][D-Ala^2-MePhe^4-Gly(ol)^5]enkephalin ([^{3}H]DAMGO) binding sites were increased by NTX treatment in saline- and PTX-pretreated groups. K_Ds were unchanged. These results indicate that PTX does not alter opioid antagonist-induced receptor upregulation. However, PTX treatment can diminish morphine potency in upregulated and control mice. Therefore, opioid analgesia in control and upregulated mice appears to be mediated by receptors linked to a common PTX-sensitive G-protein. Furthermore, in 7-day PTX-pretreated mice, NTX increased binding sites without altering morphine potency, which suggests that new binding sites can appear without being functionally coupled.

Opioid receptors   Upregulation   Supersensitivity   Morphine   Pertussis toxin   Analgesia   Naltrexone

GUANINE-NUCLEOTIDE binding regulatory proteins (G-proteins) are believed to be responsible for controlling receptor-mediated stimulation or inhibition (G$_s$ and G$_i$, respectively) of adenylate cyclase activity. These proteins are heterotrimers composed of α-, β-, and γ-subunits. The β- and γ-subunits are believed to be identical for both G-proteins with the α-subunit being structurally and functionally different (12, 13, 38, 44). Cholera toxin and PTX selectively interfere with the function of G$_s$ and G$_i$, respectively, by ADP-ribosylation of the α-subunit both in vivo and in vitro (11, 17, 22, 38, 44, 50). PTX also interferes with G$_o$, another G-protein which is believed to regulate ion flux (14). In the presence of ATP, the toxin catalyzes the transfer of an ADP-ribose moiety from NAD to an amino acid residue at the carboxy-terminus of the α-subunit of G-proteins. This covalent reaction produces essentially irreversible modification of G-protein function. Cholera toxin is an irreversible stimulator of adenylate cyclase by inhibiting the GTPase activity of G$_s$. PTX also produces increases in adenylate cyclase activity by inactivating G$_i$ which is the component that inhibits cyclase (14, 22, 50).

Opioid agonists were first demonstrated to inhibit cyclic AMP production in membranes from rat striatum (6). Now, it is gener-

ally accepted that opioids interact with stereospecific receptors that are coupled to adenylate cyclase through a G-protein (G$_i$) which inhibits adenylate cyclase activity and reduces the formation of cyclic AMP (4, 6, 7, 15, 23, 41, 42, 48, 51). Moreover, opioid tolerance and dependence have been closely linked to the function of adenylate cyclase (4, 41, 51). In recent years, several investigators have examined the effect of PTX on opioid agonist actions (5, 28, 40, 49). PTX has been demonstrated to inhibit morphine analgesia and prevent opioid dependence by interfering with the function of the inhibitory nucleotide regulatory unit (1, 18, 34, 35, 37, 39). It has also been shown that PTX treatment can reduce opioid binding (2, 7, 20, 26, 33).

Many studies have demonstrated that chronic treatment with opioid antagonists will increase the potency (supersensitivity) of opioid agonists in both in vivo (3, 30, 46, 47, 52–55) and in vitro (45) systems. In parallel with these pharmacodynamic changes, binding studies have demonstrated increased density (upregulation) of μ, δ, and κ opioid receptors in CNS (25, 31, 47, 52, 54, 56, 58) and peripheral sites (45) without affecting receptor affinity. Although upregulation and supersensitivity are well-established, the cellular events underlying the effects of chronic opioid

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antagonist treatment have never been elucidated. In the present study we examined the role of PTX-sensitive G-proteins in morphine analgesia, as well as chronic opioid antagonist induced receptor upregulation and functional supersensitivity.

METHOD

Subjects

Male, Swiss-Webster mice (22–24 g) from Taconic Farms (Germantown, NY) were used throughout. The animals were maintained 5 per cage with free access to food and water and housed for at least one day prior to experimentation. Mice were used only once.

Analgesia Assay

Analgesia (antinociception) was measured by the tailflick method of D'Amour and Smith (8). Briefly, a beam of light was focused on the dorsal tail surface approximately 2.5 cm from the tip of the tail. The intensity of the light was adjusted so that baseline flick latencies determined prior to drug administration were 2–4 s. Following morphine, if a mouse failed to flick by 10 s, the trial was terminated and a latency of 10 s was recorded. Mice that had latencies of 10 s were defined as analgesic. Mice were tested for analgesia at 30 or 45 min following morphine injection. In morphine dose-response studies the percent of mice that were analgesic was used as the dependent measure. In single dose studies the mean tailflick latency was the dependent variable. All testing was conducted in a blind manner.

Drug Administration and Pellet Implantation

IT injections (2.5 μl) were made by the method of Hylden and Wilcox (19). ICV drug administration (5 μl) was as previously described (53). Drug pellets were implanted SC in the nape of the neck. All IT, ICV drug injections and pellet implantations were made while mice were lightly anesthetized with halothane (96:4 oxygen:halothane).

Brain Opioid Binding

The opioid receptor binding assay is a modification of the procedure of Pasternak et al. (36) as described by Yoburn et al. (56). Mice were sacrificed and whole brain rapidly removed and homogenized in 20 volumes of ice-cold 50 mmol potassium phosphate buffer (pH 7.2). Homogenates were then centrifuged, the pellet resuspended and centrifuged again. Pellets were resuspended in buffer and incubated (30 min, 25°C). Homogenates were centrifuged a third time and finally resuspended in 20 volumes of buffer. An aliquot of homogenate was assayed in triplicate in tubes containing 1.4 nM [^{3}H]DAMGO or 1 nM [^{3}H]DADLE for single concentration studies. In saturation studies, tubes contained 0.156–22.4 nM [^{3}H]DAMGO or 0.095–15.0 nM [^{3}H]DADLE. Nonspecific binding was determined in the presence of 1 μM cold naloxone or levorphanol. Homogenate was incubated for 90 min at 25°C. Incubation was terminated by the addition of ice-cold buffer and samples were filtered. Filters were washed with buffer, transferred to scintillation vials and counted. Specific binding is the difference between binding determined in the absence of cold ligand and in the presence of the cold ligand. Tritiated ligands were purchased from Amersham Corporation (Arlington Heights, IL).

Effect of PTX on Morphine Analgesia

Mice were injected ICV and IT with saline or PTX (0.2 or 0.5 μg/mouse in each site). Ten days later all mice were tested for analgesia following morphine injection (4.5 mg/kg, SC, N = 11–15/group). Another group of mice was injected ICV + IT with 0.2 μg of PTX (total = 0.4 μg/mouse) or saline. Mice were tested for morphine analgesia on day 1, 2, 4, 7, 11, 16, 21, 25, 29, and 40 following SC injection of morphine (7.5 mg/kg, N = 5–11 mice/group). Different groups of mice were tested at each time point.

Effect of PTX on Upregulation and Functional Supersensitivity Induced by Chronic Opioid Antagonist Treatment

Mice were injected ICV and IT with either 0.2 or 0.5 μg PTX (total = 0.4 or 1.0 μg/mouse) or saline and 1 or 7 days later mice were implanted subcutaneously in the nape of the neck with a single pellet containing 15 mg NTX. Controls were implanted with a placebo pellet (N's are indicated in Tables and Fig. captions). Pellets were wrapped in nylon mesh before implantation to facilitate removal of the pellets at the end of treatment. NTX and placebo pellets were removed 8 days following implantation. Twenty-four hours following pellet removal some mice were sacrificed and brains removed for binding studies. Other groups of mice were tested for morphine analgesia in dose-response studies.

Data Analysis

Quantal dose-response data were analyzed using a computerized (BLISS 21) Probit Analysis (9) program, which estimates ED$_{50}$s, relative potencies and 95% confidence limits. Statistical significance between ED$_{50}$s was evaluated using Probit Analysis results. Saturation studies were analyzed using LIGAND for the PC (32). Statistical significance of parameters from saturation studies were determined using the Z-test based on the normal distribution. Single concentration binding studies and tailflick latencies were analyzed using ANOVA and post hoc tests.

Drugs

PTX (List Biological Laboratories, Inc., Campbell, CA) was dissolved in 0.9% saline for ICV and IT injections. NTX pellets (30 mg naltrexone base, 105 mg cholesterol, 15 mg tristearin) and placebo pellets were obtained from Research Triangle Institute (Research Triangle Park, NC) through the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). Pellets were cut into approximately 75 mg pieces, each of which contained an average of 15 mg NTX. All pellets were wrapped in nylon mesh prior to SC implantation. Morphine sulfate (Penick Laboratories, Newark, NJ) was dissolved in 0.9% saline for SC administration. All doses are expressed as the base.

RESULTS

Effect of PTX on Morphine Analgesia

Treatment with ICV + IT 0.2 or 0.5 μg PTX (total = 0.4 or 1.0 μg/mouse) did not modify the basal nociceptive threshold but led to a significant reduction ten days later of morphine analgesia in both PTX-treated groups compared to control (p < 0.05) (Fig. 1). There was no significant difference between the 2 doses of PTX (p > 0.05), although the higher dose produced slightly greater inhibition.

In time course studies, the baseline tailflick latencies prior to morphine did not differ significantly (p > 0.05) over time or among treatments (Fig. 2, top). Analysis of variance for latencies following morphine indicated a significant (p < 0.02) effect of treatment, and a significant interaction between treatment and time (p < 0.005). Post hoc tests indicated that PTX significantly diminished morphine analgesia on days 11–40 (Fig. 2, bottom). The blockade of morphine analgesia was maximal at 16 days
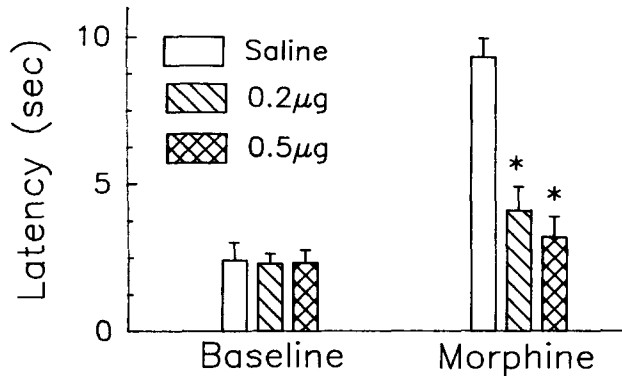
FIG. 1. The effect of PTX treatment on morphine analgesia. Mice were injected ICV + IT with 0.2 or 0.5 μg PTX in each site (total = 0.4 or 1.0 μg/mouse). Ten days later, baseline nociceptive threshold (tailflick) was determined. Mice were then tested for analgesia 30 min following SC morphine injection (4.5 mg/kg, N = 11–15/group). Data presented are means (+ s.e.). *p<0.05 significantly different from control group.

following PTX treatment and was still observed 40 days after PTX administration. Tailflick latencies following morphine were not significantly different between untreated-controls and saline-treated mice at any time.

Effect of PTX on Receptor Upregulation and Functional Supersensitivity Induced by Chronic Opioid Antagonist Treatment

Chronic NTX treatment produced a significant increase in morphine's analgesic potency 24 h following pellet removal in
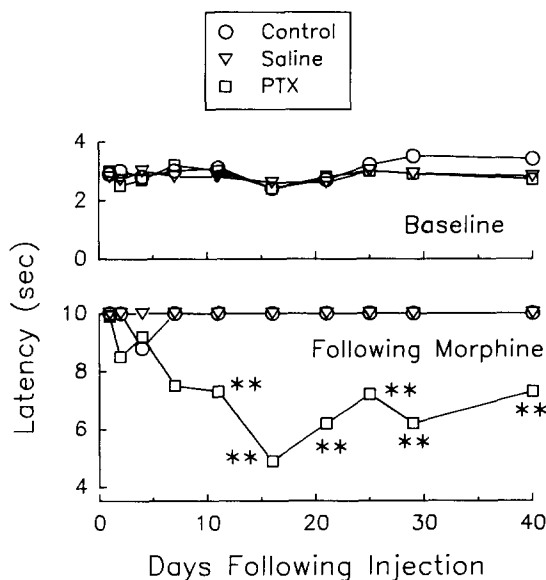
TABLE 1

COMPUTED ED_{50} VALUES AND RELATIVE POTENCY ESTIMATES FOR MORPHINE ANALGESIA FROM MICE CHRONICALLY TREATED WITH NTX OR PLACEBO 1 DAY FOLLOWING PTX OR SALINE

Treatment	ED_{50} (mg/kg) (95% Limits)	Relative Potency
Saline-Placebo	3.4 (2.9–4.0)	1.00
Saline-NTX	2.0* (1.7–2.4)	1.70
PTX-Placebo	12.6* (10.1–16.2)	1.00
PTX-NTX	4.4 (3.5–5.7)	2.86

Mice were injected with either 0.5 μg ICV + IT PTX (total = 1.0 μg/mouse) or saline and 24 h later implanted with a placebo or NTX pellet for 8 days. The pellet was then removed and 24 h later mice were injected with morphine (1–20 mg/kg, SC; N = 4–20/dose). The ED_{50}s (95% confidence limits) and relative potency estimate (compared to corresponding placebo group) were determined by computerized Probit Analysis. Similar results were obtained using 0.2 μg PTX injected into each site. *Significantly different from Saline-Placebo ($p<0.05$).

mice pretreated 1 day earlier with saline (Table 1, Fig. 3). One-day PTX pretreatment shifted to the right the morphine dose-response curve by more than 3.5-fold. In mice pretreated 1 day earlier with PTX, morphine was almost 3 times more potent in the NTX group relative to the placebo group (Table 1). NTX increased the potency of morphine in mice pretreated 7 days earlier with saline (Table 2, Fig. 4). PTX produced a 3-fold reduction in morphine potency in 7-day placebo-pretreated mice. However,

FIG. 2. The time course of the effect of PTX on morphine analgesia. Mice were injected ICV + IT with saline or 0.2 μg PTX (total = 0.4 μg/mouse). Controls were untreated. On selected days, a baseline nociceptive threshold (tailflick) was determined (top panel). Mice were then injected SC with 7.5 mg/kg morphine (n = 5–11/group) and tailflick determined 45 min following morphine (bottom panel). Different groups of mice were tested at each time point. Mean data are presented. Significantly different from control and saline **($p<0.01$).
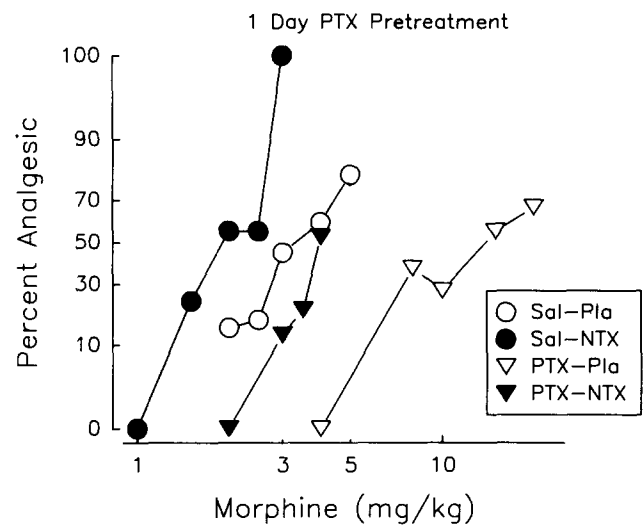

FIG. 3. The effect of 1-day PTX pretreatment on morphine analgesia in chronically NTX-treated mice. Mice were injected ICV + IT with saline (Sal) or 0.5 μg PTX (total = 1.0 μg/mouse); 24 h later mice were implanted with a single NTX or placebo (Pla) pellet for 8 days. The pellets were removed, and 24 h later mice were weighed, baseline tailflick latency determined and then injected SC with morpine (1.0–20.0 mg/kg, N = 4–20/dose) and tailflick latency determined 30 min later. Percent analgesic is presented as a function of morphine dose on log-probability axes (see Table 1). Similar results were obtained using 0.2 μg PTX injected into each site.

TABLE 2

COMPUTED ED_{50} VALUES AND RELATIVE POTENCY ESTIMATES FOR
MORPHINE ANALGESIA FROM MICE CHRONICALLY TREATED WITH
NTX OR PLACEBO 7 DAYS FOLLOWING PTX OR SALINE

Treatment	ED_{50} (mg/kg) (95% Limits)	Relative Potency
Saline-Placebo	2.6 (2.2–3.3)	1.00
Saline-NTX	1.5* (1.2–1.9)	1.73
PTX-Placebo	7.9* (6.3–9.5)	1.00
PTX-NTX	8.2* (6.3–10.3)	0.96

Mice were injected with either 0.2 μg ICV + IT PTX (total = 0.4 μg/
mouse) or saline, and 7 days later implanted with a placebo or NTX pel-
let for 8 days. The pellet was then removed and 24 h later mice were
injected with morphine (0.5–15.0 mg/kg, SC; N = 4–26/dose). The ED_{50}s
(95% confidence limits) and relative potency estimate (compared to cor-
responding placebo group) were determined by computerized Probit Anal-
ysis. *Significantly different from Saline-Placebo ($p<0.05$).

NTX treatment did not increase morphine potency in the 7-day
PTX-pretreated group compared to the PTX-Placebo group
(Table 2).

In single concentration binding studies using [³H]DAMGO
(1.4 nM) and [³H]DADLE (1.0 nM), NTX significantly ($p<0.01$)
increased specific DAMGO and DADLE binding in 1- and 7-day
saline- and PTX-pretreated groups (Figs. 5 and 6). In full satura-
tion studies using [³H]DAMGO and [³H]DADLE, computerized
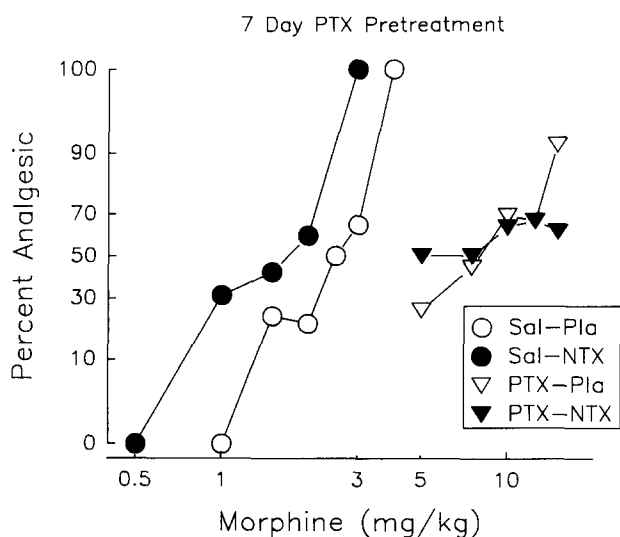analysis (LIGAND) indicated that a one-site model fit the data

FIG. 4. The effect of 7-day PTX pretreatment on morphine analgesia in
chronically NTX-treated mice. Mice were injected ICV + IT with saline
(Sal) or 0.2 μg PTX (total = 0.4 μg/mouse); 7 days later mice were im-
planted with a single NTX or placebo (Pla) pellet for 8 days. The pellets
were removed, and 24 h later mice were weighed, baseline tailflick la-
tency determined and then injected SC with morphine (0.5–15.0 mg/kg,
N = 4–26/dose) and tailflick latency determined 30 min later. Percent an-
algesic is presented as a function of morphine dose on log-probability
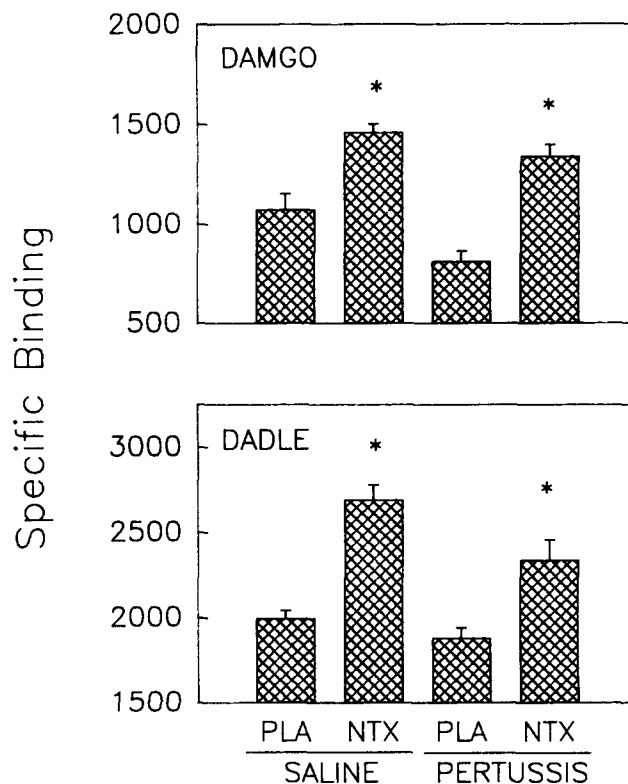axes (see Table 2).

FIG. 5. The effect of 1-day PTX pretreatment on brain opioid binding in
mice chronically treated with NTX. Mice were treated as described in
Fig. 3 except that binding studies (1.4 nM [³H]DAMGO; 1.0 nM
[³H]DADLE) were conducted 24 h following removal of pellets. Data
are presented as specific binding (cpms + s.e.m.). N = 4–5/group. *Sig-
nificantly different from saline-placebo ($p<0.01$).

most appropriately. Combined analysis of two experiments re-
vealed an increase in B_{max} for [³H]DAMGO and [³H]DADLE in
all NTX-treated groups. There were no significant changes in K_D
(Tables 3 and 4). PTX produced a small (17%) but significant
decrease in DAMGO binding in the 7-day PTX-pretreatment
group. Results from a single representative experiment for
[³H]DAMGO binding are shown in Fig. 7.

DISCUSSION

The aim of the present study was to gain insight into the
mechanisms mediating morphine analgesia, as well as receptor
upregulation and functional supersensitivity induced by chronic
opioid antagonist treatment. The results of this study indicate that
pretreatment with PTX in mice produced a time-dependent and
long-lasting reduction in morphine analgesia without affecting the
basal nociceptive threshold. These pharmacodynamic results are
consistent with those of previous reports (18, 34, 37, 39, 43)
and agree with other results that analgesia is mediated by recep-
tors coupled to adenylate cyclase via a PTX-sensitive G-protein
(4, 7, 23, 27). PTX reached its maximal blockade of morphine
analgesia on day 16 following treatment and inhibition was still
observed 40 days following PTX. This effect developed over
time, which is in agreement with findings that PTX has a lag pe-
riod of hours (in cultured cells) to days (in the intact animal)
(10, 16, 21, 24, 29, 34). This observation may reflect a slow
diffusion of the toxin molecule to sites of action in brain tissue
and the need for PTX to be activated so that it can exert its
effect.

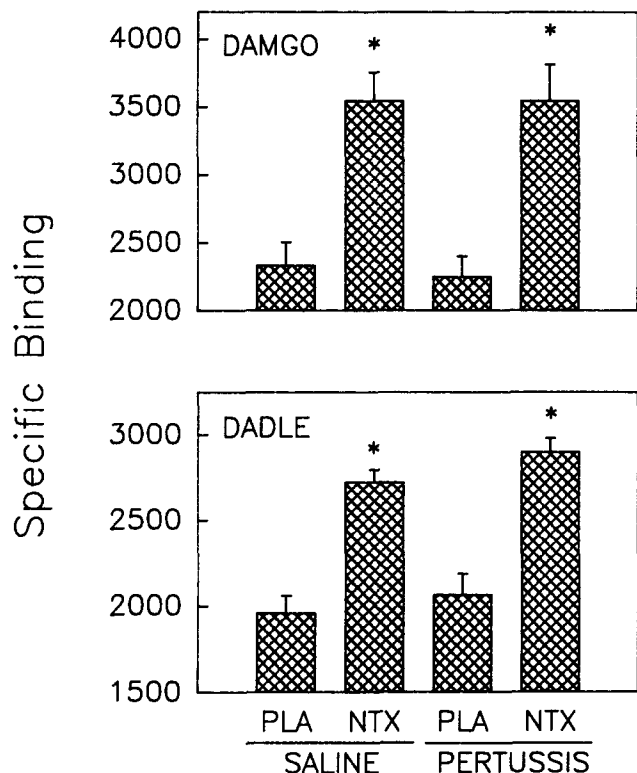In a previous binding study, it was shown that pretreatment

FIG. 6. The effect of 7-day PTX pretreatment on brain opioid binding in mice chronically treated with NTX. Mice were treated as described in Fig. 4 except that binding studies using [^{3}H]DADLE (1 nM) and [^{3}H]DAMGO (1.4 nM) were conducted 24 h following pellet removal. Data are presented as specific binding (cpms + s.e.m.). N = 5–6/group. *Significantly different from saline-placebo ($p<0.01$).

TABLE 3

COMPARISON OF BINDING PARAMETERS FOR [^{3}H]DAMGO IN WHOLE BRAIN FROM MICE CHRONICALLY TREATED WITH NTX OR PLACEBO FOLLOWING PTX OR SALINE

Treatment	B_{max} (fmol/mg)	Percent Change in B_{max}	K_D (nM)
1-Day Pretreatment			
Saline			
Placebo	8.40 ± 1.17	—	0.79 ± 0.23
NTX	13.18 ± 1.58*	+57	0.86 ± 0.22
PTX			
Placebo	8.73 ± 1.48	+4	0.78 ± 0.27
NTX	13.70 ± 1.92*	+63	0.90 ± 0.25
7-Day Pretreatment			
Saline			
Placebo	10.50 ± 0.63	—	0.61 ± 0.07
NTX	15.18 ± 0.76*	+45	0.65 ± 0.06
PTX			
Placebo	8.74 ± 0.35†	−17	0.67 ± 0.05
NTX	15.42 ± 1.23*	+47	0.66 ± 0.11

Mice were treated as described in Fig. 3 (1-day pretreatment) and Fig. 4 (7-day pretreatment) except that 24 h following pellet removal mice were sacrificed and whole brain homogenates prepared. Saturation studies were conducted using 0.156–22.4 nM [^{3}H]DAMGO (see the Method section). Results from two experiments (N = 4–5/group/experiment) analyzed simultaneously by LIGAND for the PC (32) are presented. B_{max} (± s.e.) are expressed in femtomoles per 1 mg of wet weight. Percent change is the B_{max} for each treated group divided by the B_{max} for saline-placebo multiplied by 100. The K_{DS} (± s.e.) are expressed as nanomolar. *Significantly different ($p<0.05$) from corresponding placebo value. †Significantly different from saline-placebo ($p<0.05$).
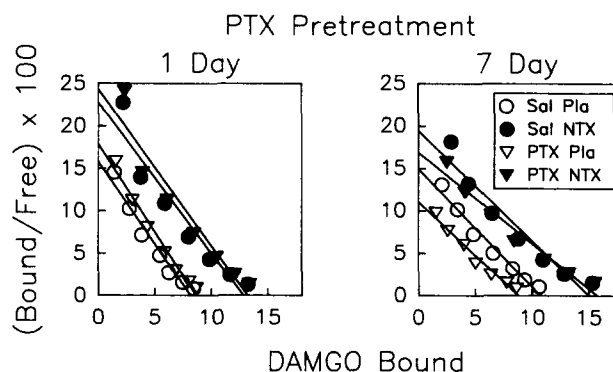
of rat brain membrane preparations with PTX resulted in selective changes in K_D or B_{max} for DADLE binding in striatum and cortex without affecting the binding in midbrain (2). Other reports have demonstrated a decrease in DADLE binding after preincubation with PTX in in vitro culture systems (7, 20, 26). In this experiment, we found that PTX either did not alter, or slightly decreased, DAMGO and DADLE binding in whole brain homogenate prepared from mice. A possible explanation for this discrepancy is the heterogeneity of neuronal cell types present in whole brain and the use of the intact animal in the present experiment as compared to cell culture and membrane preparations in other reports.

Opioid receptor upregulation and functional supersensitivity have been observed following chronic (8-day) but not acute (1-day) opioid antagonist treatment (3, 25, 46, 47, 53, 54). In the present study, we demonstrated that PTX treatment modified opioid receptor upregulation and functional supersensitivity differently. PTX treatment either for 1 day or 7 days prior to NTX implantation did not alter NTX-induced receptor upregulation. Saturation binding studies showed that both DAMGO and DADLE binding sites (B_{max}s) in whole brain homogenate were similarly increased by chronic NTX treatment in all saline- and PTX-pretreated groups without altering affinity (K_{DS}). Similar results were obtained in single concentration binding studies for the two ligands. Thus PTX does not interfere with opioid antagonist-induced receptor upregulation. However, 7-day, but not 1-day PTX pretreatment, completely blocked NTX-induced functional supersensitivity. The blockade of supersensitivity by 7-day

FIG. 7. Scatchard plots of specific [^{3}H]DAMGO binding in brain homogenate from mice chronically treated with NTX or placebo (Pla) pellet 1 day following PTX or saline (Sal) (left); and 7 days following PTX or saline (Sal) (right). Mice (N = 4–5/group) were treated as described in Fig. 3 (1 day) and Fig. 4 (7 days) except that 24 h following pellet removal binding studies were performed using 0.156–10.0 nM [^{3}H]DAMGO. Results are presented as (specific bound/free) × 10^2 versus specific DAMGO bound (fmol/mg wet weight). Data were analyzed by LIGAND for the PC (32). The K_D's (nM) and B_{max}'s (fmol/mg of wet weight) for each condition for 1-day PTX pretreatment are: Sal-Pla: 0.51 nM, 8.9 fmol/mg; Sal-NTX: 0.49 nM, 13.4 fmol/mg; PTX-Pla: 0.47 nM, 9.4 fmol/mg; PTX-NTX: 0.46 nM, 13.8 fmol/mg; for 7-day PTX pretreatment the values are: Sal-Pla: 0.64 nM, 11.3 fmol/mg; Sal-NTX: 0.62 nM, 15.1 fmol/mg; PTX-Pla: 0.74 nM, 9.3 fmol/mg; PTX-NTX: 0.78 nM, 16.1 fmol/mg. The results are representative data from a single experiment. Combined results from two experiments are shown in Table 3.

TABLE 4

COMPARISON OF BINDING PARAMETERS FOR [^{3}H]DADLE IN
WHOLE BRAIN FROM MICE CHRONICALLY TREATED WITH NTX
OR PLACEBO FOLLOWING PTX OR SALINE

Treatment	B_{max} (fmol/mg)	Percent Change in B_{max}	K_D (nM)
	1-Day Pretreatment		
Saline			
Placebo	11.16 ± 1.79	—	1.18 ± 0.30
NTX	16.68 ± 2.67*	+49	1.47 ± 0.35
PTX			
Placebo	9.51 ± 1.14	−15	1.11 ± 0.21
NTX	14.60 ± 2.19*	+31	1.39 ± 0.32
	7-Day Pretreatment		
Saline			
Placebo	9.80 ± 0.69	—	1.10 ± 0.12
NTX	13.14 ± 0.79*	+34	1.23 ± 0.11
PTX			
Placebo	9.10 ± 0.64	−7	1.20 ± 0.12
NTX	13.65 ± 1.64*	+39	1.17 ± 0.21

Mice were treated as described in Fig. 3 (1-day pretreatment) and Fig. 4 (7-day pretreatment) except that 24 h following pellet removal mice were sacrificed and saturation studies in whole brain were conducted using 0.095–15.0 nM [^{3}H]DADLE (see the Method section). Results from two experiments (N = 4–5/group/experiment) analyzed simultaneously by LIGAND for the PC (32) are presented. B_{max}s (± s.e.) are expressed in femtomoles per 1 mg of wet weight. Percent change is the B_{max} for each treated group divided by the B_{max} for saline-placebo multiplied by 100. The K_Ds (± s.e.) are expressed as nanomolar. *Significantly different ($p < 0.05$) from corresponding placebo value.

PTX pretreatment may be due to the failure of the new binding sites to be functionally coupled to adenylate cyclase. The fact that NTX-induced supersensitivity was observed in the 1-day PTX-pretreated mice compared to placebo may represent the coupling of new binding sites to functional G-proteins that have not, as yet, been deactivated by PTX.

Our data suggest that opioid receptor upregulation induced by chronic opioid antagonist treatment can be observed despite functional decoupling of the opioid receptors from adenylate cyclase by PTX. Similarly, opioid receptor downregulation induced by chronic exposure to opioid agonist in NG108-15 cells is not altered by PTX treatment (26). These results strongly suggest that alterations in receptor density in the cellular adaptation process do not appear to be related to the PTX-sensitive guanine nucleotide-binding component. It is worth noting that we have recently shown that opioid receptor upregulation is unaffected by concurrent morphine treatment that produces tolerance (57). Thus, while functional aspects of opioid receptors appear to be readily modulated, changes in receptor density are quite robust.

In summary, our results suggest that a common PTX-sensitive G-protein is important in functionally coupling opioid receptors that mediate analgesia in both control and upregulated mice. Furthermore, the ability of PTX to abolish supersensitivity without altering receptor upregulation induced by chronic opioid antagonist treatment, indicates that new binding sites can appear without being functionally coupled.

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