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Selective changes in μ opioid receptor properties induced by chronic morphine exposure

(morphine tolerance/guanine nucleotide-binding protein/down-regulation)

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ABSTRACT **Chronic infusion of morphine to guinea pigs produced selective changes in μ agonist binding properties in cerebrocortical membrane preparations. Employing the μ-selective opioid agonist [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAMGO) in direct binding studies and in competition of labeled antagonist binding, we found that the major changes were a decrease in the number of sites with high affinity for agonist, a small reduction in total receptor number, and a loss in the ability of guanosine 5′-[γ-thio]triphosphate to regulate binding. A fraction of high-affinity μ receptors appeared to retain their high affinity for agonist and their sensitivity to guanine nucleotide analogue after the induction of morphine tolerance, possibly because the morphine concentrations achieved in brain were insufficient to uncouple all μ receptors from associated guanine nucleotide-binding regulatory proteins. Some membrane preparations were treated with pertussis toxin, which has been shown to functionally uncouple μ opioid receptors from their effector systems. In these preparations, a single agonist-affinity state of the receptor was observed. The apparent dissociation constant for this affinity state in pertussis toxin-treated membranes was similar to the lower-affinity state observed in preparations from morphine-tolerant animals. In contrast to the changes observed at μ opioid binding sites, no significant changes in agonist affinity or binding density were observed for selective δ or κ agonists, consistent with the development of selective tolerance at μ receptors.**

The mechanisms by which opiate drugs induce tolerance and dependence are not clearly understood. A number of observations suggest that changes occur in the function of the receptors through which the tolerance-inducing drug acts to evoke its primary pharmacologic effect. A significant fraction of the receptors must be activated for tolerance and dependence to develop (1, 2). In tissues where more than one type of opioid receptor is present, the occurrence of receptor-selective tolerance (3–5) also suggests that receptor-specific mechanisms are critical in the tolerance process. Studies of the interaction of agonists with δ opioid receptors in NG108-15 neuroblastoma–glioma hybrid cells in culture after chronic receptor activation have demonstrated that sustained agonist exposure leads initially to a loss of the ability of δ agonists to regulate their effector system (in this case, to inhibit adenylate cyclase), followed later by a reduction in the number of δ receptors detected by radiolabeled ligand binding (6). Recently, similar results have been reported in a study of the chronic activation by morphine of μ opioid receptors in 7315c pituitary tumor cells maintained in primary culture (7). Thus, in these cell systems in which opioid receptors negatively regulate adenylate cyclase, sustained exposure to morphine resulted in an initial desensitization followed by receptor down-regulation.

Early studies examining the binding of opioid receptors in brain membranes from small mammals following treatment with morphine regimens that induce tolerance did not find evidence of any changes in the binding properties of opioid receptors (8, 9). There are several possible reasons for the apparent discrepancy between morphine-tolerance processes in cells maintained in culture and in rat brain *in vivo*. At the time of the initial studies in brain tissue, the heterogeneity of opioid receptors in brain was not understood and radiolabeled ligands with only limited receptor-type selectivity were used. Thus changes in one receptor type may not have been detected, because binding to other receptor types was unchanged. Second, the use of labeled agonists to measure binding-site properties essentially restricts the information that is obtained to possible changes in the high-affinity states of the receptors, since low-affinity agonist binding cannot be reliably quantified against a high background of nonspecific agonist binding. Recent studies have suggested that low agonist-affinity states of the receptor may be associated with or a consequence of the activation process (10, 11). Finally, the concentration of morphine that is achieved in blood in *in vivo* studies (12) is lower than has been used to induce receptor down-regulation in opiate-sensitive cells maintained in culture. Recent studies have demonstrated that receptor down-regulation upon chronic agonist exposure can be detected in brain under the proper experimental conditions. Morphine treatment produced down-regulation of μ receptors in an intact cell binding assay (13, 14), and administration of the nonselective opioid agonist etorphine to rats led to a significant decrease in the number of μ receptors in brain that could be labeled in binding assays (15).

We have therefore reevaluated the possible occurrence of changes of μ opioid receptor properties during the development of tolerance to morphine *in vivo*. Tolerance was induced in guinea pigs by the infusion of morphine from indwelling osmotic minipumps for a period of 6 days, a procedure resulting in continuous drug exposure throughout the tolerance-induction period. This induction paradigm has been shown to produce selective functional tolerance at μ receptors in guinea pig cortex as measured by a decreased ability of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAMGO) to inhibit the release of norepinephrine (5). Binding to μ, δ, and κ opioid receptors was quantified separately by the use of selective blockade of other receptor types. Multiple agonist-affinity states of the receptor were characterized by assessing the competition of unlabeled receptor-type-selective agonists

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Abbreviations: B_{max}, density of binding sites; DAMGO, [D-Ala^2, MePhe^4,Gly-ol^5]enkephalin (where Gly-ol is glycinol); DSLET, [D-Ser^2,Leu^5]enkephalinyl-Thr; G protein, guanine nucleotide-binding protein; GTP[γS], guanosine 5′-[γ-thio]triphosphate; K_{app}, apparent dissociation constant; U-50,488H, *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate.

for labeled antagonist binding sites. Finally, the ability of guanine nucleotides to reduce agonist affinity in untreated and morphine-tolerant guinea pigs was compared. The results indicate that morphine tolerance was associated with a loss of guanine nucleotide regulation of μ agonist binding and a significant reduction in the number of μ receptors in guinea pig cortex membranes. In the same membrane preparations, the properties of δ and κ receptors were unchanged by the morphine treatment.

It has been proposed that opiate tolerance involves uncoupling of the receptor from its effector system. Opioid receptor–effector uncoupling is also produced by pertussis toxin, which ADP-ribosylates the α subunit of the guanine nucleotide-binding proteins G_i and G_o (16, 17) and thereby functionally uncouples the receptor from its effector system (18–20). We therefore also examined the effects of *in vitro* treatment of membrane preparations with pertussis toxin. Opioid binding properties of the treated membranes were compared with the properties of opioid binding sites in membranes from morphine-tolerant guinea pigs.

MATERIALS AND METHODS

Preparation of Guinea Pig Cerebrocortical Membranes. Male Hartley guinea pigs (300–600 g; Camm Research Institute, Wayne, NJ) were anesthetized with halothane (2.5% in oxygen) and implanted subcutaneously in the back at the base of the neck with Alzet osmotic minipumps (Alza) containing either 0.9% saline or morphine hydrochloride at a concentration calibrated to deliver a constant infusion of 1.7 mg/hr per kilogram of body weight. After 6 days, animals were decapitated and cortical membranes were prepared as described (10), except that after the membranes were thawed, they were washed two additional times before use in the binding assay. Based upon the studies of Goldstein and Schulz (12), we estimated the maximal amount of residual morphine that could remain in the brain after 6 days of chronic infusion. Goldstein and Schulz (12) calculated that about 75 mg of morphine was delivered to each guinea pig after 1 day, but by day 6, about 15 mg/day was being delivered. This amount is comparable to our infusion rate of about 20 mg/day in a guinea pig weighing about 0.5 kg. Goldstein and Schulz reported that the highest level of morphine in the blood (2.3 μM) was observed after the first 24 hr and that the level rapidly declined to 0.5 μM after 6 days. Assuming that morphine in the blood was in equilibrium with morphine in the cerebrospinal fluid, we estimated the maximal brain concentration was unlikely ever to exceed 5 μM in our animals. We tested the efficiency of removal of radiolabeled morphine by washing. Membranes were incubated with 5 μM [^{3}H]morphine (specific activity adjusted to 4.1 Ci/mmol with unlabeled morphine; 1 Ci = 37 GBq) for 1 hr at 37°C and then washed five times to conform with the treatments performed in the standard membrane preparation. After five washes, and dilution up to the total assay volume, the fraction of remaining radioactivity was 0.00016, amounting to a maximum final morphine concentration of 0.8 nM. This concentration of morphine, which is <0.1 times its K_i at μ receptors under our assay conditions (21), should have exerted very little effect on our determination of DAMGO binding parameters.

Pertussis Toxin Treatment. To activate pertussis toxin, 50 μg was dissolved in 0.5 ml of water and dithiothreitol was added to yield a 50 mM final concentration. The mixture was incubated for 1 hr at room temperature. Membranes were thawed at room temperature and resuspended in 1/10 the storage volume, and the following reagents were added to give the indicated final concentrations: 8.0 mM dithiothreitol, 1 mM ATP, 10 mM thymidine, and 2.0 mM $MgCl_2$. The membrane preparation was divided into two portions and pertussis toxin was added at a final concentration of 10 μg/ml to one portion. The membranes were then incubated for 1 hr at 37°C. Subsequently, membranes were centrifuged at 27,000 × *g* for 15 min at 4°C and resuspended to 4% original wt/vol. The wash procedure was repeated and the membranes were resuspended at 2% original wt/vol for use in binding assays.

Binding Assay and Analysis of Binding Data. Binding assays were essentially as described (10). Saturation and displacement data were analyzed by the use of the computer program LIGAND (22) as reported (10).

RESULTS

Agonist Saturation and Competition Analysis. Parameters obtained from saturation curves for the binding of [^{3}H]-DAMGO at 0.05–15 nM are reported in Table 1. The optimal computed model for binding of the labeled μ agonist to membranes prepared from both naive and morphine-treated guinea pigs was for a single class of binding sites. The apparent equilibrium dissociation constant (K_{app}) was approximately equal in both preparations. However, the density of binding sites (B_{max}) was significantly ($P < 0.05$) reduced by about 45% in the membranes from the morphine-treated animals. We also determined the affinity of DAMGO for μ receptors by binding 5 nM [^{3}H]DAMGO and constructing homologous competition curves (Table 1). This method also demonstrated the existence of a single class of binding sites with a K_{app} of 3–4 nM in membranes from both naive and tolerant animals. There was again a small but significant ($P < 0.05$) decrease of about 25% in B_{max} for agonist binding in the membranes of the morphine-treated guinea pigs.

With membranes from morphine-treated guinea pigs, saturation curves for [^{3}H]DAMGO in the presence of guanosine 5′-[γ-thio]triphosphate (GTP[γS]) yielded binding that was too low to be modeled accurately. To determine whether agonist binding in membranes from morphine-treated animals was affected by the addition of the guanine nucleotide analogue, competition curves for DAMGO against [^{3}H]-DAMGO were constructed in the presence and absence of GTP[γS]. For these experiments, a 1 nM concentration of labeled ligand was employed to preferentially label the sites that have high affinity for DAMGO. These experiments showed that in membranes from tolerant preparations, the K_{app} for the high-affinity site was shifted from 4.0 nM to 24 ± 5.0 nM ($n = 2$). The magnitude of this shift is similar to that reported for membranes prepared from naive guinea pigs, which yielded a K_{app} of 6.6 nM in the absence of GTP and 21 nM in its presence (10).

Agonist Competition Against Labeled Antagonist Binding. To discriminate very low-affinity agonist binding states, we labeled μ sites with [^{3}H]diprenorphine, in the presence of site-selective blockers for δ and κ receptors, and constructed competition curves with DAMGO. We showed previously that [^{3}H]diprenorphine binding is not affected by the addition of 10 μM GTP[γS] (10). In membranes prepared from naive

Table 1. Parameters for DAMGO binding to μ receptors in guinea pig cortical membranes

Pretreatment	Experiment type	n	K_{app}, nM	B_{max}, fmol/mg of protein
None	Saturation	4	3.3 ± 0.8	48 ± 8
Morphine	Saturation	4	3.3 ± 0.9	26 ± 5
None	Competition	2	4.4 ± 1.0	45 ± 7
Morphine	Competition	5	4.0 ± 0.7	33 ± 4

Binding parameters were derived by the computer program LIGAND (22), which models n independent experiments simultaneously and provides an estimate of K_{app}, B_{max}, and the standard error of these estimates.

animals, competition with DAMGO yielded shallow curves, suggesting the presence of more than one class of binding sites (Fig. 1*A*). Although a two-site model provided the best fit to the experimental data (Table 2), no clear plateau was evident in the plot. When GTP[γS] was added, two affinity states of the μ receptor could clearly be discriminated, one of which has a K_{app} in the micromolar range. These findings confirm our earlier report (10).

We performed identical competition experiments on membranes prepared from morphine-tolerant animals (Fig. 1*B*). Inspection of the competition curves showed that addition of GTP[γS] to membranes from the morphine-treated animals was much less effective in shifting the curve to the right. The very low agonist-affinity state apparent in control membranes after GTP[γS] addition was not observed. Analysis of these data was performed in two ways. Initially, all parameter estimates were determined iteratively for a one-site model. The data could be fit to a single affinity site with a K_{app} of 52 ± 10 nM in the absence of added nucleotide and 65 ± 14 nM in the presence of added nucleotide, intermediate between the high- and low-affinity states discriminable in membranes from untreated animals. The plot of these data reflects this change as an apparent steepening in the competition curve (compare Fig. 1 *A* and *B*). However, this result is inconsistent with measurement of direct agonist binding, which shows that a reduced number of sites with high affinity for agonist are still present after chronic morphine treatment (Table 1). The reduced number of high-affinity sites made them less readily discriminable when labeled antagonist binding to all agonist-affinity sites of the receptor was measured. We therefore used the LIGAND program to fit the results of competition against antagonist binding to a two-site model in which the higher-affinity state was constrained to a value of 4.0 nM in the absence of GTP[γS] (Table 2). This method of analysis produced a model with a significantly improved fit ($F = 6.81, P = 0.01$). For modeling of competition data obtained in the presence of GTP[γS], the higher-affinity site was constrained to have a K_{app} of 24 nM (Table 2), the value determined by direct agonist binding in the presence of GTP[γS]. This produced a model with a slightly improved goodness of fit. This method of analysis also indicated a 20–25% decrease in total receptor number in the presence or absence of GTP[γS]. We consider these analyses to yield a better indication of the agonist-affinity states present in the membranes, since modeling of the data in this manner takes into account the findings of the direct agonist binding data and produces a better mathematical description of the binding parameters.

Agonist binding at δ and κ receptors was studied in the same membrane preparations. In marked contrast to results of competition for μ binding by DAMGO in membranes prepared from morphine-tolerant animals, competition curves for δ receptor binding by the δ-preferring agonist [D-Ser^2,Leu^5]enkephalinyl-Thr (DSLET) were not changed in this tissue. Two affinity states were clearly discriminable

Table 2. Parameters for competition of DAMGO with [^{3}H]diprenorphine for binding to μ receptors: Effects of GTP[γS] on binding to cortical membranes from morphine-tolerant guinea pigs or to membranes pretreated with pertussis toxin

Pretreatment	GTP[γS], μM	*n*	K_{app}, nM	B_{max}, fmol/mg of protein
None	0	4	11 ± 6.2	44 ± 14
			150 ± 87	38 ± 13
None	10	4	46 ± 7.4	67 ± 3.4
			2300 ± 940	23 ± 3.5
Pertussis toxin	0	5	86 ± 9.0	78 ± 3.1
Pertussin toxin	10	5	89 ± 8.9	76 ± 2.3
Morphine	0	4	4.0*	20 ± 7.2
			120 ± 41	48 ± 5.3
Morphine	10	4	24.0*	21 ± 4.1
			120 ± 26	41 ± 2.5

Parameter estimates were determined by the program LIGAND.
*Data were modeled by constraining the high-affinity-site K_{app} to 4 nM or 24 nM based upon results of agonist/agonist competition analysis in the presence and absence of added GTP[γS]. This constraint significantly improved the fit for data obtained in the absence of GTP[γS] ($P < 0.05$) and produced a model with a slightly improved goodness of fit compared to a one-site model for data obtained in the presence of GTP[γS].

in membranes from naive and morphine-tolerant guinea pigs, and both membrane preparations were sensitive to GTP[γS] (Table 3). The number of δ binding sites was unaffected by morphine treatment. We also examined the binding properties of κ receptors. To ensure that antagonist binding at κ sites was not affected by the addition of GTP[γS], we first measured competition of unlabeled diprenorphine with [^{3}H]-diprenorphine in the presence or absence of 10 μM GTP[γS]. The K_{app} for diprenorphine at κ sites was 0.42 ± 0.05 nM with or without GTP[γS], and the B_{max} was 156 ± 9.0 fmol/mg of protein ($n = 2$). Competition of U-50,488H {*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate} with [^{3}H]diprenorphine for binding to κ receptors in tissue from morphine-tolerant guinea pigs was not significantly different from that seen in tissue from untreated animals (Table 3). The competition curves were best fit by a single-site model. Competition for κ binding by U-50,488H was less affected by the addition of GTP[γS] than agonist competition for μ or δ sites (Table 3). The addition of the guanine nucleotide appeared to decrease affinity slightly. The B_{max} for κ receptors varied only marginally between tolerant and nontolerant preparations with or without added GTP[γS].

Binding in Pertussis Toxin-Treated Membranes. Some membrane preparations were treated with pertussis toxin, and binding of [^{3}H]DAMGO or [^{3}H]diprenorphine to μ sites was assayed. As with agonist saturation binding to membranes from morphine-tolerant animals in the presence of GTP[γS], the amount of saturation binding to pertussis

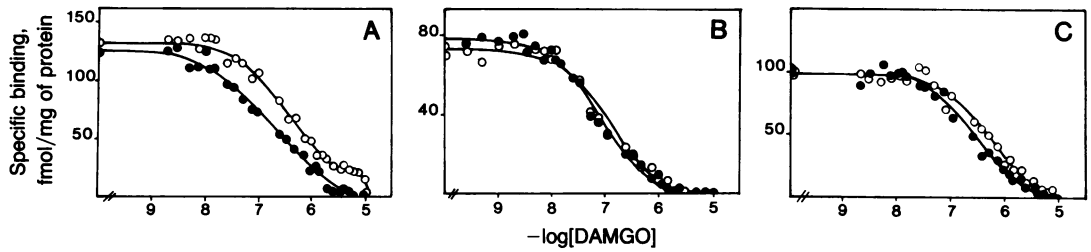

FIG. 1. Competition for binding of [^{3}H]diprenorphine to μ receptors in guinea pig cortical membranes in the presence (○) or absence (●) of 10 μM added GTP[γS]. (*A*) Membranes prepared from untreated animals. (*B*) Membranes prepared from guinea pigs that had received morphine (1.7 mg/kg per hr) for 6 days. (*C*) Membranes prepared from untreated animals and treated with pertussis toxin (10 μg/ml) for 1 hr prior to assay. Results are from single experiments which were performed 4 times each with similar results.

Table 3. Parameters for agonist competition against [^{3}H]diprenorphine binding to δ and κ receptors

Pretreatment	Agonist	Receptor	GTP[γS], μM	n	K_{app}, nM	B_{max}, fmol/mg of protein
None	DSLET	δ	0	5	4.6 ± 3.1	53 ± 11
					370 ± 550	25 ± 9.9
None	DSLET	δ	10	5	15 ± 4.7	45 ± 4.0
					2700 ± 2400	37 ± 12
Morphine	DSLET	δ	0	3	4.2 ± 1.2	37 ± 2.5
					850 ± 490	20 ± 2.5
Morphine	DSLET	δ	10	3	23 ± 5.3	39 ± 2.6
					3000 ± 2200	30 ± 5.2
None	U-50,488H	κ	0	2	15 ± 1.0	140 ± 3.0
None	U-50,488H	κ	10	2	36 ± 12	120 ± 13
Morphine	U-50,488H	κ	0	2	17 ± 1.7	160 ± 5.0
Morphine	U-50,488H	κ	10	2	28 ± 3.9	160 ± 5.0

Binding parameters were determined by the program LIGAND.

toxin-treated membranes was too low to be analyzed, indicating that no very high-affinity DAMGO binding site was identifiable in these preparations. We therefore constructed competition curves for DAMGO against [^{3}H]DAMGO in the presence and absence of GTP[γS]. These curves could be fitted to a single-site model, with a K_{app} value of 102 ± 18 nM in the absence and 111 ± 34 nM in the presence of GTP[γS] (n = 3). These curves could not be fitted to a two-site model. In competition against the labeled antagonist [^{3}H]diprenorphine, DAMGO again appeared to bind to a single affinity state of the μ receptor in the absence of GTP[γS] and in its presence (Fig. 1*C*, Table 2). The K_{app} values for this site were similar to those found for DAMGO competition against labeled [^{3}H]DAMGO. Neither agonist affinity nor the number of binding sites was changed by the addition of GTP[γS] after pertussis toxin treatment.

DISCUSSION

We have sought to reconcile a number of disparate observations regarding the effects of morphine tolerance on various aspects of agonist–receptor interactions in brain tissue. The major changes in the tolerant state observed in this study were (*i*) a decrease in the number of μ receptors with high agonist affinity, resulting in a small reduction in overall μ receptor number, and (*ii*) the loss in regulation of agonist affinity by guanine nucleotides at μ receptors. No changes were observed for agonist binding to δ or κ opioid receptors. We have also sought to correlate the changes observed for μ agonist binding to those observed after pretreatment of brain membranes with pertussis toxin, a manipulation that causes uncoupling of the μ receptor from its associated G protein.

The major change identifiable in direct binding experiments with ^{3}H-labeled agonist was a decrease in μ receptor number, without change in the affinity of the receptor for the agonist DAMGO. Our evidence suggests that the very low-affinity agonist binding sites of the μ receptor induced by GTP[γS] that can be labeled with [^{3}H]diprenorphine are no longer present after chronic morphine treatment. In cortical membranes from guinea pigs receiving a constant infusion of morphine, the very low-affinity state is conspicuously absent from competition curves for DAMGO. Since direct agonist binding studies showed that high-affinity agonist binding was still observed in the morphine tolerant state, we presumed that at least some of the receptors labeled by antagonist and competed for by agonist in the heterologous competition experiments must continue to exist in the relatively higher agonist affinity form. When the data were modeled for a two-site fit constraining one site to a K_{app} of 4.0 nM, the overall fit was significantly better than the fit to a one-site model (Table 2). Modeling for two sites also revealed the presence of a site with a K_{app} of about 120 nM. Possible assignment of various affinity forms of the μ receptor in association or dissociation with G proteins and guanine nucleotides was discussed previously (10). Results reported here suggest that the affinity state with a K_{app} of 120 nM that could be discriminated in membranes from morphine-tolerant guinea pigs was similar to that in membranes that had been treated with pertussis toxin. These sites presumably represent a state of the receptor that has been uncoupled from the G protein. We previously suggested that the high-affinity (K_{app} of 4 nM) site represents receptor coupled to G protein with no associated guanine nucleotide (10). The addition of GTP[γS] shifted the K_{app} of DAMGO for this site to about 25 nM. This site was not observed in pertussis toxin-treated membranes, suggesting that all G proteins that are coupled to μ binding sites in these membranes are capable of undergoing ADP-ribosylation. The ratio of high to intermediate affinity states of μ receptors shifted from a value of about 1:1 in untreated membranes to about 1:2 after morphine treatment. The concentrations of morphine reached during the chronic treatment may have been insufficient to cause uncoupling of all μ receptors from associated G protein.

The binding characteristics of the μ sites in membranes from brains of morphine-tolerant guinea pigs and pertussis toxin-treated guinea pig cortical membranes were similar to those from morphine-treated or pertussis toxin-treated 7315c pituitary tumor cells (10). Preparations of 7315c cells bear only the μ type of opioid receptor (7). Chronic morphine treatment of these cells resulted in a loss of the ability of μ receptor agonists to inhibit adenylate cyclase, followed by loss of the ability of GTP[γS] to regulate agonist affinity and a decrease in the number of μ receptors. The similarities between parameters for binding in 7315c cells and guinea pig cortical membrane preparations support our contention that we are successfully labeling only μ receptors under our binding conditions and therefore are able to detect very specifically μ receptor changes induced by tolerance or pertussis toxin treatment.

Our results indicate that the sum of higher and lower affinity states of μ receptors in cortical membranes was reduced ≈20% by chronic morphine treatment, representing a small down-regulation of total μ receptors. Exposure of 7315c pituitary tumor cells in culture to a high concentration of morphine for 48 hr resulted in a down-regulation of μ receptors to about 60% (7). It is possible that a larger down regulation would be observed if higher brain concentrations of morphine were achieved *in vivo*. Chronic morphine treatment appears to be associated with a decrease in the number of spare receptors. By using β-chlornaltrexamine to inactivate varying numbers of μ receptors, morphine-tolerance-inducing regimens have been shown to reduce μ receptor

reserve in guinea pig ileum myenteric plexus (23) and in rat locus coeruleus neurons (24).

No significant changes in binding parameters were detected for δ or κ receptors in cortical membranes from morphine-tolerant animals. The retained sensitivity of δ receptors to regulation by GTP[γS] and the lack of any down regulation for δ or κ receptors in membranes from the morphine-tolerant guinea pigs are consistent with the development of selective tolerance at the μ receptor. Selective tolerance to morphine in guinea pig cortex has been demonstrated at μ receptors by using the same induction paradigm employed in this study (5). Since agonist binding to δ sites was still sensitive to the effects of GTP[γS], presumably the changes induced by morphine treatment were restricted to the μ receptor itself or to a population of G proteins that are associated with μ, but not with δ opioid receptors. In contrast, pertussis toxin ADP-ribosylates G proteins associated with both μ and δ receptors, since it has been shown to uncouple the receptor from adenylate cyclase in both 7315c (25) and NG108-15 (16) cells.

The mechanism responsible for the impaired functional interaction of μ receptors and G protein(s) is not yet clear. It is possible that the ability of the receptor to activate the G protein is impaired as a result of a reversible posttranslational modification (e.g., phosphorylation). If μ receptors are associated with a unique pool of G protein(s), it is also possible that chronic morphine exposure causes a change in the properties of this G protein(s) or simply a reduction in the G-protein concentration in the μ receptor-associated pool. Christie *et al.* (24) have shown in rat locus coeruleus neurons that μ receptors and α_2-adrenergic receptors are coupled to the same potassium conductance. While morphine tolerance impaired the ability of μ agonists to activate the potassium conductance, α_2 agonists were still capable of eliciting this effect. This argues in favor of the changes produced by morphine tolerance occurring at the receptor itself instead of at the transducer level.

We cannot absolutely exclude the possibility that some residual morphine was present in membranes prepared from morphine-tolerant guinea pig brains. Although experiments with radiolabeled morphine indicated that almost all drug could be removed by the standard membrane washing procedure, morphine delivered by constant infusion over a number of days might interact differently with the receptors or effect some change in receptor conformation that renders it essentially trapped. If this were the case, however, the similarity of competition patterns with DAMGO in morphine-treated and pertussis toxin-treated tissues would be extremely fortuitous. Additionally, the loss of guanine nucleotide regulation is difficult to explain simply by the presence of residual morphine. If residual morphine were present, this could contribute to the increased K_{app} for DAMGO as a competing agent in morphine-tolerant membranes. However, it is unlikely that it would cause the complete disappearance of the very low agonist affinity state seen in membranes prepared from naive animals.

In conclusion, the results reported here suggest that morphine tolerance is associated with a loss in the ability of guanine nucleotides to regulate agonist affinity at μ receptors. This is probably the result of a functional uncoupling of the receptor from associated G protein(s). A modest decrease in receptor number also occurs.

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