Differential Cross-tolerance Between Intrathecal Morphine and Sufentanil in the Rat

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By means of a subcutaneously implanted osmotic pump, groups of rats received a constant-rate (1 μl/h), 7-day intrathecal infusion of saline or one of two μ-opioid agonists: sufentanil (0.6 nmol/h) or morphine (20 nmol/h). These concentrations of morphine and sufentanil yielded a comparable near maximal hot-plate response latency on day 1 of the infusion. On day 7, the magnitude of tolerance was assessed in each group by establishing intrathecal dose–response curves and ED₉₀ values for sufentanil and morphine given as a bolus injection. Each infused animal was used for a single bolus injection. In all cases, infusion with the opioid resulted in a rightward shift (increase in ED₉₀) for both morphine and sufentanil as compared to saline-infused animals. The magnitude of the shift, however, was different for the two drugs. Thus, in morphine-infused rats, the morphine ED₉₀ increased as compared to saline-infused animals by a factor of 44, whereas the sufentanil ED₉₀ shifted by a factor of 10. In sufentanil-infused animals, the respective shifts in the morphine and sufentanil ED₉₀ values were increased by a factor of 9 and 3, respectively. Thus, a significantly greater shift as compared to saline-infused animals was observed in morphine-infused than in sufentanil-infused animals. Conversely, regardless of the opioid to which the animal was exposed, morphine-tested animals showed a greater rightward shift than did sufentanil-tested animals. These data showing a nonsymmetric tolerance between agents acting at the same μ-opioid receptor are consistent with the argument that these two agents differ in efficacy, i.e., the fraction of the receptor population each must occupy to produce a given effect. Agents with high efficacy and a significant receptor reserve (e.g., sufentanil) will down-regulate fewer receptors than will agents with low efficacy and a smaller receptor reserve (e.g., morphine). As a result, agents with higher efficacy at a receptor show theoretically less tolerance over time as compared with less efficacious agents acting at the same receptor. (Key words: Analgesics, opioid: efficacy, morphine, sufentanil, tolerance. Anesthetic techniques: spinal.)

Several families of spinal receptors are known to modulate the processing of nociceptive stimuli after the spinal administration of receptor-selective agents. Among these are the receptors of the μ, δ, and α₂ adrenergic subclasses. These receptor systems share several common properties: they exhibit binding in the spinal dorsal horn, in part on the terminals of small primary afferents; they block the release of small afferent fiber neuropeptides; and they appear to exert their effect largely by the increase of membrane K⁺ conductance through a G protein. Continued exposure of the receptor to its respective agonist, either in vivo or in vitro, frequently will result in a reduction in the magnitude of the physiologic and biochemical effects of the agonist (e.g., inhibition of spinal reflexes; block of stimulated twitch in the guinea pig ileum; increase in K⁺ conductance; and inhibition of adenylate cyclase). This decrement of the effect produced by a given dose of drug with continuous or repeated bolus administration represents drug tolerance.

This phenomenon of spinal tolerance has three characteristics. Its onset is time- and dose-dependent; it appears reversible if the agonist is removed; and it is specific or homologous to the receptor activated by the respective agonists. Thus, animals rendered tolerant to μ-opioid agonists (morphine) in general do not show a comparable loss of response to α₂ or δ agonists, and vice versa. Conversely, it is commonly accepted that spinal drugs acting upon the same receptor, e.g., the μ site, will show cross tolerance.

The mechanism of tolerance is not known, but considerable evidence exists to suggest that the loss of effect secondary to agonist occupancy may be due to desensitization or uncoupling of the receptor from the guanosine triphosphate (GTP)-binding subunit, thereby reducing the affinity for agonist binding, or to down-regulation, in which the receptors are removed from the cell surface, resulting in a loss of binding sites. Either event (reduction in receptor number or the uncoupling of the receptor) has several potential ramifications.

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First, different agonists acting at a given receptor may require occupancy of different numbers of receptors to produce a given physiological effect; i.e., they differ in efficacy (low occupancy requirement equals high efficacy). Thus, the efficacy of the agonist is determined by the fraction of the total receptor population that the agonist must occupy (fractional receptor occupancy [FRO]) to produce the given effect. It can be appreciated that, in a given system, if a low-efficacy agent must occupy a large fraction of receptors to evoke the criterion effect, only a small fraction of receptors can be unoccupied. These are referred to as spare receptors. It also follows that if receptor uncoupling or down-regulation occurs as a function of agonist occupancy, these changes will be more substantial for a low-efficacy agent that requires a large FRO than for a high efficacy agent that needs a low FRO.

Second, based on theoretical considerations, for a given degree of receptor down-regulation or uncoupling, there will be an increase in the concentration of a given agent needed to achieve the FRO necessary to evoke a given effect. This will be manifested by a rightward shift in the agonist dose–response curve and a reduction in slope. This has been shown, for example in smooth muscle bioassays with chronic exposure to an agonist or pretreatment with an irreversible antagonist. Based on similar considerations, for any pair of agonists acting at a given receptor, the degree of shift in the respective dose–response curve—resulting from a given degree of down regulation—will be greater for the agonist having lower efficacy (i.e., having a higher occupancy requirement).

In rats receiving chronic intrathecal (i.t.) infusions for 7 days with equieffective concentrations of morphine, D-Ala\(^4\)-MePhe\(^4\)-Gly-ol\(^5\) enkephalin (DAGO), sufentanil, or alfentanil, the degree of shift in the respective dose–response curves was greater with morphine and least for alfentanil, DAGO, or sufentanil-infused animals. Based on independent studies in which the magnitude of the spinal nerve receptor population of morphine, DAGO, and sufentanil were examined by means of an irreversible antagonist, it was found that sufentanil and DAGO appeared to have a higher intrinsic efficacy than did morphine; e.g., these agents required the occupancy of fewer receptors than did morphine to produce the same degree of antinoceptive effect.

These data, in conjunction with the predicted antinoceptive properties of a system in which there is a reduction of coupled receptors, leads to the hypothesis that there should be an asymmetry in the cross tolerance between agents that act at the same receptor but differ in efficacy. The purpose of these studies was to examine the nature of the cross-tolerance between morphine and sufentanil by means of a chronic i.t. infusion in rats.

Materials and Methods

ANIMALS

This study received prior approval from the University of California–San Diego Animal Care Committee. After catheter implantation, male Sprague-Dawley rats (250–300 g) were housed individually in standard cages at room temperature on a 12-hr light–12-hr dark cycle. Tests were administered during the light cycle. Rats were used for a single experiment and then killed. In brief, each was prepared with an i.t. catheter and subcutaneous pump filled with saline or drug. The animal then received a 7-day infusion, after which it was examined for its response on the hot plate (HP) to a single i.t. injection of a given dose of morphine or sufentanil.

PREPARATION OF THE CATHETER AND IMPLANTATION

To prepare the catheter for i.t. infusion, two pieces of polyethylene tubing—a 16-cm length of PE-10 tubing and a 3-cm length of PE-60—were cut. A piece of styroflex wire was placed through the two pieces of tubing, which then were fused in a hot-air jet, and the styroflex wire was removed. The PE-10 then was doubled back on itself to form a 2-cm-long loop that was held together with a 1-cm length of silastic tubing. This loop was a modification of the Y catheter previously described.\(^24\) The PE-60 extremity of the prefilled catheter was connected to an Alzet osmotic minipump (model 2001; Alza, Palo Alto, CA) filled with 250 \(\mu\)l saline or drug solutions. This pump was designed to deliver a continuous infusion of 1 \(\mu\)l/h for 7 days. During a few hours prior to catheter implantation, the minipump was immersed in a 37°C saline bath, allowing the drug infusion to start. Catheter implantation was done while rats were anesthetized with halothane. The PE-10 stem of the catheter was advanced through an incision in the atlantooccipital membrane caudally, to position its tip at the level of the lumbar enlargement. The minipump, attached to the PE-60 arm, was tunneled subcutaneously caudally and was placed on the thoracolumbar part of the body. The loop portion of the catheter was externalized subcutaneously on the top of the head. The wound then was closed with silk sutures. Animals were fully recovered by 20 min after implantation. Animals showing any evidence of motor impairment after implantation were killed with an overdose of barbiturate.

At the end of the 7-day infusion, the externalized loop was cut to terminate the infusion and to permit the injection of the probe test dose.

DRUGS AND INJECTIONS

Morphine sulfate (Merck, Sharp and Dohme, West Point, PA) and sufentanil citrate (Janssen Pharmaceuti-
cals, Belgium) were used for continuous i.t. infusion and assessment of tolerance. Drugs were dissolved in sterile physiologic saline, and drug doses, calculated as a free base, were expressed in nanomoles per hour for the infusion concentrations or nanomoles per rat for the post-infusion tolerance dose–response curves. The pumps delivered at a constant rate of 1 μl/h, and the infusion continued for 7 days with saline (1 μl/h), morphine (20 nmol/h), or sufentanil (0.6 nmol/h). These concentrations of morphine and sufentanil were chosen on the basis of previous experiments that reliably yielded a near-maximal increase in the HP response latency on day 1 of the infusion.23

After 7 days of continuous drug infusion, an analgesic dose–response curve was constructed to assess the magnitude of tolerance. On the morning of day 7 after implantation, the external loop of the catheter was cut, and 10 μl saline was administered to clear the residual volume of opioid in the i.t. catheter. After 2–3 h (sufentanil or saline) or 3–4 h (morphine or saline) clearance time, the HP latency was measured (baseline), and the probe bolus i.t. injection was made. The probe-dose solutions were prepared by subsequent 0.5 log unit dilution of the sufentanil stock solution (0.6 nmol/μl) or morphine stock solution (20 nmol/μl); the volume of the probe injectate was always 10 μl. The dose to be administered was determined by an up–down method25: if the first dose tested in the first rat resulted in a 60-HP response latency (maximum response) after 15 min, the second rat received a dose that was 0.5 log units lower. Conversely, if the dose did not completely block the HP response, the next rat would receive a dose of 0.5 log units higher, and so on. This paradigm was repeated for all animals in the group, and the peak effects of probe testing were pooled for construction of the dose–response curves and represented four to six rats per probe dose.

**Antinociceptive Testing and Data Analysis**

Effects of i.t. agents were assessed by the HP test. The HP was maintained at 52.5°C, and licking of either hindpaw or a jump in which both hindpaws left the surface of the HP was taken as an end point. A cut-off time of 60 s was imposed to avoid tissue damage. To assess the time course, the animals were tested at 5, 15, 30, 45, and 60 min after either i.t. probe agent, and rats receiving morphine as probe agent underwent two additional tests at 90 and 120 min.

Data were expressed as: 1) maximum percent effect (MPE), and 2) area under the curve (AUC).

The MPE is calculated as follows:

\[
MPE = \frac{\text{postdrug latency} - \text{baseline}}{\text{cut-off latency} - \text{baseline}} \times 100
\]

where postdrug latency is the response measured at the particular time after injection; baseline is the predrug latency; and cut-off is 60 s.

The AUC is calculated by accumulating the effect (MPE) measured at the discrete time intervals using the trapezoidal rule.

Analysis of the dose–response curves and statistics were obtained by pharmacologic software programs of Tallarida and Murray26 and included calculation of the ED₅₀ (95% confidence intervals [CI]) test for relative potency. The tolerance ratio, (the ratio of ED₅₀ in drug-infused animal to ED₅₀ of saline-infused animal) and 95% CIs were calculated. Other comparisons between groups were carried out with a one-way analysis of variance (ANOVA) with a Newman Keul test for multiple comparisons. Differences yielding critical values corresponding to \( P < 0.05 \) were considered statistically significant.

**Results**

**Baseline**

After 7 days of infusion of saline, sufentanil, or morphine, baseline HP response latencies were 17.2 ± 0.8, 17.2 ± 0.8, and 18.2 ± 0.9 s, respectively (\( n = 92 \)). These values were not different from each other or from the response latency of naive, unimplanted rats (18.4 ± 1.1 s, \( n = 20 \)).

**Probe-Drug Effects**

**Saline-infused Animals**

In saline-infused rats, the i.t. injection of sufentanil or morphine reliably resulted in an increase in the HP response latency (fig. 1), the magnitude of which was monotonically related to dose (fig. 2). The MPE and AUC slopes (and their 95% CIs) were not statistically different in each group (\( P > 0.05 \)). The mean slopes were 56.8 (MPE, morphine), 70.0 (MPE, sufentanil) and 57.8 (AUC, morphine), and 41.8 (AUC, sufentanil). Computation of the ED₅₀ values in saline-infused animals revealed, on the basis of peak effect (MPE), that sufentanil is nine times more potent than morphine (fig. 2). Conversely, at doses that were matched for MPE, the duration of action for morphine significantly exceeded that of sufentanil (fig. 1).

**Drug-infused Animals**

Computation of the ED₅₀ values revealed that chronic infusion of morphine or sufentanil resulted in a rightward
Fentanyl in morphine-infused or sufentanil-infused animals was: morphine in morphine-infused > morphine in sufentanil-infused = sufentanil in morphine-infused > sufentanil in sufentanil-infused animals.

The duration of the probe-drug effect was diminished in morphine- and sufentanil-infused animals (fig. 1). Plotting the curves corresponding to the AUC versus probe dose revealed monotonic functions that were reliably

shift in the dose (MPE/AUC)–response curves for the respective agents. Thus, the ED₅₀ for morphine in morphine-infused animals increased from 0.65 to 30.0 nmol (44-fold increase), while for sufentanil in sufentanil-infused animals, the ED₅₀ rose from 0.07 to 0.21 nmol (3-fold increase). As indicated in table 1, the rank ordering of shift (i.e., the tolerance ratios) for morphine and sufentanil in morphee-infused or sufentanil-infused animals was: morphine in morphine-infused > morphine in sufentanil-infused = sufentanil in morphine-infused > sufentanil in sufentanil-infused animals.

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The duration of the probe-drug effect was diminished in morphine- and sufentanil-infused animals (fig. 1). Plotting the curves corresponding to the AUC versus probe dose revealed monotonic functions that were reliably
shifted to the right in drug-infused animals. The magnitude of the shift (i.e., the relative reduction in duration of action) was always greater in the morphine-infused animals.

Discussion

With chronic i.t. infusion, the infusion concentration of drugs can be matched such that the maximum effects are equal and the tissue is continuously exposed to that concentration for prolonged periods of time. This is in contrast to bolus drug administration, in which the exposure to equiactive doses of drugs differing widely in pharmacokinetics and potency results in markedly different AUC values, reflecting different time–concentration exposure curves.

In the current study, chronic exposure of the spinal cord to sufentanil of morphine resulted in a reduction in the duration of drug effect and a rightward monotonic shift in the respective dose–response curves as compared to saline infusion. Of importance in these and previous studies is that in the face of a 7-day exposure to equieffective infusion concentrations of morphine and sufentanil, the degree of shift was greater in morphine- than in sufentanil-infused animals. The potency of morphine and sufentanil in the saline-infused animals and the degree of shift observed with morphine and sufentanil in animals infused with these concentrations of the respective drugs are similar to previously reported values.

The loss of drug effect or tolerance observed in the current studies, could result from any of several possible mechanisms:

1) Behavioral conditioning to the test stimuli. Although chronic pairing of a stimulus (the HP test) with a drug has been hypothesized to account for loss of drug action, this appears unlikely in the current study, since the animals were never tested during the chronic exposure phase.

2) Pharmacokinetics. The failure of the drug to reach its spinal site of action. Chronic i.t. catheterization has been shown to result in local changes in the meninges. While it might be argued that sufentanil, a highly lipid-soluble molecule, might not be influenced by diffusion barriers, the response to morphine, a polar molecule, might be diminished. This appears unlikely: using a similar spinal infusion model, Stevens et al. have shown that animals receiving a chronic infusion with morphine show no loss of response to the hydrophilic α2 agonist ST-91 or the delta-prefering peptide D-Ala2-D-Leu5-enkephalin (DADL), and vice versa.

3) Pharmacodynamics. Investigations of the process underlying the chronic effects of opioids have consistently indicated that continued occupation of a receptor by an agonist may evoke one or both of at least two events: desensitization (the receptor becomes uncoupled from its GTP-binding subunit) or down-regulation (the receptor is removed from the cell surface). Either process yields a reduced number of coupled receptors. This phenomenon of down-regulation has been observed in a variety of in vivo and in vitro models for the mu and delta receptors.

This possible change in receptor number secondary to agonist occupancy gives rise to several testable hypotheses based on the concepts of drug efficacy and fractional receptor occupancy introduced in the beginning of this paper. First, an agent that requires occupancy of a small fraction of the total receptor population may down-regulate fewer receptors than does one that requires a higher level of occupancy (high-ta low-efficacy agonist). Second, for a given degree of receptor down-regulation, it can be shown that the degree of shift in the dose–response curve is greater for a low-efficacy than for a high-efficacy ago-
nistic. In a previous study with the irreversible antagonist β-funaltrexamine, Mjanger and Yaksh observed that for a given degree of receptor inactivation the shift in the i.t. morphine dose–response curves greatly exceeded that observed with sufentanil. This is consistent with the hypothesis that sufentanil is a higher efficacy agonist and requires the occupancy of fewer receptors than morphine to achieve the same effect.

We thus hypothesize that morphine down-regulates or uncouples more receptors than does an equipotent dose of sufentanil. Conversely, for any degree of down-regulation, morphine displays a greater rightward shift in its dose–response curve than does sufentanil. In short, the observations made in the current study are consistent with the two hypotheses predicted by the theoretical position that tolerance in this model reflects a down-regulation or uncoupling of receptors and that sufentanil, relative to morphine, is a high-efficacy agent. The observation that sufentanil displayed a greater shift in the dose–response curve in rats receiving a morphine-infusion than in those receiving a sufentanil infusion is consistent with the observation that chronic exposure to an equianalgesic concentration of morphine uncoupled more receptors than did sufentanil. Conversely, the lesser shift in the morphine dose–response curve in the sufentanil-infused animals is consistent with the possibility that the equipotent infusion concentrations of sufentanil uncoupled fewer receptors than did morphine.

Evidence for this analysis also can be gathered from other models. Thus, examining with a subcutaneous probe dose after a 5-day pellet implant, others have shown a shift for morphine that is greater than that for etorphine or heroin. These investigators concluded that a functional barrier, such as the blood–brain barrier, reduces the accessibility to the central nervous system for hydrophobic agents (morphine) but will not affect lipophilic agents (etorphine or methadone). Though not excluded, this mechanism is not applicable once the agent is located in the proximity of the receptor (as in the i.t. implanted rat). Etorphine, like sufentanil, has been shown in opiate-receptor binding studies to produce analgesia at the mu site with low FRO. We believe these results are consistent with differences in drug efficacy: high-efficacy agents (etorphine and sufentanil), are generally more lipophilic than are low-efficacy agents (morphine).

Evaluating the cross tolerance at the mu site for scheduled-controlled response of food presentation, the rightward shift after 7 weeks administration of morphine was greater for morphine than methadone. In rodents, experiments evaluating discriminative stimulus properties of drugs have shown tolerance for morphine. In contrast, rats trained to detect fentanyl did not display tolerance.

The clinical relevance of this differential magnitude of tolerance development in the acute pain animal model is not clear, although considerable shift in antinociceptive activity also has been reported in rodent arthritic models of chronic pain (but see reference 42). Nevertheless, to the degree that the down-regulation or uncoupling of receptors produced by opioid agonists is part of the tolerance process, then there is a clear role for the use of high-efficacy agonists in chronic drug administration.

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