

Studies of Morphine and D-ala²-D-leu⁵-enkephalin (DADLE) Cross-tolerance after Continuous Intrathecal Infusion in the Rat

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To determine the cross tolerance to the antinociceptive effects of μ and δ opioids in the spinal cord, rats received a 7-day infusion of one of three concentrations each of morphine (2, 6, or 20 nmol/h) or D-ala²-D-leu⁵-enkephalin (DADLE) (2, 6, or 20 nmol/h). A constant-rate (1 μ l/h), constant-dose intrathecal infusion pump was used. On day 7, the magnitude of tolerance was assessed by establishing dose-response curves for the effect of the chronic drug given as an intrathecal bolus. Cross-tolerance was assessed in separate groups of animals with identical infusions by establishing morphine dose-response curves in DADLE-tolerant animals and DADLE dose-response curves in morphine-tolerant animals. Each infused animal was used for a single bolus injection. For morphine and DADLE tolerance, a parallel rightward shift in the dose-response curve was produced with the degree of shift proportional to the log of the infusion dose. Thus, at the infusion rate of 6 nmol/h for either morphine or DADLE, the shift of the tolerance dose-response curves was 55- and 33-fold, respectively. Morphine and DADLE cross-tolerance was also detected as shown by rightward shifts of the cross-tolerance dose-response curves; however, these shifts were relatively minor compared to the shifts seen in the tolerance dose-response curves of animals tested with the same agent as infused. At the infusion dose of 6 nmol/h for either morphine or DADLE, the shift of the cross-tolerance curve for DADLE in morphine-tolerant rats was only 2.7-fold, whereas that of morphine in DADLE-tolerant rats was only 1.3-fold. These data are interpreted as supporting separate sites of spinal antinociceptive action for morphine and DADLE in the rat and emphasize the importance of a dose-dependent induction and assessment of tolerance in studies of tolerance and cross-tolerance. (Key words: Analgesics, intrathecal: cross-tolerance; morphine; tolerance. Brain: DADLE; Enkephalin. Receptors: opioid.)

BASED ON THE PHARMACOLOGY of the endogenous opioid pentapeptides, Lord and associates hypothesized the δ subclass of opioid receptors.¹ This site was distinct from the μ , κ , and σ sites originally proposed by Martin *et al.*² In these studies, the D-amino acid substituted analogue of leu-enkephalin, D-ala²-D-leu⁵-enkephalin (DADLE) was found to be a metabolically stable analogue that displayed a 20-fold δ/μ selectivity in binding studies; a

significant activity in the mouse vas deferens *versus* the guinea pig ileum; and a 10-fold difference in the naloxone pA₂ as compared to morphine.¹ These data were consistent with the existence of distinct μ and δ receptors and with a preferential but not unique effect of DADLE and morphine at the δ and μ sites, respectively.

Subsequent pharmacologic studies revealed that the antinociceptive effects produced by intrathecally administered opioids were mediated in the spinal cord at both μ and δ opioid sites. Thus, agents with high μ affinity, such as sufentanil or D-ala²-met-phe⁵-gly-ol-enkephalin (DAMGO) and the metabolically stable, δ -preferring peptide D-pen^{2,5}-enkephalin (DPDPE) yield potent, dose-dependent effects following spinal administration in a variety of species.³ Systematic examination of competitive δ antagonists reveals that agents such as naltrindole or ICI-174816 will selectively block the spinal effects of the δ , but not the μ agonists.⁴⁻⁶ Secondly, in keeping with the lower affinity of naloxone for δ *versus* μ sites, the pentapeptide DADLE was found to display a lower sensitivity to naloxone antagonism than morphine after spinal administration.⁷ The above observations suggested that while neither morphine nor DADLE is absolutely selective for μ and δ sites, at the concentrations effective to produce a robust analgesia, each was acting at a distinct spinal opioid site.

In addition to selective opioid antagonists and the ability of naloxone to block agonist effect, a third characteristic commonly used to define the sites acted upon by two agents is that of cross-tolerance. The chronic spinal administration of μ (morphine, DAMGO, or sufentanil) or δ (DPDPE or DADLE) opioids will result in a progressive loss of effect that is unrelated to behavioral or pharmacokinetic variables and is receptor-selective.⁸⁻¹² Initial descriptions of cross-tolerance between μ and δ agonists in the spinal cord were derived from chronic systemic morphine treatment followed by the intrathecal administration of DADLE. Thus, in rats or monkeys made tolerant to systemic morphine, no decrement in the antinociceptive effects of spinal δ opioid agonists was observed.¹³⁻¹⁵ In contrast, early reports using an intrathecal catheter and osmotic minipump implanted in rats did demonstrate a partial cross-tolerance between morphine and DADLE,^{16,17} between the μ -specific morphiceptin analogue PLO-17 and DADLE or the highly specific δ analogue DPDPE.⁸ It was noted, however, that these rightward shifts in the δ cross-tolerance dose-response curves

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were not as large as those seen in the μ tolerance dose response curves. More recently, this same group has shown that there is little cross-tolerance seen when a spinal administration stratagem is used whereby μ and δ opioids are sequentially alternated in rats on the tail-flick test.¹⁸

DADLE may be an anomaly, given what appears to be the current opinion that its effects are in large part dependent on its interaction with the μ receptor.¹⁹ The data outlined above clearly suggests that it does not yield a symmetrical cross-tolerance. The absence of systematic examination of the cross-tolerance characteristics of morphine and DADLE over a wide range of effective concentrations, however, prevents any firm conclusion. Such studies are of more than a simple pharmacologic interest, considering that DADLE, aside from β -endorphin,²⁰ is the only peptide that has been systematically used for chronic spinal administration in terminal cancer patients who have displayed an apparent tolerance to morphine.²¹⁻²⁴

In the present study, we sought to determine the degree of cross-tolerance of the antinociceptive effects between morphine and DADLE. We have used a 7-day intrathecal infusion model²⁵ in which the rat spinal cord is exposed to one of several concentrations of either morphine or DADLE. Similar studies have shown that there is complete cross-tolerance between morphine and the μ -preferring peptide dermorphin⁹ and little cross-tolerance between morphine and the α_2 -agonist ST-91.²⁶ In the present studies, the magnitude of the cross-tolerance induced by continuous intrathecal infusions of either morphine and DADLE is subsequently defined by the efficacy of a bolus intrathecal injection of either morphine or DADLE on the hot plate (HP) test.

Materials and Methods

Studies were carried out according to a protocol approved by the Institutional animal care committee of the Mayo Clinic.

ANIMALS

Male Sprague-Dawley rats (250–300 g; Harlan Industries, Indianapolis, IN) were maintained in group cages at room temperature on a 12-h photoperiod (lights on at 7:00 AM). After Y-catheter implantation (see below), rats were housed individually in standard cages. Animals had free access to rat chow and tap water at all times.

PREPARATION OF Y-CATHETER AND IMPLANTATION

Complete procedures for the manufacture and testing of Y-catheters have been fully described previously.²⁵ In brief, three pieces of polyethylene tubing (PE-10, 10 and 6 cm; PE-60, 4 cm) were joined under a hot-air jet and

the junction reinforced with a drop of dental cement. Alzet osmotic minipumps (model 2001, rate = 1 μ l/h; Alza Corporation, Palo Alto, CA) were filled with saline or drug solutions and attached to the likewise prefilled Y-catheter.

The Y-catheter and pump were implanted in the animal according to procedures originally described for chronic catheterization of the rat spinal cord²⁷ with the added modification to accommodate the subcutaneously implanted minipump. In brief, animals were anesthetized with halothane and placed in a stereotaxic head holder, and a midline incision was made to expose the occipital crest. The atlantooccipital membrane was exposed and pierced, and the PE-10 stem of the Y-catheter, trimmed to 8.5 cm, was threaded down intrathecally to the level of the thoracolumbar junction. The PE-10 arm of the catheter was externalized forward (for subsequent administration of the probe drug); the PE-60 arm attached to the minipump and tunneled intrascapularly; and the wound sutured. Animals fully recovered 15–30 min after implantation, and those few showing obvious signs of motor impairment were euthanized.

DRUGS AND INJECTION

The following drugs were used for continuous spinal infusion and assessment of tolerance and cross-tolerance: morphine sulfate (Merck, Sharp and Dohme, West Point, PA), and DADLE (Burroughs Wellcome, Research Triangle Park, NC). Drugs were dissolved in sterile physiologic saline, and drug doses, calculated as the free base, were expressed as nanomoles per hour for the infusion concentrations or nanomoles per rat for the postinfusion tolerance and cross-tolerance dose–response curves.

Animals were normally prepared in groups of 12; 6 randomly selected animals received drug infusions and the remaining 6 received saline. This procedure was repeated until enough animals were obtained for the construction of a full probe dose–response curve. Solutions (saline vehicle or one of three log-spaced doses of morphine: 2, 6, or 20 nmol/h, or DADLE: 2, 6 or 20 nmol/h) were infused at a constant rate of 1 μ l/h for 7 days. These doses were selected on the basis of our previous work with these compounds and were chosen so that the highest infusion concentration reliably yielded a near-maximal increase in the HP response latency 1 day after pump implantation.^{10,26} These previous studies also showed parallel infusion concentration–response curves and the use of the half-log spaced infusion doses resulted in the high, medium, and low infusion doses of each tolerogen that produced about the same degree of antinociceptive effect.

After the acute recovery from the Y-catheter and pump implantation, rats were returned to their individual cages.

For assessment of tolerance or cross-tolerance on the morning of day 7 after implantation, the stylet plug of the external arm of the Y-catheter was removed and 15 μ l saline administered to clear the residual volume of tolerogen in the intrathecal catheter. After a 3–4-h clearance time, the HP latency was measured to obtain baseline values, and a probe bolus intrathecal injection of the tolerogen was made. The dose to be administered was determined by the “up-down” method.^{11,28} In this method, the first rat received a dose of the probe agent. If that dose resulted in a 60-s HP response latency after 10 min, the subsequent rat received a dose that was 0.5 log unit less. Conversely, if the dose did not completely block the HP response, the next rat received a dose 0.5 log unit higher. This paradigm was repeated for all animals in the group, and the peak effect of all animals receiving similar infusion doses and probe doses were pooled for construction of the dose-response curves.

SPINAL MORPHINE CONCENTRATIONS

To determine if levels of morphine in tissue were stable over the 7-day period of drug delivery, rats were prepared with intrathecal catheters and pumps filled with morphine (20 nmol/ μ l). After 24 h (day 1), or on day 7, the rats were anesthetized with halothane and the catheters/pump removed. The rats were then decapitated and the spinal cords (sacral to cervical) were removed by hydraulic extrusion. The cords were then rinsed in cold saline and blotted dry. Cords were then frozen until the assay. Morphine was extracted after homogenizing in a sodium borate buffer. The homogenate was filtered on a CE1003 column. Drug was eluted with aliquots of chloroform:2-propanol. Extracts were dried and redissolved in phosphate buffer:methanol:acetonitrile. Morphine was then determined using high-performance liquid chromatography coupled to electrochemical detection. Assay sensitivity was 0.5 ng/g tissue. These assays were performed by courtesy of Dr. Harlan Hill (Fred Hutchinson Cancer Research Center, Seattle, Washington).

ALGESIOMETRIC TESTING AND DATA ANALYSIS

Agonist effects of intrathecal agents were assessed by the HP test. The metal surface of the enclosed HP was maintained at 52.5° C, and the licking of either hindpaw, or in a few cases extreme agitation or jumping, was taken as the nociceptive endpoint. A cut-off time of 60 s was imposed to avoid tissue damage to the hindpaws. Data are expressed as maximum percent effect (MPE) by the equation:

$$\text{MPE} = \frac{(\text{postdrug latency} - \text{baseline})}{(\text{cut-off latency} - \text{baseline})} \times 100$$

Analysis of the dose-response curves and statistics were obtained by pharmacologic software programs²⁹ and in-

cluded calculation of the ED₅₀ (\pm 95% confidence intervals) and tests for parallelism and relative potency, where ED₅₀ is the dose that produces an MPE = 50%. Tolerance and cross-tolerance ratios were defined as the ED₅₀ of animals treated with tolerogen divided by the ED₅₀ of rats treated with saline. The tolerance ratio analysis was performed by a microcomputer program written from published procedures³⁰ and calculated the standard error of the ED₅₀ ratios and 95% confidence limits. Differences with probability of $P < 0.05$ were considered statistically significant.

Results

As we have previously shown, continuous spinal infusion of morphine (2, 6, or 20 nmol/h) or DADLE (2, 6,

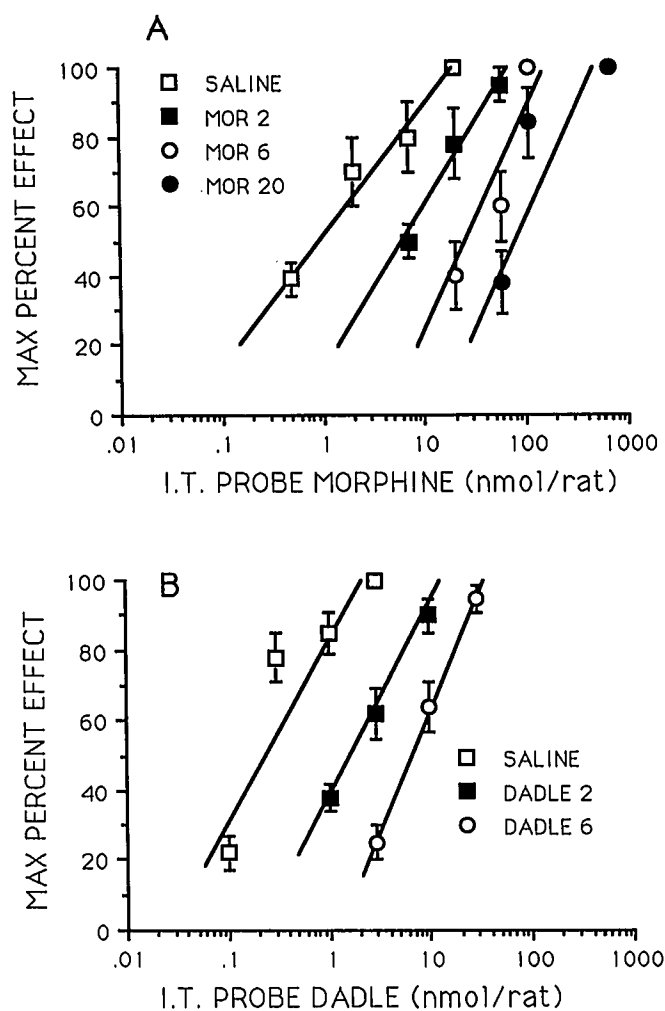


FIG. 1. Tolerance dose-response curves of probe intrathecal morphine or DADLE. Animals treated with continuous infusion for 7 days with either (A) saline or morphine (2, 6, or 20 nmol/h) and probed with intrathecal morphine, or (B) saline or DADLE (2 or 6 nmol/h) and probed with intrathecal DADLE. See table 1 for hot-plate ED₅₀ values.

or 20 nmol/h) produces a dose-dependent increase in HP latency on day 1 that returns to saline control levels by day 4.¹⁰ Thus, at the time of probe drug injection on day 7, animals were tolerant to the antinociceptive effect of all tolerogen doses infused.

DEGREE OF MORPHINE AND DADLE TOLERANCE

The degree of tolerance on HP tests produced by a 1-week spinal infusion of morphine or DADLE (both at 2, 6, or 20 nmol/h) has been previously reported¹¹ and is shown in figure 1 for comparison to cross-tolerance data. Injection of the intrathecal probe doses of morphine in morphine-tolerant rats and rats treated with saline revealed a significant, dose-dependent and rightward shift in the tolerant animals (fig. 1A). These tolerance dose-response curves did not differ statistically in slope ($P > 0.05$), and the degree of shift was proportional to the infusion concentration of morphine (table 1). Thus, as compared to saline controls, tolerance ratios of 4.4, 55.0, and 117.2 were observed for the low, medium, and high infusion doses of morphine.

The continuous intrathecal infusion of saline or DADLE (2 or 6 nmol/h) resulted in dose-dependent, rightward shifts of tolerance dose-response curves of DADLE-tolerant animals given intrathecal probe doses of DADLE (fig. 1B). The ED₅₀ values were 0.2, 1.2, or 6.6 nmol/rat for saline, and the low and medium infusion doses of DADLE, respectively. These shifts of the tolerance dose-response curve were parallel; *i.e.*, they did not differ statistically in slope ($P > 0.05$) with respect to the saline-infused control curves and exhibited tolerance ratios of

6.1 and 33.2. The assessment of DADLE-tolerant animals at the highest infusion concentration (20 nmol/h) was precluded by a reversible motor impairment (waxy flaccidity) observed after the saline flush of the residual tolerogen on day 7.

DEGREE OF MORPHINE AND DADLE CROSS-TOLERANCE

Cross-tolerance dose-response curves for probe DADLE in the separate groups of animals treated with saline or morphine (2, 6, or 20 nmol/h) showed a dose-dependent rightward shift (fig. 2A). The probe DADLE dose-response curves did not differ significantly from parallelism ($P > 0.05$) and gave cross-tolerance ratios of 3.6, 2.7 and 29.4, respectively (table 1).

Animals treated with spinal saline or DADLE (2 or 6 nmol/h) and probed with spinal morphine produced relatively minor shifts in the morphine cross-tolerance dose-response curves (fig. 2B), corresponding to ED₅₀ values of 0.71, 0.80, and 0.94, respectively. ED₅₀ ratios were 1.1 and 1.3 (table 1).

COMPARISON OF MORPHINE AND DADLE TOLERANCE AND CROSS-TOLERANCE

Each infusion dose is plotted against the shift in the probe dose-response curve (ED₅₀ ratio) to compare the degrees of shift produced by the different infusion treatments (fig. 3). Comparing the degrees of shift for each of the treatments, it is clear that for a given infusion con-

TABLE 1. Tolerance and Cross-tolerance ED₅₀ Values from Morphine and DADLE Dose-Response Curves in Continuously Infused Rats

Toleragen	Dose	Probe	Hot-plate ED ₅₀ (95% confidence interval)	ED ₅₀ Ratio
Tolerance studies				
Saline	—	Morphine	0.71 (0.32–1.91)	—
Morphine	2	Morphine	3.10 (0.41–24.20)	4.4
	6	Morphine	39.04 (20.8–72.7)*	55.0
	20	Morphine	83.10 (48.6–142.0)*	117.2
Saline				
DADLE	2	DADLE	0.20 (0.10–0.32)	—
	6	DADLE	1.22 (1.12–3.12)*	6.1
	20	DADLE	6.64 (4.62–9.64)*	33.2
Cross-tolerance studies				
Morphine	2	DADLE	0.71 (0.25–2.06)	3.6
	6	DADLE	0.53 (0.13–2.20)	2.7
	20	DADLE	5.88 (4.03–8.58)*	29.4
DADLE	2	Morphine	0.80 (0.61–1.06)	1.1
	6	Morphine	0.94 (0.13–7.06)	1.3
	20	Morphine	—†	—

DADLE = D-al²-D-leu⁵-enkephalin; ED₅₀ ratio = ED₅₀ of toleragen-treated groups over ED₅₀ of saline treated groups.

* Significant shift of the dose response curve as compared to saline-infused control group ($P < 0.05$).

† ED₅₀ values not obtained due to motor effects at high infusion doses (see text for details).

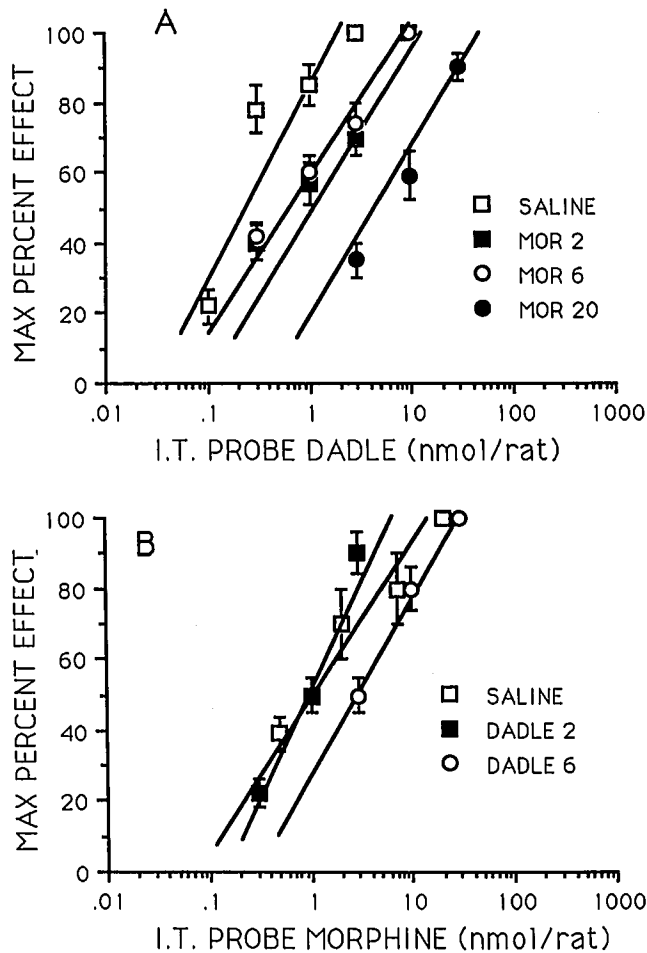


FIG. 2. Cross-tolerance dose-response curves of probe DADLE or morphine. Animals treated with continuous infusion for 7 days with either (A) saline or morphine (2, 6, or 20 nmol/h) and probed with intrathecal DADLE, or (B) saline or DADLE (2 or 6 nmol/h) and probed with intrathecal morphine. See table 1 for hot-plate ED_{50} values.

centration of DADLE, the magnitude of shift for intrathecal probe DADLE was greater than for intrathecal probe morphine, whereas in morphine-infused animals, a greater shift was observed in probe-morphine animals compared to those subsequently administered probe DADLE (fig. 3).

STABILITY OF MORPHINE LEVELS IN SPINAL CORD OVER 7-DAY INFUSION INTERVALS

In spinal cords removed from rats at day 1, the concentrations of morphine in whole cord were observed to be 12 ± 4 ng/mg of tissue (mean and SE for $n = 5$ rats). On day 7, cord concentrations were found to be 16 ± 5 ng/mg ($n = 5$). These differences were not statistically significant ($P > 0.20$).

Discussion

In the present study, animals receiving 7 days of spinal morphine or DADLE infusion showed concentration-dependent shifts in the probe dose-response curves of the respective agonist. This shift in the dose-response curve was not accompanied by a decrease in the maximum achievable effect up to infusion doses at which deleterious side effects were observed, such as notable motor weakness or waxy flaccidity after high doses of DADLE¹⁴ and cutaneous hyperesthesia after high doses of morphine.³¹

TOLERANCE STUDIES

We believe that the mechanism underlying the rightward shift of the spinal tolerance dose-response curves in the present spinal infusion model does not reflect either distributional changes or behavioral conditioning. Thus, continuous spinal infusion, in contrast to systemic tolerance induction by repeated bolus injections or implantation of subcutaneous pellets, avoids the peak and trough or exponentially decreasing concentrations of tolerogen at the receptor during the exposure period. Direct intrathecal administration avoids issues pertinent to peripheral drug distribution and the blood-brain barrier, which may alter the central availability of the tolerogen and permits systematic comparison of drugs varying in ability to cross the blood-brain barrier, e.g., opioid alkaloids versus opioid peptides and peptide analogues. As reported in the present study, for continuously infused spinal morphine, it was found that the levels of infused agent in spinal tissue remains essentially unchanged between day 1 and day 7 of the infusion. This suggests first that steady state is probably reached by 24 h of the initiation of the intrathecal infusion and secondly that the concentrations remain constant for the remainder of the infusion period.

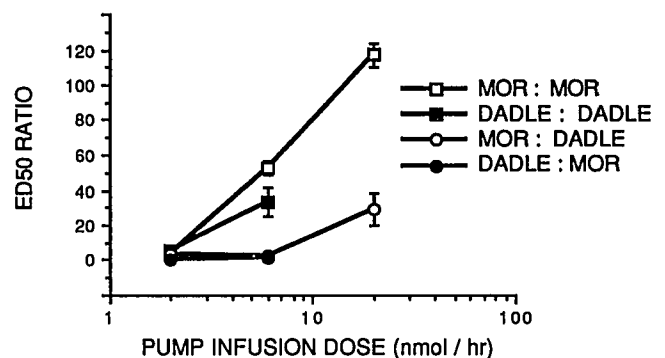


FIG. 3. ED_{50} ratios for morphine and DADLE tolerance and cross-tolerance. Calculated ED_{50} ratios (from table 1) are plotted as a function of the infusion dose (2, 6, or 20 nmol/h) of the respective drug. The ED_{50} ratio is equal to the ED_{50} of the toleragen-infused group divided by the ED_{50} of the saline-infused group. DADLE was not infused at 20 nmol/h due to motor dysfunction (see text).

It thus appears unlikely that the loss of effect at 7 days relates to a failure of drug delivery or a decline in drug-tissue diffusion.

With regard to behavioral conditioning, the changes in response to the spinally administered drugs occurred in the absence of prior exposure to the test condition (the HP) or environmental cues. Importantly, rats treated either with morphine or DADLE received infusion doses that were approximately equieffective as assessed 1 day after pump implantation,¹⁰ so all animals at corresponding infusion levels (*i.e.*, low, medium, or high doses) were exposed to an equianalgesic state for 7 days.

Based on the above considerations, we believe that the prominent dose-dependent shifts in the tolerance dose-response curves observed in morphine-infused animals given probe morphine and DADLE-infused animals given probe DADLE reflect a pharmacodynamic event that occurs secondary to receptor occupation. This may be due to either a down-regulation in the number of receptors or receptor affinity changes, or an alteration in the receptor effector coupling, or both. Insofar as the down-regulation is proportional to receptor occupancy, it follows from the law of mass action that the degree of shift should be proportional to the log of the tolerogen dose—a finding supported by the results in figure 1. The failure to see a reduction in the maximum achievable effects in tolerant animals indicate that the population of receptors necessary to produce a maximum effect, in this case a 60-s HP latency, is relatively small compared to the total available opioid receptors mediating the measured effect. This represents a condition in which morphine and DADLE may be said to possess spare receptors in this particular assay. It follows that the smaller the pool of spare receptors for a given agonist, the greater will be the rightward shift in the tolerance dose-response curve for that drug. Thus, in recent studies we have shown that in the presence of a fixed dose of intrathecal β -funaltrexamine, a noncompetitive μ opioid selective antagonist, the degree of reduction for intrathecal administered μ agonists is: morphine \gg sufentanil = DAMGO.³² In continuous spinal infusion studies as carried out here, the rank order of the degree of rightward shift in the tolerance dose-response curves after 7 days of infusion of the respective agonist was: morphine $>$ DADLE $>$ sufentanil = DAMGO.¹¹ Thus, these data are consistent with morphine being a drug with a relatively small receptor reserve for producing an antinociceptive effect in the rat spinal cord.

Higher infusion concentrations of DADLE could not be used either for the infusion dose or for the probe drug because of the reversible motor dysfunction induced by these concentrations. As we have previously reported, spinal DADLE given as a one-time bolus injection in doses exceeding 50 nmol (approximately 30 μ g) resulted in blockade of the hind limb placing/stepping reflex.¹⁴ Nev-

ertheless, with infusion of DADLE at concentrations that produce antinociceptive effects comparable to those of the intermediate concentrations of morphine,¹¹ an approximately equal shift in the probe DADLE dose-response curve to that observed with the intermediate infusion concentration of morphine was observed. Because approximately equieffective infusion doses were used, we interpret the DADLE tolerance dose-response curves as suggesting that, like morphine, spinally administered DADLE has a relatively small spare receptor reserve.

CROSS-TOLERANCE

While chronic morphine or DADLE infusion resulted in a rightward shift in their respective probe dose-response curve that was proportional to the log of the infusion concentration, this was not the case for probe DADLE in morphine-tolerant animals or *vice versa*. Thus, at the intermediate infusion concentrations of morphine and DADLE yielding a 55- and 33-fold increase in the ED₅₀ of morphine and DADLE, respectively, the comparable shifts for morphine in a DADLE-tolerant animal was 1.3-fold and for DADLE in a morphine-tolerant animal was 2.7-fold. At the highest morphine dose, which resulted in a 110-fold shift in the probe morphine ED₅₀, probe DADLE displayed only a 29.4-fold increase in the resulting ED₅₀. Whether a higher dose of DADLE would have resulted in a parallel increase in probe morphine ED₅₀ could not be assessed. These results are in partial agreement with previous reports by Russell *et al.*⁸, who found that in rats treated spinally with an infusion of DADLE and a μ opioid agonist, PLO17, equal degrees of shift in the dose-response curves for PLO17 and DADLE in PLO17 were observed. In contrast, animals treated with DADLE showed a significantly greater shift in the probe DADLE curves than in the PLO17 probe dose-response curves. Earlier studies by Tseng^{16,17} using cumulative dose-response curves (*i.e.*, each animal receiving multiple sequential doses) in animals infused spinally with morphine and DADLE demonstrated a significant shift after probe morphine subsequent to a single infusion concentration of DADLE-tolerant animals, but not the reverse. All that can be said at this time is that different times of infusion and different paradigms with different agonists have been used. Moreover, both of these earlier studies examined a spinal nociceptive reflex (tail flick), whereas the present experiments used the supraspinally mediated HP test. Still, we believe that the present failure to see a shift in the respective dose-response curves at multiple concentrations that do not produce motor dysfunction in saline-injected animals provides very strong evidence that, under these conditions, a significant amount of cross-tolerance was not observed at doses that were pharmacologically effective. Moreover, these results are in accord with the

lack of significant cross-tolerance seen in μ - and δ -tolerant rats maintained on a schedule of alternated spinal infusion.¹⁸

This observed lack of cross-tolerance at pharmacologically effective doses under the conditions of the present study is consistent with the hypothesis that the two drugs are exerting their antinociceptive effect, at the doses used by their respective action on the μ and δ receptors. The affinity of DADLE for a naloxone-binding site and the evidence that DADLE has efficacy at a μ site as defined by antagonist experiments^{33,34} has led to the conclusion that DADLE will invariably exert significant effects as a μ agonist. We note, however, that this need not necessarily occur. Activity at a receptor depends upon affinity as well as intrinsic activity (efficacy) of the agonist at that site. Ward and colleagues noted that treatment of the guinea pig ileum with the highly μ -selective β -FNA resulted in a 10-fold shift in the dose-response curves for opioid alkaloids, such as morphine, and a 30-fold shift for DADLE.³³ This observation is consistent with the possibility that if DADLE has an action at a μ opioid receptor, it has a higher occupancy requirement, *i.e.*, a lower intrinsic activity, and a smaller receptor reserve than morphine at the receptor population upon which morphine acts.

Clinically, the present results suggest that DADLE would produce effective spinal analgesia in patients tolerant to morphine. Although systematic studies have not been accomplished, the analgesic activity of intrathecal DADLE in humans has been confirmed.²¹⁻²⁴ Our data suggest that spinal morphine would be effective in a patient rendered tolerant to DADLE.

We thus conclude that exposure of the spinal cord to morphine and DADLE at pharmacologically active concentrations for 7 days results in rightward shifts in their respective dose-response curves with little evidence of cross-tolerance. These data, in conjunction with the corollary pharmacology, argue that these two drugs act upon two fundamentally distinct spinal sites to yield analgesia.

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