DIFFERENTIATION OF SPINAL AND SUPRASPINAL OPIOID RECEPTORS BY MORPHINE TOLERANCE*

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Summary

Mice treated for 72hrs with morphine (subcutaneously implanted pellets) were tested with a variety of opioid receptor agonists to examine the development of tolerance and cross-tolerance to their analgesic action. The development of spinal and supraspinal tolerance following morphine treatment was evaluated by administering compounds systemically (sc), intrathecally (IT) and intracerebroventricularly (ICV). Following morphine treatment, tolerance to morphine analgesia was observed following IT, ICV and sc administration. Chronic morphine treatment also produced cross-tolerance to the analgesic effects of the selective δ opioid receptor agonist [D-Pen², D-Pen⁵]enkephalin (DPDPE) following IT and ICV administration. However, morphine treatment selectively produced cross-tolerance to ICV [D-Ala², NMePhe⁴, Gly-ol⁵]enkephalin (DAGO) (μ receptor agonist) analgesia, without altering IT DAGO analgesia. These results suggest that brain and spinal cord receptors mediating the effects of DAGO differ in terms of the development of cross-tolerance to morphine; and suggest that tolerance to systemic morphine may be due to changes in spinal δ and brain μ and δ receptor mechanisms.

Opioid agonists can produce analgesic effects when administered both spinally and supraspinally (e.g., 1,2,3,4, 5,6,7,8,9,10). However, studies indicate the different nature of the opioid receptors that mediate analgesic effects at the spinal and supraspinal level (e.g., 2,3,11). For example, supraspinal analgesia following the ICV administration of morphine or the μ selective agonist DAGO is blocked by pre-treatment with the long-lasting μ receptor antagonist naloxonazine; while spinal analgesia following DAGO and morphine is not altered (12). Furthermore, using an acute cross-tolerance paradigm, morphine pre-treatment produces cross-tolerance to the analgesic effect of ICV DAGO and IT DPDPE (δ selective agonist); however, cross-

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tolerance to ICV DPDPE was not observed (3).

In an effort to determine changes in opioid receptor types, we have examined the extent of analgesic tolerance and cross-tolerance to morphine and μ and δ selective peptides following chronic (72hr) morphine treatment in mice. The development of both spinal and supraspinal tolerance has been studied in full dose-response experiments.

**Methods**

**Procedure.** Male, Swiss-Webster mice (22-24g, Taconic Farms, Germantown, NY) were employed in all experiments. At least 24hr following arrival, mice were anesthetized and implanted with a single 75mg morphine or placebo pellet at the nape of the neck. The pellets were not removed after implantation. Seventy-two hrs following implantation, mice were weighed and a baseline nociceptive response (tailflick) was determined. The tailflick apparatus was adjusted so that a focused beam of light applied to the dorsal surface of the tail produced baseline tailflicks typically between 2-3sec. Animals that did not respond within 5sec during baseline testing were discarded from the experiment.

Following baseline testing, mice were injected sc; or briefly (approximately 2min) anesthetized and then injected into the spinal lumbar intrathecal (IT) space (1μl) or in the lateral ventricle (ICV) (5μl). IT injections were made by the method of Hylde and Wilcox (13). ICV drug administration was by a modification of a previously described method (9). All pellet implantations, IT and ICV drug injections were made while mice were lightly anesthetized with halothane (96:4 Oxygen:Halothane). Mice were tested for analgesia (tailflick) 30min following morphine administration and 15min following peptides (see below). If an animal did not flick by 10sec, the test was terminated and the mouse was defined as analgesic. All testing was conducted by someone who was not informed as to the dose injected or the pre-treatment of the animals.

**Drugs.** Morphine pellets (75mg morphine base) and placebo pellets were obtained from the Research Technology Branch of the National Institute on Drug Abuse. Each pellet was wrapped in nylon mesh prior to implantation. Morphine sulfate was obtained from Penick Laboratories (Newark, NJ) and was dissolved in 0.9% saline for sc, IT and ICV administration. All doses are expressed as the base. DPDPE and DAGO were purchased from Penninsula Laboratories and were dissolved in 0.9% saline.

**Data Analysis.** Quantal dose-response data were analyzed using a computerized (BLISS 21) Probit Analysis (14) program, which estimates ED50s, relative potencies and 95% confidence limits. Statistical significance was evaluated using Probit Analysis results. Dose-response data are plotted on log-probit axes with the fitted regression line. Since doses that produce 0% or 100% response cannot be plotted on probit coordinates, the response estimated by Probit analysis was plotted. Estimated data points are indicated in the figures by an asterisk and the actual response is disclosed in the figure legend.
Results

In placebo-treated mice, analgesic potency following spinal administration was equal to or greater than potency following supraspinal administration (Table I). Chronic morphine treatment produced tolerance to the analgesic effect of morphine (Table I; Figure 1), regardless of the route of administration. Chronic morphine also produced cross-tolerance to DPDPE administered IT and ICV (Table I; Figure 2). However, morphine treatment produced site-selective cross-tolerance to DAGO. The analgesic dose-response function for ICV DAGO was significantly shifted to the right by more than 2.5-fold, while there was no significant decrease in the potency of IT DAGO (Table I, Figure 3).

Discussion

Chronic morphine treatment produced tolerance to morphine administered by the subcutaneous, IT and ICV route. The development of tolerance to morphine following chronic morphine

![Graphs showing analgesic effects](image-url)
FIG. 2
The effect of 72hr morphine treatment on IT (0.5-4.0 µg/mouse, N=6-9/dose/group) and ICV (2.0-10.0 µg/mouse, N=5-12/dose/group) administered DPDPE-induced analgesia. For IT and ICV data points with asterisks, actual response was 100% and 0%, respectively (see methods).

FIG. 3
The effect of 72hr morphine treatment on IT (2-20 ng/mouse, N= 5-29/dose/group) and ICV (5-40 ng/mouse, N=5-8/dose/group) administered DAGO-induced analgesia. Data are plotted on log-probit axes. For ICV data points with asterisks, the actual response was 0% (see methods).
was anticipated, although other investigators have reported that such treatment in the mouse produces tolerance only to systemically administered morphine and not to spinally and supraspinally administered morphine when the morphine pellet is left in place during testing (15,16,17). However, our results in the mouse are consistent with those of Porreca et al. (3) and Vaught and Barrett (18) using an acute tolerance protocol, as well as demonstrations of tolerance to spinal and brain injections of morphine following chronic morphine in the rat (e.g.,19,20,21).

Mice treated chronically with morphine were cross-tolerant to the analgesic effect of ICV DAGO and ICV DPDPE. Since these peptides are selective for μ and δ opioid receptors, respectively, these findings suggest that brain μ and δ receptor

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Analgesic ED₅₀ Estimates for Placebo and 72hr Morphine Treated Mice</th>
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</thead>
<tbody>
<tr>
<td>Agonist and Route of Administration</td>
<td>ED₅₀</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
</tr>
<tr>
<td>Morphine SC (mg/kg)</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>(2.0-2.8)</td>
</tr>
<tr>
<td>Morphine (μg/mouse) IT#</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>(0.3-0.9)</td>
</tr>
<tr>
<td>ICV</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>(0.5-1.3)</td>
</tr>
<tr>
<td>DPDPE (μg/mouse) IT</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>(0.1-1.3)</td>
</tr>
<tr>
<td>ICV</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>(1.0-5.6)</td>
</tr>
<tr>
<td>DAGO (ng/mouse) IT</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>(2.0-6.2)</td>
</tr>
<tr>
<td>ICV</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>(8.7-33.0)</td>
</tr>
</tbody>
</table>

Mice were implanted with a single 75mg morphine or placebo pellet. Seventy-two hrs following implantation, mice were injected subcutaneously (SC), intrathecally (IT) or intracerebroventricularly (ICV) and then tested for analgesia (tailflick) at the time of peak effect. ED₅₀s and 95% confidence limits were estimated by computerized Probit Analysis (Bliss 21). * significantly different from placebo (p<.05). # IT morphine data are from Yoburn et al. (29). † Ratio is ED₅₀ for morphine-treated divided by ED₅₀ for placebo-treated. See Fig. 1, 2 and 3 for N's and doses.
mechanisms undergo changes during chronic morphine treatment. The cross-tolerance between systemic morphine and ICV DAGO as well as IT DPDPE is in agreement with the findings of Porreca et al. (3). However, Porreca et al., (3) did not find cross-tolerance between morphine and ICV DPDPE. A possible explanation of this discrepancy is the use of a chronic morphine treatment in the present experiment as compared to an acute morphine treatment in the previous report. Although morphine has greatest affinity for the μ receptor and has been correctly characterized as principally a μ opioid receptor ligand, it does display affinity and activity at the δ receptor, (e.g., 22,23,24,25). Chronic, but not acute, treatment with morphine may be sufficient to produce tolerance at δ receptors as a result of sustained binding of morphine at δ receptors.

Unlike DPDPE, chronic morphine produced selective cross-tolerance to ICV DAGO. Our finding of cross-tolerance following chronic morphine for ICV DAGO agrees with the results of Porreca et al. (3) using the acute cross-tolerance paradigm. Similarly, Vaught and Barrett (18) demonstrated that chronic and acute morphine treatment produce cross-tolerance to ICV administration of the μ receptor preferring agonists [D-Ala², Pro⁵]-enkephalinamide and morphiceptin. The lack of cross-tolerance to IT DAGO following morphine suggests that the receptors mediating analgesia in the cord and in the brain are quite different. This suggestion is supported by previous studies that have shown that naloxonazine antagonized ICV DAGO analgesia, but not IT DAGO analgesia (12). The naloxonazine results (12) and our current differential cross-tolerance results in the cord and brain with DAGO are in agreement with recent studies (28).

Based upon the results in these studies, the possible spinal and supraspinal mechanisms that are involved in morphine analgesia may be suggested. Since IT morphine and IT DPDPE analgesia display tolerance, it may be inferred that IT morphine tolerance is based upon tolerance at δ (DPDPE) receptors. The residual analgesic action of IT morphine in morphine treated mice may be accounted for by action at non-tolerant μ (DAGO) sites and diminished analgesic activity at tolerant δ sites. In the brain, however, ICV morphine tolerance may be based upon diminished activity at both δ and μ receptors with each supraspinal receptor type contributing some portion of the tolerance; or ICV morphine tolerance may be due to tolerance at either the DAGO or DPDPE site independently. Alternatively, tolerance to ICV morphine may be related to a possible single high affinity receptor for DAGO and DPDPE; namely the μ₁ site postulated by Pasternak and colleagues (26). On the basis of the data in the present experiment it is difficult to assign the relative contribution of DAGO and DPDPE sites to ICV morphine tolerance since all three agonists produce comparable ICV tolerance (approximately 2.5-fold reduction in potency, see Table I). By analogy, tolerance to systemically administered morphine which acts both spinally and supraspinally (6,7,27), may be due to tolerance at spinal δ receptors as well as tolerance at supraspinal δ and/or μ sites. Regardless of the mechanism of morphine tolerance, it remains clear that spinal μ (DAGO) receptors are functionally distinct from supraspinal μ receptors, since brain sites, but not spinal sites, display cross-tolerance.
Acknowledgments

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References

27. S. IRWIN, R.W. HOUDE, D.R. BENNET, L.C. HENDERSHOT and M.H.