

Analgesic tolerance to microinjection of the μ -opioid agonist DAMGO into the ventrolateral periaqueductal gray

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Abstract

Repeated administration of the relatively low-efficacy μ -opioid receptor agonist morphine induces tolerance to its antinociceptive effects. High-efficacy agonists such as D-Ala²NMePhe⁴,Gly-ol⁵ (DAMGO) have been shown to be less effective at producing tolerance, suggesting that different neural mechanisms underlie tolerance to these agonists. However, the correlation between agonist efficacy and tolerance development has not been examined within the ventrolateral periaqueductal gray (vPAG), a brain area known to be crucial for the development of morphine tolerance. The current studies examined whether tolerance to DAMGO occurs within the vPAG, and whether repeated treatment with DAMGO into the vPAG alters the development of morphine tolerance. The results showed that repeated vPAG microinjections of DAMGO induced robust tolerance and cross-tolerance to morphine. Further, co-administration of a low dose of DAMGO with morphine potentiated morphine tolerance. These findings indicate that similar mechanisms underlie tolerance to morphine and DAMGO within the vPAG.

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Chronic administration of opioids such as morphine results in tolerance to their antinociceptive effects. The ability of these drugs to induce tolerance may be related to their intrinsic efficacy at the μ -opioid receptor. Studies using continuously administered opioids either systemically or into the spinal cord have observed that low-efficacy agonists such as morphine are more effective in inducing tolerance than high-efficacy agonists, including fentanyl, sufentanil, D-Ala²,NMePhe⁴,Gly-ol⁵-enkephalin (DAMGO), and etorphine (Duttaroy and Yoburn, 1995; Paronis and Holtzman, 1992; Sosnowski and Yaksh, 1990; Stevens and Yaksh, 1989; Tiano et al., 1998; Walker and Young, 2001). Furthermore, intrathecal co-administration of DAMGO with morphine blocked the development of morphine tolerance (He et al., 2002), suggesting that DAMGO protects against the development of morphine tolerance. However, other studies have shown substantial tolerance to high-

efficacy μ -opioid receptor agonists. Repeated intracerebroventricular (i.c.v.) injection of DAMGO produced pronounced tolerance (Mattia et al., 1991), and substantial cross-tolerance to morphine (Tseng et al., 1993). These findings suggest that there may be similar mechanisms underlying tolerance to morphine and DAMGO in the brain.

Studies in our laboratory and others have found that the ventrolateral periaqueductal gray (vPAG) is crucial for the development of antinociceptive tolerance to morphine (Jacquet and Lajtha, 1974; Lane et al., 2005; Morgan et al., 2006a; Tortorici et al., 1999). Microinjection of morphine or DAMGO into the vPAG produces antinociception (Fang et al., 1989; Jacquet and Lajtha, 1974), and repeated intra-vPAG administration of morphine produces tolerance (Morgan et al., 2006b; Tortorici et al., 1999). However, it is unknown whether repeated intra-vPAG DAMGO administration results in tolerance. The current studies examined whether repeated injections of DAMGO into the vPAG results in tolerance, as well as cross-tolerance to subsequent morphine injections. If the mechanisms underlying opioid tolerance to morphine and DAMGO are similar in the vPAG, tolerance to DAMGO and cross-tolerance to morphine

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should be observed. Moreover, repeated co-administration of DAMGO and morphine into the vPAG should facilitate the development of antinociceptive tolerance to morphine. In contrast, if opioids act on vPAG neurons in a manner consistent with spinal actions, then a high-efficacy μ -opioid agonist like DAMGO should be resistant to tolerance and possibly attenuate morphine tolerance. To test these hypotheses, DAMGO and morphine were microinjected into the vPAG and the development of antinociceptive tolerance was measured.

1. Methods

1.1. Subjects/surgery

Male Sprague–Dawley rats (230–375 g) were anesthetized with equithesin (3 ml/kg, i.p.) and implanted with a guide cannula (23 gauge; 9 mm long) aimed at the right vPAG using stereotaxic techniques. The cannula was affixed to the skull using two screws and dental cement. A stainless steel stylet was placed into the cannula to prevent occlusion. Rats were housed individually after surgery and allowed to recover for one week prior to testing. During this recovery period, rats were handled daily. Food and water were available ad libitum except during testing. Experiments were conducted in accordance with the animal care and use guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain, and were approved by the Animal Care and Use Committee of Washington State University.

1.2. Procedures

Drugs were injected directly into the vPAG using a 31 gauge stainless steel injection cannula that extended 2 mm beyond the tip of the guide cannula. One day prior to testing, a microinjector was inserted into the guide cannula but no drug was injected. This sham injection habituated rats to the microinjection procedure and reduced the effects of mechanical stimulation of neurons during subsequent microinjections. Morphine sulfate was a gift from the National Institute of Drug Abuse and diluted in saline for injection. DAMGO was obtained from Sigma (St. Louis, MO) and diluted in saline. Drugs were injected in a volume of 0.4 μ l over a period of 40 s using a 1 μ l syringe. The microinjector remained in place for an additional 20 s to minimize backflow of the drug into the guide cannula. The tolerance induction procedure consisted of twice daily injections (0930 h and 1600 h) for two consecutive days (Trials 1–4). Rats were returned to their cages immediately after each injection. On the third day (Trial 5), cumulative dose–response curves were generated as described below.

Nociception was assessed using the hot plate test on Trials 1 and 5. This test measures the latency to lick the hindpaw or attempt to jump out of the apparatus, when the rat is placed on a 52.5 °C surface. The rat was removed if it did not respond within 40 s. Testing was conducted during the dark phase of a 12:12 h reverse light/dark cycle (lights off at 0700 h) in a dimly illuminated room.

1.3. Experiment 1: tolerance to DAMGO

This study examined whether tolerance would develop to intra-vPAG injections of DAMGO. Rats were microinjected with either saline or 500 ng DAMGO into the vPAG on Trials 1–4. This dose of DAMGO was chosen from preliminary studies in our laboratory based on its ability to produce maximal antinociception within 15–25 min (unpublished data). On the third day (Trial 5), both groups received DAMGO microinjections of 0.03, 0.07, 0.2, and 0.7 μ g/0.4 μ l into the vPAG, resulting in cumulative half log doses (0.03, 0.1, 0.3, and 1 μ g/0.4 μ l). Injections were spaced 20 min apart, and nociception was assessed at baseline and 15 min after each injection. The doses and interdose intervals used in this cumulative dosing procedure were adapted from previous studies (Duttaroy et al., 1997; Kalant et al., 1971; Morgan et al., 2006a,b; Wenger, 1980).

1.4. Experiment 2: cross-tolerance to morphine in DAMGO-pretreated rats

If high-efficacy and low-efficacy μ -opioid receptor agonists differ in ability to induce tolerance, then rats pretreated with DAMGO should not show cross-tolerance to morphine. This hypothesis was tested by assessing cross-tolerance from repeated vPAG injections of DAMGO to vPAG injection of morphine. As in Experiment 1, rats were microinjected with either saline or 500 ng DAMGO into the vPAG on Trials 1–4. The magnitude of morphine tolerance was assessed the following day (Trial 5) by microinjection of morphine (cumulative quarter log doses of 1, 1.8, 3.2, 5.6, and 10 μ g/0.4 μ l) into the vPAG. Rats were injected every 20 min and tested on the hotplate at baseline and 15 min after each injection.

1.5. Experiment 3: effects of DAMGO on the development of morphine tolerance

Co-administration of DAMGO and morphine into the spinal cord has been shown to prevent the development of morphine tolerance (He et al., 2002). The objective of this experiment was to determine whether a similar effect occurs with morphine and DAMGO microinjections into the vPAG. To this end, rats were given intra-vPAG injections of morphine (5 μ g) or a combination of morphine (5 μ g) and DAMGO (70 ng) twice daily for 2 days (Trials 1–4). The 70 ng dose of DAMGO used in this experiment was the half-maximal dose determined from preliminary experiments. The 5 μ g morphine dose was also chosen from previous experiments demonstrating maximal antinociception within 20 min and significant tolerance upon repeated administration (Morgan et al., 2006a; Tortorici et al., 1999). The magnitude of morphine tolerance was assessed the following day (Trial 5) as described for Experiment 2. Saline pretreated rats from Experiment 2 were used as the control to measure the magnitude of morphine tolerance.

1.6. Histology

Upon completion of the experiment, 0.3 μ l cresyl violet was microinjected to mark the injection site. Rats were killed, and brains were removed and stored in 10% formalin. The vPAG was sectioned coronally, and the injection sites were plotted (Paxinos and Watson, 2005) using light microscopy.

1.7. Statistics

D_{50} values, defined as the dose that results in a half-maximal response in a graded dose–effect relationship (Tallarida, 2000), and their confidence intervals were calculated for all groups using nonlinear regression (GraphPad Prism 4, San Diego). Differences in morphine and DAMGO potency (D_{50} values) were evaluated using one-way analysis of variance (ANOVA). Data are presented in the figures as mean \pm standard error of the mean (SEM).

2. Results

The locations of microinjectors for all three experiments are presented in Fig. 1. Only rats with microinjector placements within or immediately adjacent to the vPAG were included in data analyses. Each treatment group contained 6–9 rats.

2.1. Experiment 1: tolerance to DAMGO

Microinjection of 500 ng DAMGO on Trial 1 caused an increase in hot plate latency (38.6 ± 1.4 s) compared to microinjection of saline (17.8 ± 3.7 s). On Trial 5, increasing cumulative doses of DAMGO caused a dose-dependent increase in

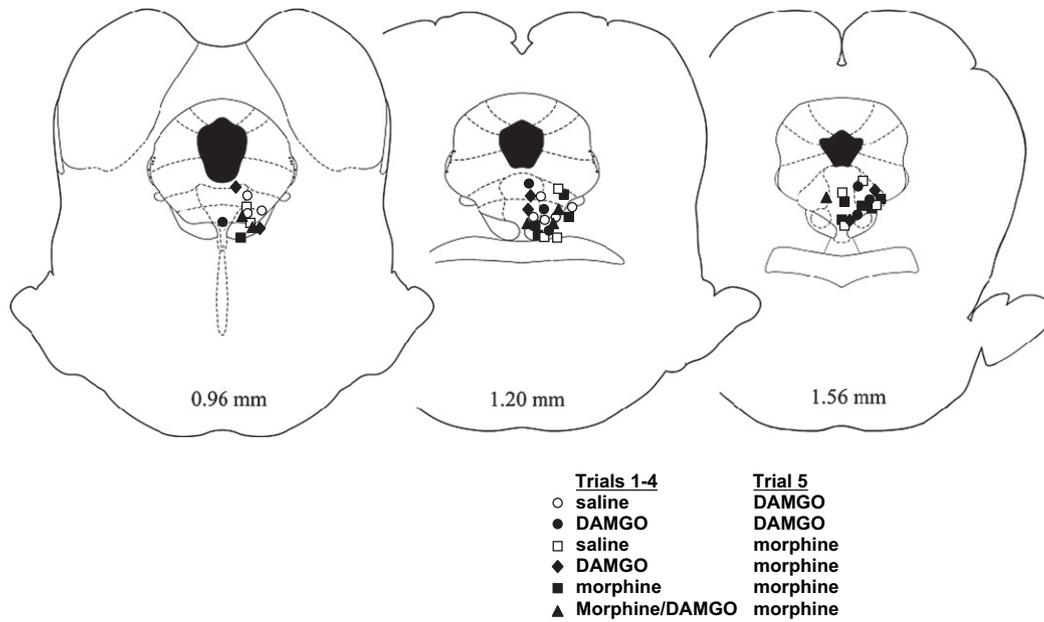


Fig. 1. Location of microinjectors for all experiments. Only rats with microinjector placements within or immediately adjacent to the vPAG were included in data analyses. Placements are relative to the interaural line (Paxinos and Watson, 2005). Symbols refer to drug treatments on Trials 1–4 and Trial 5, which are indicated in the legend. No difference in the location of the microinjections sites between groups was evident.

hot-plate latency (Fig. 2). DAMGO potency was greatly reduced in rats pretreated with DAMGO ($D_{50} = 190$ ng; 95% CI 110–270 ng) compared to saline pretreated controls [$D_{50} = 70$ ng; 95% CI 40–90 ng; $F(1,71) = 5.3, p < 0.05$]. This rightward shift in the DAMGO dose–response curve demonstrates tolerance to repeated vPAG DAMGO microinjections.

2.2. Experiment 2: cross-tolerance to morphine in DAMGO-pretreated rats

As in Experiment 1, microinjection of 500 ng DAMGO on Trial 1 caused an increase in hot plate latency (36.0 ± 3.1 s) compared to microinjection of saline (20.3 ± 1.9 s). Repeated

intra-vPAG administration of DAMGO resulted in cross-tolerance to subsequent intra-vPAG morphine administration on Trial 5 (Fig. 3), as indicated by a shift in the morphine D_{50} from 1.5 μ g (95% CI 1.2–1.8 μ g) in saline pretreated rats to 6.6 μ g (95% CI 4.4–8.9 μ g) in DAMGO pretreated rats [$F(1,85) = 29.4, p < 0.01$]. These data indicate that repeated intra-vPAG injections of DAMGO result in cross-tolerance to morphine.

2.3. Experiment 3: effects of DAMGO on the development of morphine tolerance

Microinjection of morphine (5 μ g) into the vPAG on Trial 1 caused an increase in hot plate latency (39.2 ± 0.8 s)

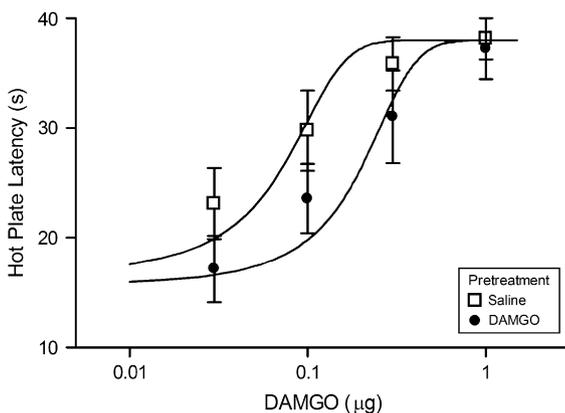


Fig. 2. Antinociceptive tolerance to intra-vPAG injections of DAMGO. Rats were microinjected with 500 ng DAMGO or saline into the vPAG on Trials 1–4. The figure depicts the responses to DAMGO, which was administered in a cumulative dosing procedure to both groups on Trial 5. Tolerance is indicated by a rightward shift of the DAMGO dose–response curve in DAMGO-pretreated rats, compared to saline pretreated rats.

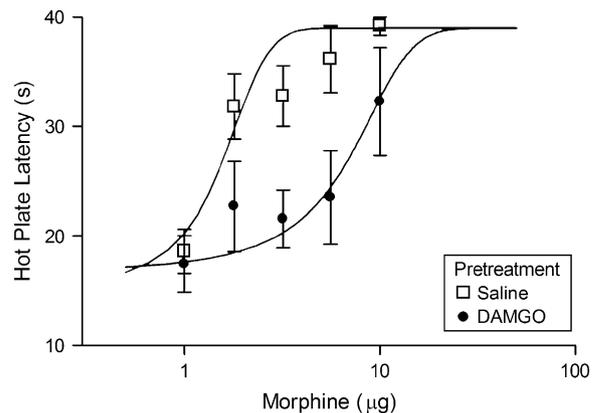


Fig. 3. Cross-tolerance to morphine in rats pretreated with vPAG injections of DAMGO (500 ng). Rats were microinjected with 500 ng DAMGO or saline into the vPAG on Trials 1–4. The figure depicts the response to morphine, which was administered in a cumulative dosing procedure to both groups on Trial 5. Cross-tolerance is indicated by a rightward shift of the morphine dose–response curve in DAMGO-pretreated rats.

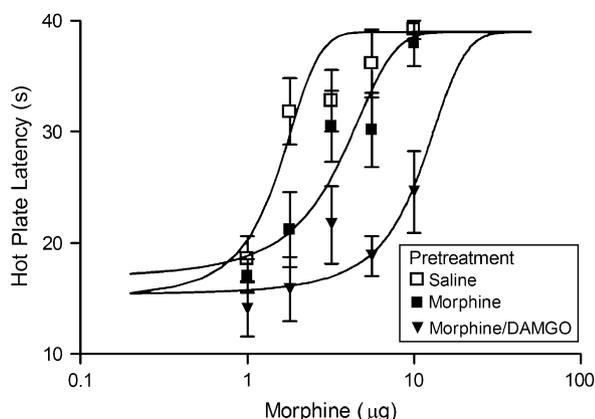


Fig. 4. Coadministration of DAMGO (70 ng) and morphine enhances the development of antinociceptive tolerance to subsequent morphine administration. Rats were microinjected with morphine (5 µg) alone, in combination with DAMGO (70 ng), or with saline into the vPAG on Trials 1–4. The figure depicts the responses to morphine, which was administered in a cumulative dosing procedure to all groups on Trial 5. Facilitation of morphine tolerance is indicated by the rightward shift of the morphine dose–response curve in morphine/DAMGO pretreated rats, compared to rats pretreated with morphine alone.

compared to saline microinjection (20.3 ± 1.9 s). Co-administration of a low dose of DAMGO (70 ng) and morphine produced a similar increase in hot plate latency (34.0 ± 3.9 s). Pretreatment with morphine resulted in tolerance to morphine microinjections (Fig. 4), as indicated by an increase in the morphine D_{50} from 1.5 µg (95% CI 1.2–1.8 µg) in saline pretreated rats to 3.4 µg (95% CI 2.3–4.5 µg) in morphine pretreated rats. The D_{50} was increased further to 10.9 µg (95% CI 8.4–13.3 µg) in rats pretreated with DAMGO and morphine [$F(2,119) = 34.2$, $p < 0.01$]. This finding indicates that DAMGO enhanced, rather than blocked, tolerance to intra-vPAG morphine administration.

3. Discussion

These experiments demonstrate that tolerance develops to the antinociceptive effect of repeated vPAG DAMGO microinjections, cross-tolerance develops to morphine after repeated vPAG DAMGO injections, and combined vPAG microinjection of morphine and DAMGO produced the greatest tolerance to subsequent morphine microinjection. These results support the existence of a common mechanism for morphine and DAMGO-induced tolerance within the vPAG.

In contrast, studies using systemic and spinal opioid treatments have suggested that tolerance induced by low-efficacy and high-efficacy agonists of the μ -opioid receptor are subserved by different mechanisms (He et al., 2002; Roerig et al., 1985; Tseng et al., 1993). The incongruence of our findings with those using systemic or spinal cord treatments may be caused by differences in tolerance mechanisms within distinct regions of the central nervous system (Porreca et al., 1987). The development of tolerance to systemic administration of opioids is complex, and usually involves synergism between multiple sites within the nervous system (Fairbanks and

Wilcox, 1999; Kolesnikov et al., 1996; Rossi et al., 1993; Siuciak and Advokat, 1989).

The differential antinociceptive profiles of supraspinal versus spinally administered drugs may be due to the presence of different opioid receptor subtypes. The opioid antagonist naloxonazine has allowed the distinction of two opioid binding sites, μ_1 and μ_2 (Pasternak, 2001). There may be a difference between the presence of the μ_1 and μ_2 subclass of receptors within the spinal cord and vPAG. Previous studies have suggested that the μ_2 binding site mediates spinal antinociception (Ling et al., 1983; Paul et al., 1989; Pick et al., 1991), while the μ_1 binding site mediates antinociception following i.c.v. microinjections (Ling et al., 1986, 1983; Moskowitz and Goodman, 1985). Ling et al. (1989) further suggested that tolerance develops more readily to responses mediated through the μ_1 binding site, including antinociception and prolactin release, relative to μ_2 -mediated responses, including respiratory depression and gastrointestinal transit. Since the cloning of the μ -opioid receptor, several splice variants of the receptor have been identified. These splice variants may correspond to pharmacologically identified μ_1 and μ_2 receptors (Pasternak, 2001). Differences in expression of μ -opioid receptor variants within spinal and supraspinal sites may also explain differences in the findings in the current studies and those of He et al. (2002).

The vPAG is of particular interest in the development of tolerance. Repeated microinjections of morphine into the vPAG, but not in adjacent or output targets, produce tolerance (Morgan et al., 2006a, 2005; Tortorici et al., 1999). It is unlikely that the tolerance observed in our studies is caused by damage from repeated intra-vPAG injections. This was controlled for by repeated saline injections in the current studies, and in previous studies from our laboratory which demonstrated that morphine analgesia differs in saline- and morphine-pretreated rats (Morgan et al., 2005; Tortorici et al., 1999). Moreover, blocking opioid receptors in the vPAG is sufficient to prevent tolerance to systemic administration of morphine (Lane et al., 2005). The advantage of microinjecting DAMGO and morphine directly into the vPAG is that confounds produced by opioid effects in the spinal cord and other brain areas are avoided. The current studies indicate that, when a high-efficacy agonist such as DAMGO is administered directly into the vPAG, pronounced tolerance can be readily observed. In addition, the finding that intra-vPAG DAMGO administration results in cross-tolerance to morphine suggests that the expression of morphine and DAMGO-induced tolerance involve similar mechanisms within the vPAG. As suggested by behavioral and in vitro recording studies, this mechanism may involve a change in opioid-sensitive GABAergic neurons within this brain area (Chieng and Christie, 1996; Ingram et al., 1998; Morgan et al., 2003).

The development of tolerance may also depend on the pattern of drug administration. In a study examining different methods of drug administration, continuous (osmotic minipump) drug administration produced robust tolerance to morphine, while continuous administration of two high-efficacy drugs, fentanyl and sufentanil, resulted in less tolerance. However, when these drugs were administered intermittently (via subcutaneous injection), the degree of tolerance induced by

all drugs was similar (Duttaroy and Yoburn, 1995). Based on this finding, the intermittent nature of the intra-vPAG DAMGO injections may contribute to the discrepancy between the current studies showing tolerance to DAMGO and other studies in which tolerance to DAMGO is not evident (He et al., 2002).

When opioids are administered systemically, the degree of tolerance and cross-tolerance appears to be inversely related to agonist efficacy (Paronis and Holtzman, 1992; Roerig et al., 1985; Tiano et al., 1998; Walker and Young, 2001). Studies using cellular models of tolerance suggest that the distinct signaling and internalizing efficacies of μ -opioid receptor agonists underlie this differential susceptibility to tolerance. That is, an agonist's ability to internalize the μ -opioid receptor is inversely related to its tolerance liability (Kieffer and Evans, 2002; Whistler et al., 1999). Low-efficacy agonists such as morphine are poor internalizing agents, while DAMGO and other high-efficacy agonists of the μ -opioid receptor cause substantial internalization. The few studies that have examined this idea in whole animals (He et al., 2002; He and Whistler, 2005; Stafford et al., 2001) show that low doses of DAMGO can block tolerance by facilitating the internalization of μ -opioid receptors by morphine (He et al., 2002). Contrary to this, our findings demonstrate that intra-vPAG DAMGO administration enhances the development of tolerance to morphine, suggesting that tolerance is either independent of internalization or that DAMGO does not promote internalization in the vPAG. Our laboratory and others have found that the cellular responses to high efficacy agonists such as met-enkephalin and DAMGO desensitize rapidly within the vPAG and locus ceruleus (Alvarez et al., 2002; Dang and Williams, 2005). Inasmuch as desensitization is a result of internalization, the most likely explanation for our current data is that tolerance is independent of internalization. Together, these data suggest that both morphine and DAMGO promote tolerance despite differences in their ability to promote receptor desensitization and/or internalization within the vPAG.

In summary, these data demonstrate that antinociceptive tolerance occurs readily when a high-efficacy agonist such as DAMGO is administered directly into the PAG. Further, morphine tolerance was facilitated by DAMGO in cross-tolerance and co-administration studies. Together with previous studies, these data suggest that the relationship between agonist efficacy and tolerance that has been found systemically and in the spinal cord is not maintained in the vPAG. From a clinical perspective, these experiments suggest that supplementation of morphine administration for pain treatment with additional opioid agents may enhance or inhibit the development of tolerance, depending on the route of administration.

Acknowledgments

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