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Modifications of Social Conflict-Induced Analgesic and Activity Responses in Male Mice Receiving Chronic Opioid Agonist and Antagonist Treatments

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TESKEY, G. C. AND M. KAVALIERS. *Modifications of social conflict-induced analgesic and activity responses in male mice receiving chronic opioid agonist and antagonist treatments.* PHARMACOL BIOCHEM BEHAV **38**(3) 485–493, 1991.—This study examined the effects of chronic (7 day) administrations of opioid agonists and antagonists, via osmotic minipumps (20 μg/μl/h, or 2 mg/kg/h for each agent) on: 1) nociception and activity, and 2) the analgesic and locomotor responses of subordinate male mice experiencing social conflict (aggression without defeat) and defeat in a "resident-intruder" paradigm. Chronic infusion of the mu opioid antagonist, naltrexone, resulted in a hypoanalgesic response and a decrease in basal locomotor activity on days 3–7 postimplantation which returned to the basal levels of saline-implanted control mice after termination of the infusions on day 9. Naltrexone reduced defeat-induced analgesia on the second day after implantation, but had no consistent effects on analgesia on test days 6 and 9 or on the aggression-induced (nondefeat) analgesia and increases in activity. The delta opioid antagonist ICI-154,129, while having no significant effects on basal nociception or locomotor activity, augmented nondefeat-induced analgesia (day 2) and reduced the defeat-induced increases in activity (days 2 and 6). The mu agonist, levorphanol, resulted in a significant analgesia on the first two days after infusion, followed by the development of tolerance to the analgesic effects over days 3–7. On day 9, a hypoanalgesic response indicative of withdrawal was evident. Levorphanol also induced a marked decrease in locomotor activity over days 3–7 postimplantation, with no evidence of the development of tolerance or withdrawal following termination of infusion. Levorphanol also enhanced the social conflict-induced analgesia on the second day of administration and attenuated defeat-induced analgesia and activity on day 6, with a return to basal response levels on day 9. The kappa opiate agonist, U-50,488, resulted in initial analgesic responses and decreases in activity, followed over days 3–7 by the development of tolerance to both the analgesic and locomotor effects. On day 9, a hyperalgesia and increased activity, indicative of withdrawal were evident. On test day 6, during the tolerance phase, chronic U-50,488 reduced the defeat-induced analgesic and locomotor responses with no consistent effects on the other test days. These results 1) demonstrate the differential nociceptive and locomotor consequences of chronic activation or blockade of mu, delta and kappa opiate receptors, and 2) indicate that mu, delta and kappa opioid systems are variably and likely synergistically involved in the expression of the analgesic and locomotor consequences of social conflict and defeat.

Mu, delta, kappa opioids Chronic opioid agonists and antagonists Levorphanol U-50,488 Naltrexone
ICI-154,129 Tolerance Withdrawal Aggression Defeat Analgesia Activity Mice Social conflict

SOCIAL conflict, intraspecific agonistic encounters and aggression, all of which are key facets of natural behavior (54), have been used as biologically and ecologically relevant means of examining central opioid activation and its behavioral and physiological consequences (26, 38, 39, 46–49). In the typical laboratory study a small male intruder mouse is introduced into the home cage of a larger dominant, isolated resident animal in a "resident-intruder" paradigm and the ensuing agonistic encounter is monitored. In both wild and laboratory-bred mice, intraspecific aggressive interactions, which are made up of a number of components including threats, attacks, and fighting, can result in the display of a specific defeat posture by the vanquished individual (23,26). This defeat behavior is considered to represent a generalized natural biological response to the stress of social confrontation (47). Both the aggressive encounter and the subsequent defeat experience obtained in a "resident-intruder" interaction have been shown to induce opioid-mediated analgesic, ingestive and locomotor responses in the subordinate, intruder mice (48,49). These behaviors are analogous to the responses obtained after central administrations of either endogenous opioid peptides or exoge-

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nous opiate agonists such as morphine (48,49). The behavioral responses can be similarly blocked by the prototypic exogenous opioid antagonist, naloxone (27, 38, 48, 49), the long-lasting alkylating antagonist, β-chlornaltrexamine (10,22), and putative opioid modulating peptides (16, 17, 46). These observations, coupled with the increased levels of the opioid peptide, β-endorphin, observed in discrete brain areas of defeated mice (20), along with the cross tolerance between chronic defeat and the analgesic effects of morphine (28,38), provide further support for a direct activation of endogenous opioid systems during social conflict.

A variety of studies have confirmed the existence of multiple opioid peptides and opioid receptors (25). To date at least five different types of opioid receptors (mu, delta, kappa, sigma and epsilon) have been postulated. Results of studies involving acute and chronic administrations of specific opioid agonists and antagonists have indicated that these opioid receptors and their endogenous ligands are involved in the modulation of a variety of behavioral and physiological functions (25). Delta, kappa and mu opiate receptors have also been implicated in the display of aggression and its behavioral consequences [see reviews in (2–4, 48)]. Acute administrations of specific delta, kappa and mu opioid agonists and antagonists to an intruder mouse in a "resident-intruder" pairing have been shown to have differential effects on both the aggressive encounters and the subsequent analgesic, locomotor and ingestive responses of the subordinate intruder (48). Relatively little is known, however, about the effects of either long-term activation or blockade of different opioid receptors by chronic treatments with opiate agonists or antagonists on the expression of defeat and its analgesic consequences.

The present study describes the effects of chronic administration of various opiate agonists and antagonists via implanted osmotic minipumps to intruder male CF-1 mice in a "resident-intruder" pairing. The effects of these agonists and antagonists on the analgesic and locomotor responses of the intruder mice undergoing the aggressive encounter (nondefeat) and experiencing defeat are described. In addition, the effects of chronic opioid agonist and antagonist administrations on the nociceptive and locomotor responses on control unpaired mice not experiencing social conflict are reported.

The agonists and antagonist examined include: the prototypic mu opiate-directed agonist, levorphanol (25), the specific kappa agonist, U-50,488 (35,52), the relatively specific delta antagonist, ICI-154,129 (12,36), and the prototypic mu-directed antagonist, naltrexone (25,42).

METHOD

Animals

Experimentally naive, small (two months of age, 20–25 g) and large (8–14 months, 40–45 g) male CF-1 mice (Charles River, Quebec) were used. Mice were housed either individually (large mice) or in groups of five under a 12-hour light:12-hour dark cycle (LD 12:12, L: 0700–1900 h, 20 $\mu W/cm^2$, D<0.01 $\mu W/cm^2$) at 22 ± 1°C. Mice were held under these conditions for at least two weeks before experimentation. Food (Purina mouse chow 5015) and water were available ad lib. All experiments were carried out at mid-photophase.

Implantation of Pumps

Osmotic minipumps (Alzet Model 2001, Palo Alto, CA) were implanted intraperitoneally into the abdominal cavity of mice under Avertine anesthetic (15). By 0.50–2.0 h after surgery the mice engaged in apparently normal behavioral activities. The pumps were filled with either levorphanol tartrate, naltrexone hydrochloride, ICI-154,29, or U-50,488 hydrate (20 μg/μl, in each case) or isotonic saline vehicle. The 2001 series of pump delivers 0.99 μl/h for 7 days (20 μg/μl/h or approximately 2 mg/kg/h, for each agonist and antagonist). Operation of the pumps was confirmed several days after surgery in a number of control implanted small mice by surgical removal of the pumps and measurement of volume released. In addition, the amount of fluid (dye) released in an in vitro condition over a measured time period was determined.

Experimental Procedures

Agonistic encounters. On postimplant days two, six and nine the same groups of mice (n = 5, for defeated and nondefeated mice for each drug) were at mid-photophase paired in a "resident-intruder" paradigm with a large, individually housed mouse in the home cage of the large mouse. After the smaller implanted mouse had received a specified number of bites to lead to the defeat of half of them [EB_{50}, defined as the number of bites required to produce defeat in half of the individuals in a particular resident-intruder paradigm (49)] or 10 minutes the encounter was terminated. Determinations were then made of the locomotor activity levels and nociceptive responses of the intruder mice. On postimplantation days three and seven determinations were made of the basal locomotor and nociceptive responses of these mice. Defeat was characterized by an upright position, limp forefeet, an upward angled head and retracted ears (23,26). On the basis of results of previous studies (48,49), as well as from the results of pilot studies, the EB_{50} was determined to be 35 bites. This EB_{50} was used distinguish the effects associated with defeat versus those related to just social conflict and the aggression experienced by the subordinate mice. The resident mice used displayed equivalent high levels of aggressiveness. These animals were reused, with no evident changes in the level of aggression displayed between or within the "resident-intruder" encounters. Although the assessment of aggression (bites) and defeat was not carried out in a blind manner, control determinations with a number of observers revealed that there was minimal interobserver variability or bias in the assessment of the aggressive encounters (47). In addition, there was no evidence for an increase in the propensity to undergo defeat over the test periods.

Locomotor activity. Mice were individually placed in a glass aquarium (20 × 35 cm) that was provided with a wood chip (Hardwood Laboratory Bedding, NY) substrate and positioned on top of an activity sensor (Varimex Activity Meter, Columbus, OH). Total locomotor activity was recorded for 30 seconds. Control determinations of the activity levels of unpaired implanted mice (n = 5, for each treatment) not experiencing any aggressive interactions were also made. Sensitivity was adjusted so that only locomotor activity was recorded. Results of preliminary studies showed that the amount of activity per unit time measured by the present procedure was the same over 30–120 seconds, and not significantly different from that recorded over a longer 10-min period. Results of previous investigations with CF-1 and other strains of mice have shown that the 30-second determinations gave a reliable and consistent index of the locomotory effects of acute administrations of various opiate agonists and antagonists (18,48).

Nociceptive responses. Immediately following activity measurements, the thermal response latencies of the defeated, nondefeated and unpaired mice experiencing no aggression were determined. Mice were individually placed onto a warm surface (50 ± 1°C, hot-plate, Thermo-Electric, NY) and the time to either foot-licking or jumping (or a predetermined limit of 120 s which was never reached) was recorded. After displaying an aversive

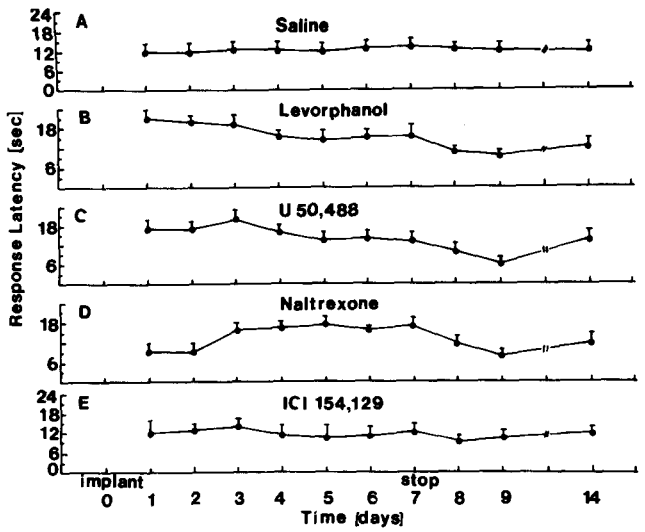

FIG. 1. (A–E) Effects of continuous seven-day infusion of (A) saline vehicle (1 μl/h), (B) levorphanol, (C) U-50,488, (D) naltrexone and (E) ICI-154,129 (20 μg/μl/h or 2 mg/kg/h, in all cases) via implanted osmotic minipumps on the nociceptive responses (thermal response latencies) of individual mice. Multipumps were implanted (implant) on day 0 and considered to have stopped (stop) delivery of the various agonists and antagonists on day 7. Vertical lines represent a standard error of the mean. N = 5, in all cases.

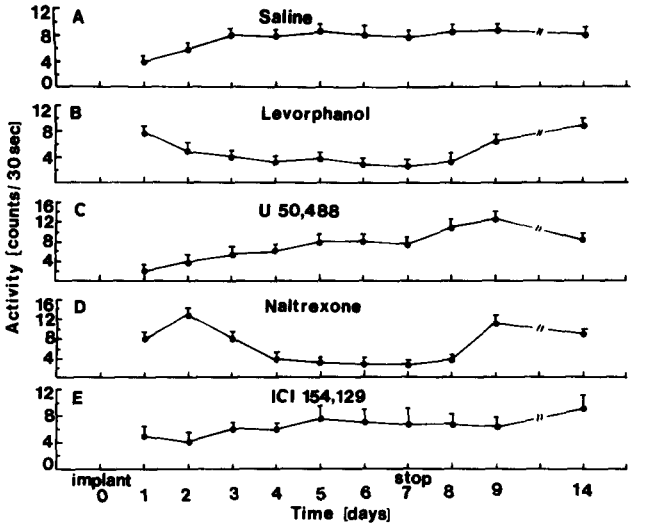

FIG. 2. (A–E) Effects of continuous seven-day infusion of (A) saline vehicle (1 μl/h) (B) levorphanol, (C) U-50,488, (D) naltrexone and (E) ICI-154,129 (20 μg/μl/h or 2 mg/kg/h, in all cases) via implanted osmotic minipumps on the locomotor activity levels of individual mice. Minipumps were implanted (implant) on day 0 and considered to have stopped (stop) delivery of the various agonists and antagonists on day 7. Vertical lines represent a standard error of the mean. N = 5, in all cases.

response, mice were immediately removed from the surface and returned to their home cages.

Activity levels and nociceptive responses of other control-implanted animals (n = 5, for each agonist, antagonist and saline) were also recorded on a daily basis for days 1–9 and on day 14 postimplantation.

The effects of the agonist and antagonists were separately analyzed by repeated measures analysis of variance and Student-Newman-Keuls multiple range tests. The significance level for hypothesis testing was set at the 0.05 level.

RESULTS

Effects of Chronic Opioid Agonist and Antagonist Administration on Nociception and Locomotor Activity

Saline. Control saline implants had no significant effects on the nociceptive responses of the small mice (Fig. 1A). Their thermal response latencies were not significantly different from each other on postimplant days 1–9 and 14, or from the previously (48) determined response latencies of similarly housed mice that received either acute saline or no treatment. On the first two days after implantation, the activity levels of mice receiving chronic saline were significantly ($p<0.05$) lower than the locomotory responses recorded on days 3–9 and day 14 (Fig. 2A). Activity levels on days 3–9 and day 14 were not significantly different from each other, or from the activity recorded previously (48) from similarly housed mice that received either acute saline or no treatment.

Levorphanol. On days 1–3 postimplantation, mice receiving chronic levorphanol were analgesic (Fig. 1B). Their thermal response latencies were significantly ($p<0.05$) greater than those of control mice receiving chronic saline. However, on days 4–9 and 14, the thermal response latencies of the levorphanol administered mice were not significantly different from those of saline controls.

On day one, the levorphanol-administered mice displayed significantly ($p<0.05$) greater locomotor activity than saline-treated animals (Fig. 2B). The activity levels of the levorphanol-treated mice were not, however, significantly different from the previously (48) determined activity levels of mice that did not receive any implants. On days 2, 9 and 14 postimplantation the activity levels of mice receiving chronic levorphanol treatment were not significantly different from animals that received saline. On days 3–8 postimplantation the levorphanol-administered mice displayed significantly ($p<0.05$) lower activity than saline controls.

U-50,488. On days 1–3 postimplantation, mice receiving chronic U-50,488 were analgesic (Fig. 1C). Their thermal response latencies were significantly ($p<0.05$) greater than those of mice receiving chronic saline. On days 4–8 and day 14 the thermal response latencies of the mice receiving chronic U-50,488 were not significantly different from those receiving saline, while on day 9, the U-50,488-implanted mice displayed significantly ($p<0.05$) lower response latencies than the saline controls.

On days 1–3 postimplantation, mice receiving chronic U-50,488 displayed significantly ($p<0.05$) lower activity levels than saline controls (Fig. 2C). On days 4–7 and 14 the activity levels of the mice receiving chronic U-50,488 were not significantly different from those of saline controls, while on days 8 and 9, U-50,488-implanted mice displayed significantly ($p<0.05$) greater activity than saline controls.

Naltrexone. The thermal response latencies of mice receiving chronic naltrexone were significantly ($p<0.05$) greater than those of the saline-treated control on days 4 and 5 postimplantation, and significantly ($p<0.05$) lower on day 9 (Fig. 1D). On all other days, there were no significant differences between the thermal response latencies of the saline- and naltrexone-implanted mice.

Mice receiving chronic naltrexone displayed significantly ($p<0.05$) greater activity levels than the saline controls on days

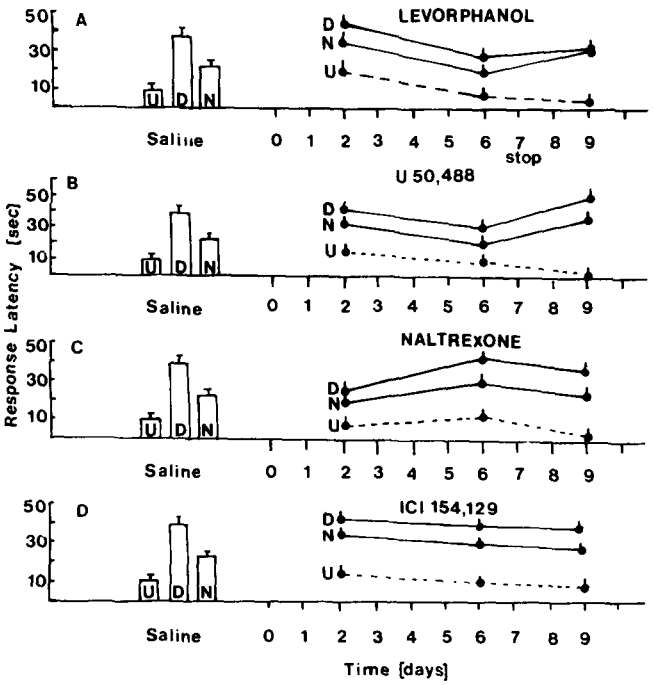

FIG. 3. (A–D) Effects of continuous seven-day infusion of (A) levorphanol, (B) U-50,488, (C) naltrexone and (D) ICI-154,129 (20 μg/μl/h or 2 mg/kg/h, in all cases) via implanted osmotic minipumps on nociceptive responses (thermal response latencies) recorded following aggressive encounters from defeated (D) and nondefeated (N) individual mice and unpaired control animals experiencing no aggressive encounters (U). Determinations were made on days 2 and 6 of infusion and on day 9 after infusion had stopped. Saline-infused (1 μl/h) mice served as a control, with nociceptive responses from day 6 being shown in the histograms in each row. Vertical lines represent a standard error of the mean. N = 5, in all cases.

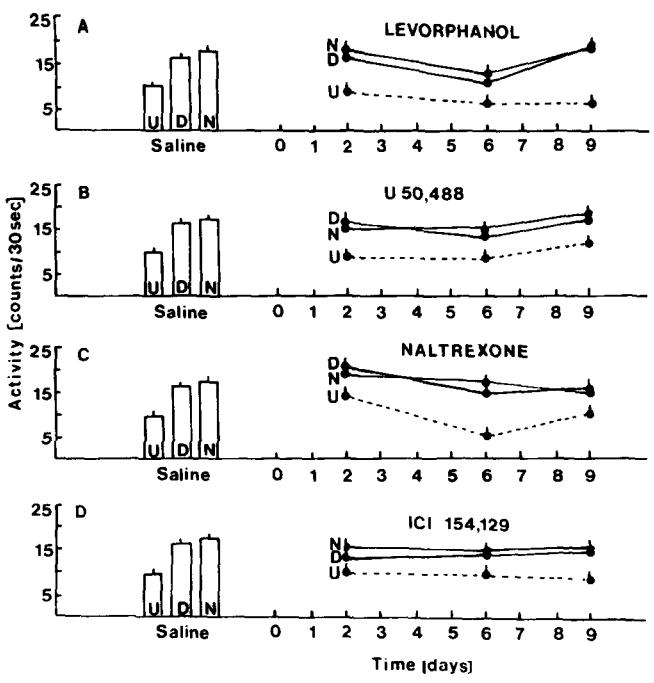

FIG. 4. (A–D) Effects of continuous seven-day infusion of (A) levorphanol, (B) U-50,488, (C) naltrexone and (D) ICI-154,129 (20 μg/μl/h or 2 mg/kg/h, in all cases) via implanted osmotic minipumps on the locomotor activity responses recorded following aggressive encounters from defeated (D) and nondefeated (N) individual mice and unpaired control animals experiencing no aggressive encounters (U). Determinations were made on days 2 and 6 of infusion and on day 9 after infusion had stopped. Saline-infused (1 μl/h) mice served as a control, with activity responses recorded on day 6 being shown in the histograms in each row. Vertical lines represent a standard error of the mean. N = 5, in all cases.

1, 2 and 9 postimplantation (Fig. 2D). The activity levels of the naltrexone-implanted animals on days 1 and 2 were not, however, significantly different from previously (48) determined activity levels of similarly housed mice which did not receive any implants.

ICI-154,129. The thermal response latencies of mice receiving chronic ICI-154,129 were on all days similar to those of the saline controls (Fig. 1E). Activity levels of mice chronically treated with ICI-154,129 were, however, significantly ($p<0.05$) lower than those of saline-treated controls (day 3) on days 2, 4 and 9 postimplantation (Fig. 2E). There were no significant differences in activity levels on the other test days.

Effects of Chronic Opioid Agonist and Antagonist Administration and Defeat on Nociception and Locomotor Activity

Chronic saline-treated mice, which displayed the characteristic defeat posture, were analgesic displaying a significantly ($p<0.01$) greater latency of response to the aversive thermal stimulus than saline-treated unpaired mice not experiencing any aggression (Fig. 3, for day 6). Those individuals which did not display the characteristic defeat posture, but still received the equivalent 35 bites [EB_{50}, as described previously (48)], also showed a significant ($p<0.05$) increase in their thermal response latency relative to saline controls not undergoing aggressive interactions. However, the degree of analgesia was significantly ($p<0.05$) greater in the defeated than nondefeated mice. These responses of the defeated, nondefeated and unpaired mice were not significantly different across days 2, 6 and 9 postimplantation (in all cases only responses from day 6 are shown). The thermal response latencies recorded from the 3 implanted groups were also not significantly different from the response latencies that were previously (48) recorded from defeated, nondefeated and unpaired control mice that received either acute saline treatment or no treatment.

Chronic saline-treated defeated and nondefeated mice had significantly ($p<0.05$) higher activity levels than unpaired controls (Fig. 4, with only activity for day 6 shown). The elevated activity levels of the defeated and nondefeated were not significantly different from one another (Fig. 4). The activity levels recorded from the three saline-implanted groups on day 6 were also not significantly different from the activity responses previously (48) recorded from defeated, nondefeated and unpaired control mice that received either acute saline or no treatment.

Basal thermal response latencies and activity levels (measured on days 3 and 5 postimplantation) of the saline-treated mice undergoing aggressive interactions were not significantly different from those of saline-implanted (day 6; for activity) animals not experiencing any aggression (Figs. 1 and 2).

Levorphanol. On day 2 postimplantation, levorphanol had no significant effect on the analgesic responses of the defeated mice, but significantly ($p<0.05$) increased the antinociceptive responses of the nondefeated mice (Fig. 3A). However, the defeated mice still displayed a significantly ($p<0.05$) greater analgesic response than the nondefeated animals. On day 6 postimplantation, the levels of defeat-induced analgesia displayed by the levorphanol-

treated mice were significantly ($p<0.05$) lower than those of control saline-treated mice, while the analgesic responses of the nondefeated mice were not significantly changed, relative to the saline controls. On day 9 the level of analgesia displayed by the defeated levorphanol-treated mice was significantly ($p<0.05$) lower, while that of the undefeated mice was significantly ($p<0.05$) greater, than the antinociceptive responses of their respective saline-treated control. As well, on day 9, there were no significant differences between the thermal response latencies of the levorphanol-treated defeated and nondefeated mice.

On day 2 postimplantation, levorphanol treatment had no significant effects on the increased activity levels of the defeated and nondefeated mice (Fig. 4A). On day 6 postimplantation, the levorphanol-treated defeated and nondefeated mice displayed significantly ($p<0.05$) lower activity levels than the saline controls, while on day 9 the levorphanol-treated mice displayed significantly ($p<0.05$) greater activity levels than the saline-treated mice.

U-50,488. On day 2 postimplantation, U-50,488 had no significant effects on the analgesic responses of the defeated and nondefeated mice (Fig. 3B). The thermal response latencies of the U-50,488-implanted mice were not significantly different from the responses of the corresponding saline-treated control animals. However, on day 6 postimplantation, the mice receiving U-50,488 displayed significantly ($p<0.05$) lower levels of defeat- and nondefeat-induced analgesia than the saline controls. On day 9 postimplantation, the U-50,588-treated mice displayed significantly ($p<0.05$) greater levels of defeat- and nondefeat-induced analgesia than the saline controls. On each test day, the U-50,488-treated defeated mice displayed significantly ($p<0.05$) greater thermal response latencies than the similarly treated nondefeated animals.

On days 2 and 9 postimplantation, the activity levels of the U-50,488 implanted defeated and nondefeated mice were not significantly different from the increased activity levels recorded from the saline-treated control animals (Fig. 4B). On day 6 postimplantation, the U-50,488-treated defeated and nondefeated mice displayed significantly ($p<0.05$) lower activity levels than the saline-treated controls. The activity levels of the U-50,488-treated mice were, however, still significantly ($p<0.05$) greater than those of undefeated saline or U-50,488-implanted animals.

Naltrexone. On day 2 postimplantation, the naltrexone-treated defeated and nondefeated mice displayed significantly ($p<0.05$) lower thermal response latencies than the corresponding saline-treated control animals (Fig. 3C). The defeated and nondefeated mice receiving the chronic naltrexone treatments were, however, still analgesic, displaying significantly ($p<0.05$) greater thermal response latencies than either naltrexone- or saline-treated unpaired mice. The naltrexone-treated defeated mice also displayed significantly ($p<0.5$) greater thermal response latencies than the nondefeated naltrexone-implanted animals. On days 6 and 9 postimplantation, there were no significant differences between the thermal response latencies of the naltrexone-implanted defeated and nondefeated mice and their corresponding saline-treated controls.

On days 2 and 6 postimplantation, the activity levels of the naltrexone-treated defeated and nondefeated mice were not significantly different from the activity levels recorded from the corresponding saline-treated animals (Fig. 4C). However, on day 9 postimplantation, the naltrexone-treated defeated and nondefeated mice displayed significantly ($p<0.05$) lower activity levels than those of the corresponding saline controls. The activity levels of the naltrexone-treated defeated and nondefeated mice were, however, still significantly ($p<0.05$) greater than those of either the naltrexone- or saline-treated unpaired animals experiencing no aggressive encounters.

ICI-154,129. The analgesic responses recorded from the ICI-154,129-treated defeated (days 2, 6, 9) and nondefeated (days 6, 9) mice (Fig. 3D) were not significantly different from the responses of the corresponding saline-treated controls (Fig. 1E). However, on day 2 postimplantation, the nondefeated mice displayed a significantly ($p<0.05$) greater analgesic response than the saline controls.

On days 2 and 6 postimplantation, the activity levels of the ICI-154,129-treated defeated and nondefeated mice were significantly ($p<0.05$) lower than those of the corresponding saline-treated control animals (Fig. 4D). The ICI-154,129-treated defeated and nondefeated mice, however, still displayed significantly ($p<0.05$) greater activity levels than unpaired mice that were chronically treated with either saline or ICI-154,129. On day 9 postimplantation, there were no significant differences between the activity levels of the saline- and ICI-154,129-treated defeated and nondefeated mice.

Basal thermal response latencies and activity levels (measured on days 3 and 5 postimplantation) of the opioid agonist and antagonist implanted mice undergoing the aggressive interactions were not significantly different from those of implanted mice not experiencing any aggression (Figs. 1 and 2).

DISCUSSION

Effects of Chronic Opioid Agonists and Antagonists on Nociception and Locomotor Activity

The results of the present study show that chronic administrations of mu, delta and kappa opioid agonists and antagonists via implanted osmotic minipumps have significant effects on the nociceptive and locomotor responses of mice. Chronic administration of either the mu agonist, levorphanol, or the kappa agonist, U-50,488, resulted in significant analgesic responses for the first 2–3 days after implantation. The initial analgesic responses obtained were similar to the antinociceptive effects observed after acute treatments with the respective agonists (48). By the fourth day, tolerance to the analgesic effects of the mu and kappa agonists was evident, with the nociceptive responses of both the levorphanol- and U-50,488H-implanted mice being similar to that of the saline-treated control. On days 8–9, when release of the agonists had stopped, a hypoanalgesic response indicative of withdrawal (8) was observed in both the levorphanol- and U-50,448-implanted mice. By day 14, seven days after cessation of treatment, the nociceptive responses of the implanted mice were similar to those of the saline-treated controls. It should be noted that the reduction in nociceptive response over time (days 3–7) is not due to loss of pump function or mechanical failure. This osmotic infusion pump has been widely documented as producing reliable delivery over a 7-day period (51,53). Degradation of the infused agent within the minipump is also unlikely, as a number of studies have documented the stability of peptides and alkaloids in this preparation (19).

In contrast to the similar profiles for nociception, there were marked differences between the effects of chronic administration of levorphanol and U-50,488 on the locomotor response of the mice. Continuous levorphanol induced a sustained decrease in activity (days 3–7) similar to that observed after acute administration of levorphanol to CF-1 mice (48). There was no evidence of the development of tolerance to the locomotor-depressing effect of levorphanol. With the termination of the release of levorphanol, the locomotor responses returned to the same levels as those of the saline controls. In contrast, mice chronically treated with U-50,488 developed over days 4–7 tolerance to the initial locomotor depressing effects of U-50,488. Moreover, following cessation of the release of U-50,488, the mice displayed an increase in activity, suggestive of withdrawal and paralleling the

hypoanalgesic responses evident at this time. The initial decrease in activity was similar to that observed after acute administration of a relatively low dose of U-50,488 (48).

The present findings with levorphanol and U-50,488 are consistent with, and extend, the results of previous investigations of the effects chronic administrations of mu and kappa agonists on nociception. Similar time courses for the development of tolerance have been described for the analgesic effects of peripheral and spinal administrations a number of other mu opioid agonists including, heroin, morphine, sufentanil and the highly specific agonist D-Ala^2-MePhe^4-Gly-ol^5-enkephalin (DAGO) (8, 30, 31, 43). In addition, after discontinuation of chronic mu opioid agonist treatment a number of behavioral responses symptomatic of withdrawal have also been previously reported (8).

The development of tolerance, and likely withdrawal, following chronic administration of mu opiates, may be associated with the upregulation of mu opiate receptors. Upregulation of central mu opiate receptors has been documented in vivo in a number of autogradiographic studies with rats exposed to chronic treatment with muopiates such as morphine (30, 31, 41). It should, however, be noted that there is also some evidence for either down-regulation (37) or no change (9) in mu opiate receptors after chronic mu agonist treatment.

There have been no systematic examinations of the effects of continuous administrations of mu agonists on locomotor responses. The lack of apparent development of tolerance to the locomotory effects of levorphanol observed in the present study may reflect the involvement of different categories or subtypes of mu receptors than those associated with the expression of analgesia. There have been proposed to be two subtypes of mu receptors, each associated with the mediation of different behavioral responses (34). These subtypes are apparently differently affected by chronic administrations of mu agonists (41). There is also evidence that levorphanol is an agonist at both mu and delta receptors, with the latter receptors being involved in the mediation of locomotor responses (25). Whether or not tolerance develops to behavioral effects of delta agonists is, however, at present not clear.

Rapid development of tolerance to the hypothermic and analgesic effects of the specific kappa agonist, U-50,488, has been previously reported (5, 6, 52). This is, however, the first report of tolerance to the locomotor depressing effects of U-50,488. Moreover, in those previous studies of analgesia, high doses of U-50,448 were intraperitoneally injected 2–4 times daily over 2.5–4 days, while in the present study a low dose of U-50,488 was continuously infused over 7 days. As well, in the previous shorter-term studies, there was no direct consideration of withdrawal after cessation of treatment with U-50,488. Chronic treatment with U-50,488 and another less specific kappa agonist, bremazocine, has been reported to result in a down-regulation of kappa opioid receptors in the rat spinal cord, pons medulla, midbrain and cortex, but an upregulation in the corpus striatum (5,31). How these changes in kappa opiate receptor numbers are related to tolerance and to, and/or withdrawal from, chronic U-50,488 administration remains to be determined. As well, possible differences in the involvement and responses of the various postulated subtypes of kappa receptors need to be considered.

The results of the present study also showed that chronic administration of the mu opioid antagonist, naltrexone, has significant effects on murine nociceptive and locomotor responses. On the first two days of chronic administration of naltrexone there was a decrease in nociceptive sensitivity and an increase in locomotor activity. Following this, over days 3–7, chronic infusion of naltrexone resulted in a significant increase in response latency, suggestive of a hyperalgesia, and a marked decrease in locomotor activity. On days 8 and 9 following cessation of the release of naltrexone there was a hypoanalgesic response and increase in locomotor activity. These response patterns were qualitatively similar to the responses observed after termination of chronic levorphanol treatment. By day 14, the nociceptive and locomotor responses of the animals that had received chronic naltrexone treatments were similar to those of the saline control animals.

Results of previous investigations have shown that long-term blockade of mu opioid receptors by antagonists such as naloxone and naltrexone can result in a sustained reduction of ingestive behavior, body weight and core temperature (24,29). The present results extend these effects of chronic naltrexone treatment to reductions of locomotor activity. Consistent with the reported effects on body weight and temperature (29), there was a rebound in activity after discontinuation of long-term treatment with naltrexone, with no evidence for the development of tolerance to the depressant actions. These inhibitory effects of naltrexone on activity may be associated with the selective blockade and upregulation of mu, delta and kappa opiate receptors that have been observed after chronic treatment with equivalent [3.0 mg/kg/h in (32)] doses of naloxone (1, 11, 21, 30–33) or naltrexone (41, 45, 55–57). It should be noted that lower doses (0.50 mg/kg/h) of naloxone have been reported to be more selective, upregulating only mu receptors (32).

Results of previous studies have suggested that there are minimal effects of chronic administrations of opioid antagonists such as naloxone and naltrexone on basal nociceptive responses (11, 14, 24, 29, 55, 56). Those previous investigations have, however, been primarily concerned with modifications of responses to exogenous opiates following chronic opioid antagonist treatment, rather than systematic examinations of basal nociception during and following antagonist treatment, as described in the present study. It has also been indicated that the nociceptive effects of opioid agonists in rats receiving chronic naloxone can be affected by the test used to measure nociception (30). Whether or not these differences in test sensitivity also extend to basal measures of nociception in animals receiving chronic naloxone and naltrexone needs to be determined. In addition, it has been reported that repeated measurements of nociception under naloxone or naltrexone may result in the display of a paradoxical "naloxone-induced analgesia" (13). Whether or not this "naloxone-induced analgesia" contributes to the present naltrexone-induced hypoanalgesia also remains to be examined.

Results of previous investigations have reported minimal effects on basal nociception and activity of delta opioid antagonists (32). This is consistent with the results of the present study and investigations of the effects of acute administrations of the delta antagonist ICI-154,129 (7, 12, 18, 36). These variable effects may relate to the reported dose-dependent agonist and antagonist properties of ICI-154,129 (7,12).

Effects of Chronic Opioid Agonists and Antagonists on the Behavioral Consequences of Agonistic Encounters

Chronic administrations of mu, delta and kappa opioid agonists and antagonists also selectively affected the analgesic and locomotor consequences of social conflict and defeat. These results, in conjunction with the effects of acute administrations (48), provide further support for a differential activation of endogenous opioid systems during and/or following aggressive encounters and defeat. The present findings also reinforce the likelihood that the stress of defeat may lead to either a generalized or synergistic global opioid activation.

The demonstration of a significant analgesic response following the agonistic encounters is consistent with previous findings of the existence of defeat-induced analgesia (26, 38–40, 47–49). It also confirms prior observations that the agonistic encounter it-

self has a significant analgesic effect in the intruder animal (47–49). In addition, as previously reported (49), the level of defeat-induced analgesia was significantly greater than the antinociceptive response observed following just the aggressive interaction, even though the levels of physical aggression (number of bites experienced) were equivalent.

The defeat-induced analgesia, which was significantly reduced by naltrexone, on the second day of chronic administration, and was insensitive to ICI-154,129, may be interpreted as being mediated by mu opioid receptors. This does not, however, preclude the involvement of kappa opioids in the mediation of the analgesic responses (2). This antagonistic effect of chronic naltrexone on defeat-induced analgesia is consistent with the inhibitory effects of acute injections of naloxone (28, 38, 49), as well as the longer-term effects of administration of the irreversible mu receptor alkylating agent β-chlornatrexamine (10,22). Both peripheral and central injections of naloxone into the periaqueductal grey area and arcuate nucleus have been shown to block analgesia in defeated mice, supporting central opioid involvement in the mediation of these responses (27).

It was also observed that on the second day of continuous administration of levorphanol and U-50,448, there was an enhancement of aggression (nondefeat)-induced analgesia. This is consistent with the effects of acute injections of levorphanol and U-50,488 on the analgesic responses of mice undergoing agonistic encounters (48), and supports mu and kappa opioid involvement in the mediation of aggression-induced analgesia (2,48). In addition, during the tolerance phase, on the sixth day of chronic administration of either levorphanol or U-50,488, there was reduction in the levels of defeat-induced analgesia. This is suggestive of cross-tolerance to mu and kappa opioid-mediated components (or subcomponents of multiple components) of aggression-induced analgesia. Cross-tolerance has been previously shown between morphine and chronic defeat (28,38). Following the termination of chronic treatment with either levorphanol or U-50,488, there was an enhancement of defeat and nondefeat analgesia. This is also consistent with the reported changes in sensitivity to morphine and U-50,488 during withdrawal (5, 8, 29).

It should, however, be noted that while acute peripheral injections of naloxone blocked both defeat- and nondefeat-induced analgesia (46), after two days of chronic treatment with naltrexone only the defeat-induced analgesia was significantly reduced. Moreover, on the sixth day of chronic naltrexone treatment, as well as on day 9 after termination of the infusion of naltrexone, there were no significant effects on either defeat- or nondefeat-induced analgesia. These latter findings are at variance with the demonstrations that chronic naltrexone and naloxone upregulate and increase the number of mu, and in several cases, kappa opiate receptors (1, 14, 21, 30, 31, 56, 57), significantly enhancing the analgesic effects of morphine and U-50,488 (5, 43, 44, 55). The present findings are, however, consistent with the brief report that chronic naltrexone administered via pellet implantation enhanced morphine, but not defeat-induced analgesia in B6AF1 mice (50). Together, these observations suggest that the analgesia produced by the stress of the aggressive encounter, as well as by defeat, may involve different and/or additional subpopulations of opioid receptors than those associated with morphine and U-50,488 induced analgesia. The present findings also raise the possibility that defeat and nondefeat aggression related analgesia may each be associated with, or involve, different opioid components. These observations also suggest that other nonopioid mechanisms (39,40) may be associated with the mediation of the defeat- and/or nondefeat-induced analgesic responses of CF-1 mice. Whether or not these effects of chronic naltrexone are unique to the analgesic consequences of aggression and defeat, or are evident with other sources of opioid-mediated naturalistic stress-induced analgesia, needs to be determined before further conclusions are drawn.

The present results also show that the locomotor activity levels of the intruder mice were increased after the aggressive encounters and, moreover, that the occurrence of defeat had no additional effect on this heightened activity. The increased levels of activity recorded after the agonistic encounter were reduced by chronic administration of ICI-154,129 and were unaffected by naltrexone after compensating for the latter's affects on basal activity. This is consistent with the effects of acute administrations of naloxone and ICI-154,129 (48), though it should be noted that acute administration of ICI-154,129 completely blocked the increase in activity, whereas chronic administrations only partially reduced the increases. These differences between chronic and acute effects may reflect the mixed agonist-antagonist properties of higher doses of ICI-54,129 (7,12). These effects of chronic ICI-154,29 are, however, still suggestive of delta opioid involvement in the mediation of the aggression-induced increases in activity. This is consistent with the results of previous studies with various species and strains of rodents in which it was observed that stress-induced increases in activity were reduced by ICI-154,129 and were insensitive to antagonism by low doses of naloxone (18). This does not, however, preclude the involvement of other classes of opioid receptors in the mediation of the aggression-induced increases in locomotor activity. This proposal is supported by the decreased aggression-induced activity responses observed during the tolerance phases (day 6) of chronic levorphanol and U-50,488. These findings with the kappa and primarily mu agonists are suggestive of cross-tolerance and raise the possibility of mu, delta and kappa opioid contributions to the aggression-induced changes in locomotory activity.

CONCLUSIONS

In summary, chronic administrations of mu, delta and kappa opioid agonists and antagonists differentially and selectively affected the nociceptive and locomotor responses of male mice. Chronic treatments with exogenous opioid and antagonists also selectively modified the opioid-mediated analgesic and locomotory consequences of aggressive encounters and defeat in subordinate male mice in a "resident-intruder" pairing. These findings provide further support for the activation and involvement of mu, delta and kappa opioid systems in the mediation of the behavioral consequences of aggression and defeat.

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