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Effects of Opiate Agonists and Antagonists on Aggressive Encounters and Subsequent Opioid-Induced Analgesia, Activity and Feeding Responses in Male Mice

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TESKEY, G. C. AND M. KAVALIERS. *Effects of opiate agonists and antagonists on aggressive encounters and subsequent opioid-induced analgesia, activity and feeding responses in male mice.* PHARMACOL BIOCHEM BEHAV 31(1) 43–52, 1988.—The effects of peripheral administration of the mu, kappa and sigma opiate agonists, levorphanol (1.0 mg/kg), U-50,488 (1.0 and 10.0 mg/kg), (±) SKF-10,047 (10.0 and 30.0 mg/kg), respectively, as well as the delta opiate antagonists, ICI-154,129 (10.0 mg/kg), and the prototypic antagonist, naloxone (1.0 mg/kg), on the agonistic behaviors and subsequent analgesic, locomotory and ingestive responses of subordinate mice were examined in a "resident-intruder" paradigm. The latter behaviors were examined in both defeated and nondefeated mice that had received an equivalent level of aggression. The mu and delta opiate antagonists decreased, while the mu, kappa, and sigma opiate agonists selectively increased aggressive behavior (number of bouts of aggressive interactions, number of bites to defeat, time to defeat). Both naloxone and the delta antagonist suppressed defeat- and aggression-induced activity and feeding, while only naloxone blocked the analgesic response. Levorphanol enhanced, U-50,488 had variable dose related effects, and SKF-10,047 decreased the defeat and aggressive-induced responses. These results indicate that various opioid systems and opiate receptors are differentially involved in the mediation of various components of the agonistic encounters and in the expression of the consequences of social conflict and defeat-induced opioid activation.

Mu, kappa, delta, sigma opioids Naloxone U-50,488H ICI-154,129 SKF-10,047 Levorphanol
Aggression Defeat Feeding Activity Analgesia Mice

THERE is substantial evidence that after exposure to either physically or psychologically stressful situations, endogenous opioid activity is increased. In laboratory rodents endogenous opioids systems can be activated after a variety of physical stresses including footshock, centrifugal rotation, immobilization and restraint [1, 5, 30, 51]. These artificial stresses can lead to a number of behavioral and physiological alterations, including the induction of analgesia and increases in feeding [1]. These responses are similar to the effects obtained after administration of either endogenous opioid peptides [39] or exogenous opiate agonists such as morphine [38].

Relatively recently, social conflict and intraspecific aggression, which are key facets of natural behavior [56], have been used as a more biologically and ecologically relevant means of examining central opioid activation and its behavioral and physiological consequences [35, 46, 52]. In the typical laboratory "resident-intruder" paradigm a small male intruder mouse is introduced into the home cage of a larger dominant, isolated resident animal 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, may result in the display of a specific defeat posture by the vanquished individual [32,35]. This defeat behavior is considered to represent a generalized natural biological response to the stress of social confrontation [35,46]. Both the aggressive encounter and the subsequent defeat experiences obtained in a "resident-intruder" interaction have been shown to induce analgesic and ingestive response in the subordinate, intruder mice [52]. These behaviors, which are analogous to the responses obtained after central administrations of either opioid peptides or morphine, could be similarly blocked by the exogenous opiate antagonists, naloxone, [52] as well as by putative endogenous opioid antagonists Phe-Met-Arg-amide (FMRFamide) or N-terminal extended FMRFamide related peptides and Prolyl-leucyl-glycinamide

(PLG or MIF-1) [25,53]. These observations provide additional support for a direct activation of endogenous opioid systems during social conflict.

Results of a variety of studies have confirmed the existence of multiple opiate receptor types and opioid peptides [32]. Delta, mu, and kappa opiate receptors have been implicated in the display of aggression (see reviews in Benton *et al.* [4]; Benton [2] and Rodgers and Randall [47]). However, relatively less attention has been paid to the roles of various opioid receptors in modulating (1) the behavioral components of the agonistic interaction, and in particular those of the defeated individual and (2) the opioid-mediated behavioral consequences of the aggressive interactions and defeat.

The present study describes the effects of peripheral administration of various opiate agonists and antagonists to intruder mice on murine aggressive interactions in a "resident-intruder" pairing. In addition, the effects of these agonists and antagonists on the analgesic, ingestive and locomotory responses of the intruder mice undergoing the aggressive encounter and experiencing defeat are described. The agonist and antagonist examined include: the prototypic mu opiate-directed agonists levorphanol [11], the specific kappa agonists, U-50,488 [42,55], the sigma agonists, (±) SKF-10,047 [28,29], the relatively specific delta antagonist, ICI-154,129 [13,43], and the prototypic mu-directed antagonist, naloxone [49].

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 or in groups of five under a 12 hour light:12 hour dark cycle (LD 12:12 L:0700–1900 hr, 20 $\mu W/cm^2$, D<0.01 $\mu W/cm^2$) at 22±1°C. Grouped mice were held in cages 30×19 cm, while isolated mice were held in cages 30×13 cm. Mice were held under the above 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 midphotophase.

Experiment I

Dose and time dependent effects of the various opiate agonists and antagonists on basal nociceptive responses, locomotor activity, and food intake of control undefeated small mice were established in previous and pilot studies [26,54]. On the basis of these results, specific doses of the drugs and times after administration (in brackets after dose) were chosen for analysis of their effects on the aggressive interactions and their behavioral consequences. Small intruder mice were injected intramuscularly (IM) with either naloxone hydrochloride (1.0 mg/kg, 30 min), ICI-154,129 (10.0 mg/kg, 60 min), levorphanol tartate (1.0 mg/kg, 30 min), U-50,488 hydrate (1.0 and 10.0 mg/kg, 60 min), (±) SKF-10,047 hydrochloride (30.0 and 10.0 mg/kg, 90 min), or saline (10 ml/kg, 30 min) or received control handling.

At the designated time after receiving the drug, social conflict was initiated by placing the young mouse with an older mouse in a "resident-intruder" pairing in the home cage of the latter. The latency to first attack by the resident, number of bites to produce defeat in half the animals tested (EB_{50}, effective number of bites to produce defeat in half of the individuals in this particular resident-intruder paradigm [52]) or 10 minutes, time to defeat, and number of bouts to defeat were recorded. Although this procedure was not carried out in a blind manner, control determinations with a number of observers revealed that there was minimal interobservation variability or bias in the assessment of the aggressive encounters.

A bout of fighting was defined as a continuous series of threats and attacks over an interval of time (30–60 sec) [32]. A bout was considered to be ended when both mice engaged in nonthreatening activities such as grooming. Use of the EB_{50} permitted the analysis of the subsequent behavioral responses of intruder mice that had experienced equivalent levels of aggression without undergoing defeat. In all cases intruder mice were only used once (n=10 for defeated and nondefeated mice, for each drug and dose). 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 [54].

Experiment II

Small, grouped mice were paired with large, singly housed individuals in the home cage of the larger individual after injection at the previously specified times. After the smaller mouse had received a specified number of bites to lead to the defeat of half of them (EB_{50}, for dose and drug, from Experiment I) the encounter was terminated. Determinations were then made of the locomotor activity levels, and nociceptive responses of the mice. Other mice were used to examine the effect of the aggressive encounter and defeat on the ingestive responses of the intruder (n=10, for defeated and nondefeated mice, for each drug and dose).

Locomotor activity. Mice were individually placed in a glass aquarium (20×35 cm) provided with a wood chip (Hardwood Laboratory Bedding, NY) substrate on top of the activity sensor (Varimex Activity Meter, Columbus, OH) and total locomotory activity was recorded for 30 seconds. Sensitivity was adjusted so that only locomotory activity was recorded. Results of preliminary studies showed that the amount of the activity per unit time was the same over 30–120 seconds. Control determinations of the activity levels of mice not experiencing any social conflict were also made. Results of previous investigations with CF-1 and other strains of mice had shown that the 30 second determinations gave a reliable and consistent index of the locomotory effects of various opiate agonists and antagonists [25].

Nociceptive responses. Immediately following activity measurement the thermal response latencies of individual mice were determined using a modified hot-plate technique [8]. Mice were individually placed onto the warm surface (50±1°C, Thermo-Electric hot-plate, NY) and the time to either foot-licking or jumping was recorded. After displaying an aversive response, mice were immediately removed from the surface and returned to their home cages. Locomotory activity levels and nociceptive responses of control animals (n=10) experiencing no social conflict were also recorded. Control determinations showed that the intervening activity measurements had no apparent effects on thermal response latencies.

Ingestive responses. Mice were individually placed in elevated 20 cm diameter (7 cm high) clear plastic small rodent metabolism units that were provided with a wire mesh floor (E-1100 Econo-Metabolism Unit, Maryland Plastics, NY). An aluminum ring in front of the food hopper restricted entry

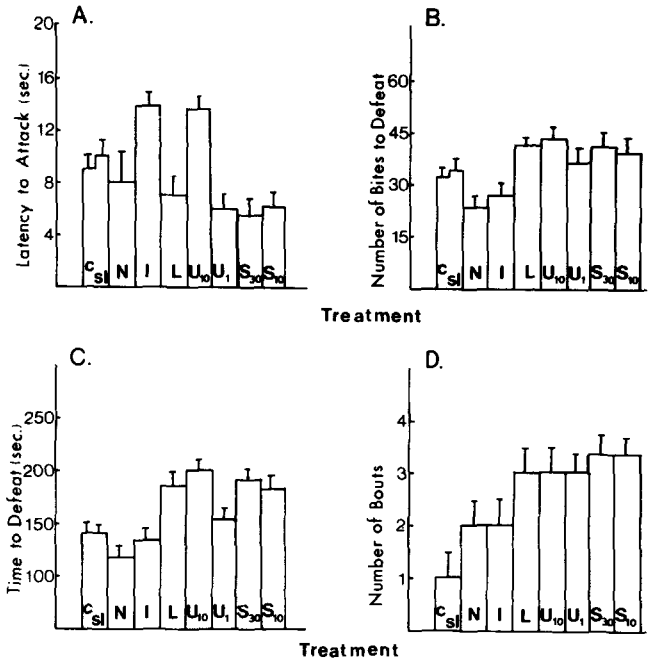

FIG. 1. (A–D) Effects of intramuscular injections of various opiate agonists and antagonists on the behavioral components during the agonistic encounter between large-isolated and small-grouped housed mice in a "resident-intruder" pairing. The behavioral components recorded were (A) latency to first attack, (B) number of bites to defeat, (C) time to defeat and (D) number of bouts, all in the smaller mouse. Data are presented for animals undergoing defeat. Sl: saline (1.0 mg/kg); N: naloxone (1.0 mg/kg); I: ICI-154,129 (10.0 mg/kg); L: levorphanol (1.0 mg/kg); U: U-50,488 (1.0 and 10.0 mg/kg); S: SKF-10,047 (10.0 and 30.0 mg/kg). c represents unpaired control animals not experiencing the aggressive encounter. Vertical lines represent standard error of the mean ($n=10$, in all cases, all 4 behavioral measurements were made with each submissive individual).

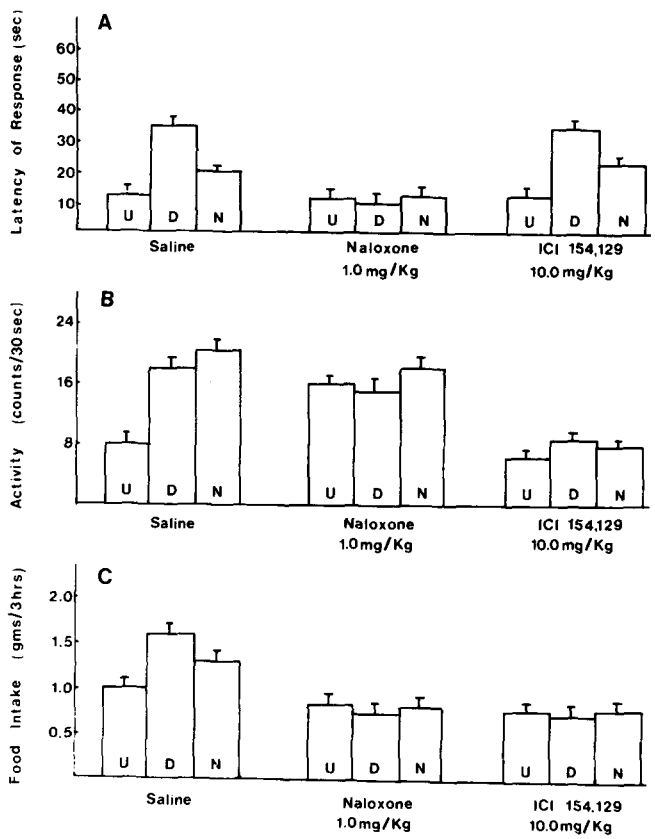

FIG. 2. (A–C) Effects of intramuscular (IM) injections of naloxone (1.0 mg/kg), ICI-145,129 (10.0 mg/kg) and saline (1.0 ml/kg) vehicle on (A) thermal response latencies, (B) activity and (C) food intake over 3 hours following agonistic encounters in (D) defeated and (N) nondefeated mice. U represents unpaired control animals. Naloxone and ICI-154,129, were injected 30 and 60 minutes respectively before the pairing while saline was injected either at 30 or 60 minutes before the pairing. Vertical lines represent standard error of the mean (n=10 in all cases).

to only the head and prevented the animals from placing their feet in the food. A short (3 cm) aluminum tunnel provided access to a food hopper in which a preweighed quantity of powdered food (Purina mouse chow 5015) was provided (approximately 2 g) in a plastic tray. The animals readily consumed the powdered food by licking. Determinations were made of food consumed at the end of each hour, for three hours following social conflict. Fresh food was provided each hour. Any food that was lost by scatter or spillage was collected and corrected for in the hourly food intake determinations. Water was provided in a plastic graduated tube which was placed directly across from the food hopper. Control determinations were made using ten small mice that did not experience any social conflict.

Data for both experiments were analyzed by analysis of variance and Student-Newman-Keuls multiple range tests. Significance level for hypothesis testing was set at the 0.05 level.

RESULTS

Experiment I

Latency to attack. Levorphanol (1.0 mg/kg), U-50,488 (1.0 mg/kg), and (±) SKF-10,047 (30.0 and 10.0 mg/kg) when compared to saline treatment, significantly ($p<0.05$) decreased the latency to first attack on the intruder, mice. Naloxone (1.0 mg/kg) did not have any significant effect on the latency to first attack, while ICI-154,129 (10.0 mg/kg), and U-50,488 (10.0 mg/kg), significantly ($p<0.05$) increased the latency to being attacked (Fig. 1A). Saline-injected mice did not significantly differ from being attacked (Fig. 1A). Saline-injected mice did not significantly differ from untreated mice in the latency to receiving their first attack.

Number of bites to defeat. Mice treated with naloxone received significantly ($p<0.05$) fewer bites than did the saline-injected controls before demonstrating the defeat posture. Levorphanol, U-50,488 (10.0 mg/kg), and (±) SKF-10,047 (30.0 and 10.0 mg/kg) caused the subordinate mouse to receive significantly ($p<0.05$) more bites before undergoing defeat. U-50,488 (1.0 mg/kg) and ICI-154,129 had no effect on the number of bites received by the subordinate mice (Fig. 1B). The EB_{50} values were as follows: saline 35, naloxone (1.0 mg/kg) 23, ICI-154,129 (10.0 mg/kg) 28, levorphanol (1.0 mg/kg) 40, U-50,488 (10.0 and 1.0 mg/kg) 43 and 37, respectively, and (±) SKF-10,047 (30.0 and 10.0 mg/kg) 41 and 39, respectively.

Time to defeat. Naloxone significantly ($p<0.05$) decreased the time to defeat. Levorphanol, U-50,488 (10.0

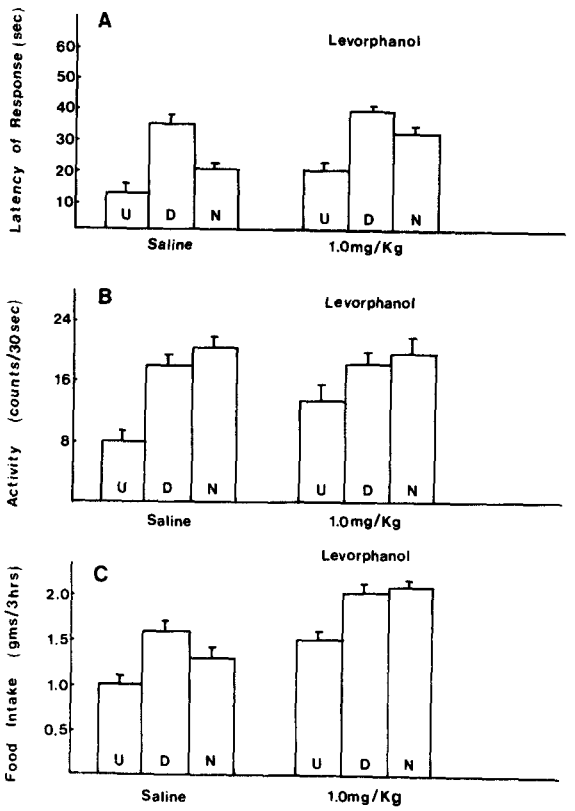

FIG. 3. (A–C) Effects of intramuscular (IM) injections of levorphanol (1.0 mg/kg) and on (A) thermal response latencies, (B) activity and (C) food intake over 3 hours following agonistic encounters in (D) defeated and (N) nondefeated mice. Levorphanol and saline were injected 30 minutes before the agonistic encounter. U represents unpaired control animals. Vertical lines represent standard error of the mean (n=10, in all cases). Saline- (1.0 ml/kg) injected groups are repeated as an aid in interpretation of the data.

mg/kg), and (±) SKF-10,047 (30.0 and 10.0 mg/kg) significantly ($p<0.05$) increased the time to defeat. U-50,488 (1.0 mg/kg) and ICI-154,129 had no effect on the time to defeat (Fig. 1C).

Number of bouts to defeat. A significantly ($p<0.05$) greater number of bouts was displayed after levorphanol, U-50,488, and (±) SKF-10,047 treatments, as compared to naloxone and ICI-154,129 treatment. However, administration of the latter antagonists did lead to significantly ($p<0.05$) greater number of bouts than that observed following saline or untreated controls (Fig. 1D).

Experiment II

Saline-treated mice, which displayed the characteristic defeat posture, were analgesic displaying a significantly greater ($p<0.001$) latency of response to the aversive thermal stimulus than nondefeated mice (Fig. 2A). The response latencies of the saline-treated defeated mice were not significantly different from those of uninjected control defeated mice. Those individuals which did not display the characteristic defeat posture, but still received the equivalent 35 bites (EB_{50}, as described previously), also showed a significantly ($p<0.05$) increase in the latency of thermal response relative to saline treated controls not undergoing agonistic interactions (Fig. 2A). However, the degree of analgesia was significantly ($p<0.05$) greater in the defeated than nondefeated mice. Results from various studies using CF-1 mice has established a high level of consistency in the response of control uninjected nondefeated mice [52,53]. Thus the mice can be reliably considered to have equivalent thermal response latencies prior to experiencing defeat.

Saline-treated defeated and nondefeated mice had significantly ($p<0.05$) higher activity levels than unpaired controls. The elevated activity levels of the defeated and nondefeated mice were not significantly different from one another (Fig. 2B). In addition the activity levels of the saline-treated animals were, in all cases, not significantly different from those of uninjected control animals.

During the three-hour period following the aggressive interaction, saline-treated defeated mice consumed significantly ($p<0.05$) more food than did nondefeated mice, which in turn, consumed significantly ($p<0.05$) more food than did unpaired saline-treated mice (Fig. 2C). The food intake levels of the saline-treated defeated mice were not significantly different from those of uninjected defeated animals.

Effects of Naloxone

Pretreatment with naloxone blocked both the defeat and nondefeat aggression-induced analgesia (Fig. 2A). The thermal response latencies of the defeated, nondefeated, and unpaired mice were not significantly different from one another, or from saline-treated unpaired mice (Fig. 2A). Naloxone significantly ($p<0.05$) increased the activity levels of unpaired mice, while having no significant effect on the activity levels of defeated, or nondefeated mice. There were no significant differences between activity levels of defeated, nondefeated and unpaired mice pretreated with naloxone (Fig. 2B). Naloxone blocked both the defeat- and aggression-induced increases in food intake. The food intakes of the naloxone-treated defeated, nondefeated and saline-treated unpaired mice were not significantly different from one another. Naloxone had no significant effect on the total food intake of unpaired control mice (Fig. 2C).

Effects of ICI-154,129

ICI-154,129 had no significant effect on defeat- and aggression-induced analgesia. ICI-154,129 also did not affect the thermal response latencies of unpaired animals (Fig. 2A).

ICI-154,129 significantly ($p<0.05$) blocked the defeat- and aggression-induced increases in activity levels. The activity levels of the ICI-154,129-treated defeated and nondefeated mice were not significantly different from those of unpaired mice. ICI-154,129 did not have any significant effect on the activity levels of unpaired mice, their activity not being significantly different from that of the saline-injected controls (Fig. 2B).

ICI-154,129 also suppressed the defeat- and aggression-induced increases in food intake. The food intake levels of the ICI-154,129-treated defeated, nondefeated, and saline-treated unpaired controls were not significantly different from one another. ICI-154,129 had no significant effect on the food intake levels of unpaired control mice (Fig. 2C).

Effects of Levorphanol

Levorphanol significantly ($p<0.05$) increased the thermal response latencies of defeated, nondefeated and unpaired

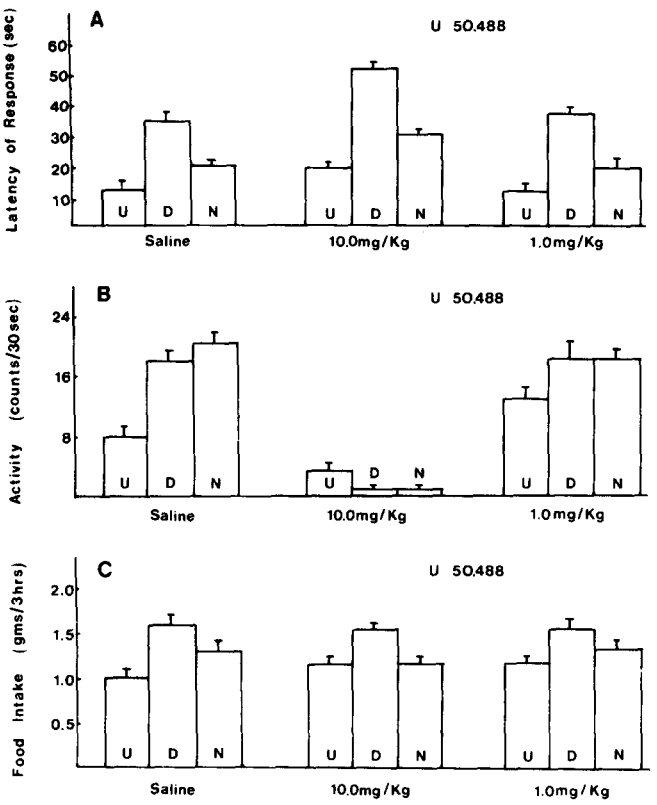

FIG. 4. (A–C) Effects of intramuscular (IM) injections of U-50,488 (1.0 and 10.0 mg/kg) and saline (1.0 ml/kg) vehicle on (A) thermal response latencies, (B) activity (30 sec) and (C) food intake over 3 hours following agonistic encounters in (D) defeated and (N) nondefeated mice. U-50,488 and saline were injected 60 minutes before the agonistic encounter. U represents unpaired control animals. Vertical lines represent standard error of the mean (n=10, in all cases). Saline (1.0 mg/kg) injected groups are repeated from Fig. 2.

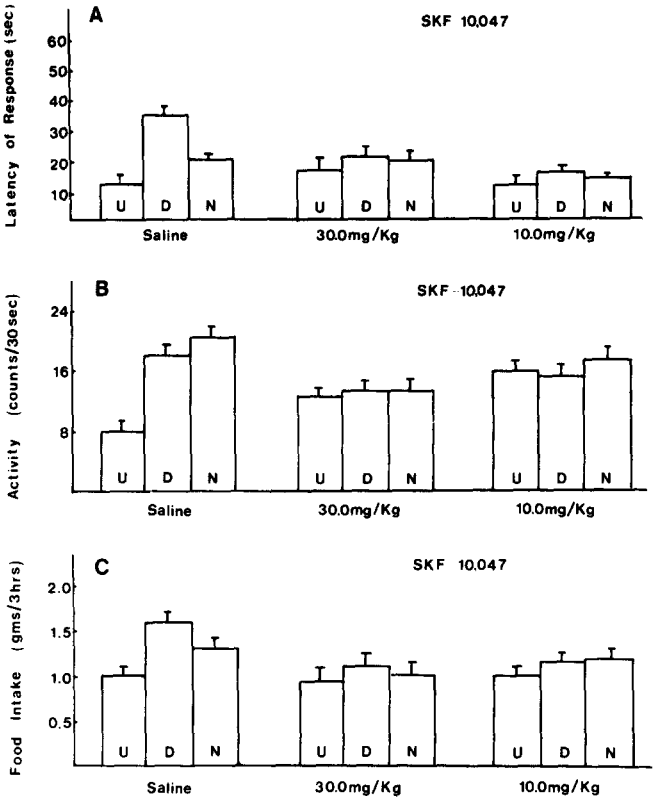

FIG. 5. (A–C) Effects of intramuscular (IM) injections of SKF-10,047 (30.0 and 10.0 mg/kg) and saline (1.0 ml/kg) vehicle on (A) thermal response latencies, (B) activity (30 sec) and (C) food intake over 3 hours following agonistic encounters in (D) defeated and (N) nondefeated mice. SKF-10,047 and saline were injected 90 minutes before the agonistic encounter. U represents unpaired control animals. Vertical lines represent standard error of the mean (n=10, in all cases). Saline- (1.0 mg/kg) injected groups are repeated from Fig. 2.

mice. Defeated, levorphanol-treated mice had significantly ($p<0.05$) higher response latencies than nondefeated levorphanol-treated mice, which in turn had significantly ($p<0.05$) higher response latencies than levorphanol treated unpaired mice (Fig. 3A). Levorphanol significantly ($p<0.05$) increased the activity levels of unpaired mice. Levorphanol had no significant effect on the elevated activity levels of defeated and undefeated mice, their activity levels not being significantly different from those of the defeated and nondefeated saline-injected animals (Fig. 3B).

Levorphanol-treated defeated, nondefeated and unpaired mice displayed significantly ($p<0.05$) greater food intake than their saline-treated counterparts (Fig. 3C). There were no significant differences over 3 hours between the food intake levels of levorphanol-treated defeat and undefeated mice. The food intakes of the latter were significantly ($p<0.05$) greater than those of the unpaired levorphanol-treated animals.

Effects of U-50,488

U-50,488 (10.0 mg/kg) significantly ($p<0.05$) increased the thermal response latencies of unpaired, defeated and nondefeated mice, while having no significant effects on the thermal response latencies of unpaired mice experiencing no aggressive encounter (Fig. 4A). Defeated mice that were treated with U-50,488 (10.0 mg/kg) displayed significantly ($p<0.01$) higher response latencies than nondefeated mice treated with U-50,488 (10.0 mg/kg), which in turn has significantly ($p<0.05$) higher response latencies than unpaired U-50,488 (10.0 mg/kg) treated mice (Fig. 4A). Defeated, nondefeated and unpaired treated with U-50,488 (1.0 mg/kg) displayed no significant differences in thermal response latencies relative to their saline-treated counterparts (Fig. 4A).

U-50,488 (10.0 mg/kg) significantly decreased the activity levels of unpaired, defeated and nondefeated mice. Defeated and nondefeated U-50,488- (10.0 mg/kg) treated mice had significantly lower activity levels than U-50,488 (10.0 mg/kg) treated unpaired mice (Fig. 4B). U-50,488 (1.0 mg/kg) caused a significant ($p<0.05$) increase in the activity levels of unpaired mice. U-50,488 (1.0 mg/kg) had no significant effect on the activity levels of defeated and nondefeated mice (Fig. 4B).

U-50,488 (10.0 and 1.0 mg/kg) had no significant ($p<0.05$) effect on food intake levels over three hours in unpaired mice. A significant ($p<0.05$) effect was observed, however, over four hours in unpaired, defeated and nondefeated mice. U-50,488 (10.0 and 1.0 mg/kg) had no significant effect on food intake levels over three hours in defeated and nondefeated mice (Fig. 4C).

Effects of (±) SKF-10,047

(±) SKF-10,047 (30.0 mg/kg) caused a significant ($p<0.05$) increase in the thermal response latencies in unpaired mice. The response latencies of (±) SKF-10,047- (30.0 mg/kg) treated defeated and nondefeated mice were not significantly different from those of unpaired (±) SKF-10,047- (30.0 mg/kg) treated mice. (±) SKF-10,047 (30.0 mg/kg) also significantly ($p<0.05$) reduced the response latencies of defeat mice (Fig. 5A). (±) SKF-10,047 (10.0 mg/kg) significantly ($p<0.05$) reduced the thermal responses of defeated and nondefeated mice such that they displayed thermal response latencies which were not significantly different from those of saline- or (±) SKF-10,047- (1.0 mg/kg) treated unpaired mice (Fig. 5A).

(±) SKF-10,047 (30.0 and 10.0 mg/kg) caused a significant ($p<0.05$) increase in the activity levels of defeated and nondefeated mice (Fig. 5B).

(±) SKF-10,047 (30.0 and 10.0 mg/kg) had no significant effect on the food intake levels of defeated, nondefeated and unpaired mice (Fig. 5C).

DISCUSSION

Opiate Agonists, Antagonists and Agonistic Encounters

The present results show that the mu, delta, kappa, and sigma opiate-directed agonists and antagonists differentially affect both the various components of aggressive interactions and the subsequent behavioral consequences of the agonistic encounters between male mice. These observations provide support for the proposal that aggressive behavior is influenced by endogenous opioids. Moreover, these findings suggest that the opioid systems differentially affect various aspects of aggression and its behavioral consequences.

Results from a number of studies have suggested that endogenous opioid systems are involved in the mediation of aggressive behaviors [2, 46, 47]. The present results extend these proposals, showing that differential opioid activation and blockade, in the subordinate animal in a "resident-intruder" paradigm, can selectively affect various components of an agonistic encounter. Previous studies examining the effect of opioid antagonists on aggressive and social behavior have yielded equivocal results. Naloxone, the prototypic mu opiate antagonists, has been reported to enhance [9,12], to inhibit [31], to have biphasic [10,45] or to have no [31] effects on shock-induced defensive fighting in various species and strains of laboratory rodents. These highly variable results may, at least in part, relate to differences in naloxone dosage. In this regard, there are proposed to be two subtypes of mu opiate receptors which may be differentially sensitive to naloxone [41]. The variable effects of naloxone may reflect differential effects on the mu subtypes as well as nonselective effects on other classes of opiate receptors, or possibly other systems implicated in the mediation of aggression [48]. Facilitatory effects of naloxone are generally observed at doses below 5 mg/kg, with inhibition of defensive fighting evident at higher doses. Interestingly, neither the facilitatory nor inhibitory effect of naloxone on shock-induced fighting can be explained by a hyperalgesic action of the antagonist [46]. In addition, naloxone reduces offensive behaviors displayed by resident rats in response to intruding conspecifics [40], but importantly it does not modify their defensive reactions. In mice, shock-induced fighting, an offensive behavior in this species, is potentiated by low doses of naloxone [44], while in resident-intruder interactions, biphasic effects have been reported. In the present study naloxone, at 1.0 mg/kg, administration to the subordinate animals altered the agonistic encounter, reducing the number of bites required to obtain defeat (EB_{50} from 35 to 25 bites) and increasing the number of bouts, while not significantly affecting other measures of aggressive behaviors. These observations substantiate previous proposals that the level of defeat-induced analgesia observed is independent of the number of bites received by the vanquished mouse [35, 52, 54].

The relatively specific delta opiate antagonist, ICI-154,129, slightly reduced the number of bites required to obtain defeat (EB_{50} from 35 to 28 bites) and increased the latency of first attack, without significantly affecting other parameters. Interestingly, and contrary to what was observed with naloxone, ICI-145,129 had no significant effect on defeat-induced analgesia. Although the degree of central penetrance of the ICI-154,129 compound has not been established, its antagonistic effects on opioid-mediated activity and feeding are consistent with central actions. Indeed the latter effect parallels the antagonistic effects of centrally administered ICI-154,129 on delta-opiate-induced feeding [21].

The present results also suggest that the effect of the delta antagonist, and presumably delta opioids, on aggression differ from that of naloxone (1.0 mg/kg) and by implication, mu opioids and may not involve "pain" perception or nociceptive mechanisms. These differences in the effects of naloxone and ICI-154,129 on agonistic encounters are consistent with previous reports of differences in the actions of the two opiates on other opiate and stress-induced behaviors [3].

It is conceivable that some of the effects of the opiate antagonists and agonists on the aggressive interactions may be associated with or secondary to, alterations in the behavioral activity and nociceptive responses of the intruder animal. In unpaired control mice the mu opiate agonist, levorphanol, had a significant analgesic effect, though the prototypic mu opiate antagonist, naloxone, did not have any significant effects. These results support previous findings that prototypical mu agonists, such as morphine and levorphanol, have analgesic effects [11], and are consistent with the overall lack of effect of naloxone on basal nociceptive responses [25]. It should be noted, however, that at higher doses, naloxone has been reported to alter the thermal response latencies, though this action may involve non-opiate-mediated effects of naloxone [49]. Both levorphanol and naloxone caused an increase in locomotor activity. Generally, mu opiates, at the relatively low doses administered here, are considered to have little effect on locomotory activity [23]. However not all measurements of activity are equivalent, and it may well be that the type of activity measure employed affects the opiate-mediated response obtained. It has also been proposed that there are two different types of mu receptors (mu_1 and mu_2), which may be differentially affected by levorphanol and naloxone, and may mediate different behavioral and physiological responses [41]. Enkephalins, endogenous delta opioid receptor directed peptides, have also been shown to affect locomotory activity [23]. Whether or not the locomotory effects of levorphanol and naloxone are mediated through the delta receptor remains to be determined. ICI-154,129, a delta opiate antagonist, at a dose of (10.0 mg/kg) caused a decrease in activity, while at higher doses (30.0 mg/kg) it has been shown to decrease activity in CF-1 mice ([6], Teskey unpublished). These results support the suggestions that ICI-154,129 has both agonist and antagonist dose-dependent properties.

ICI-154,129 at 10.0 mg/kg also had a slight analgesic effect which could be attributed to either a nonspecific, and/or nonopioid effect [19]. The specific kappa opiate agonist U-50,488 had a dose-dependent analgesic effect of CF-1 mice as well as in other rodents [20]. A low dose of U-50,488 (1.0 mg/kg) caused an increase in activity, while at a high dose (10.0 mg/kg) a general sedative effect was evident, also supporting previous observations [55]. The sigma opiate agonist, (±) SKF-10,047 produced less clear-cut effects. The mixed isomer of (±) SKF-10,047 produced severe debilitating effects on the animals for approximately 60 minutes (Teskey, unpublished). Although the thermal responses and activity levels during this period were equivalent to saline-treated animals, the mice did display marked disruptions of their normal coordinated movement. This debilitating effect of (±) SKF-10,047 lasted for approximately 60 minutes, after which both analgesia and activity levels increased, peaking at 90 minutes postinjection, then declining to basal levels. This suggests that the mixed isomer of (±) SKF-10,047 may be exerting its effect through two different modes of action. Results of previous studies indicate that (−) isomer has opioid actions while the (+) isomer possess psychotogenic properties [28,29].

These modulatory effects of the opiate agonists and antagonists on the basal activity and nociceptive responses of the intruder animal could have at least two major effects on the aggressive encounters. Firstly, increases in the activity of the intruder could shorten the latency to first attack by the intruder. Secondly, increases in pain sensitivity (lower nociceptive responses) could lead to a more rapid defeat of the intruder. Conversely, a decrease in pain sensitivity might increase the time to defeat.

With regards to the first hypothesis, there is no consistent relationship across treatments between activity level of the intruder and the latency to first attack by the resident. The delta antagonist, ICI-154,129 (10.0 mg/kg) and the high dose of the kappa agonist, U-50,488 (10.0 mg/kg), both of which decreased activity, increased the latency to first attack; while levorphanol, the low dose of U-50,488 (1.0 mg/kg) and (±) SKF-10,047, which had no effect on basal activity, decreased the latency to first attack. Moreover, naloxone (1.0 mg/kg), which increased activity, had no effect on the latency to first attack.

With regards to the second hypothesis, there is some evidence for a direct relation between the level of pain perception (basal nociceptive responses) in the intruder mouse and time taken to be defeated by the resident. The mu, sigma, and kappa opiate agonists, levorphanol, (±) SKF-10,047 and U-50,488 (10.0 mg/kg), respectively, all of which had analgesic effects, increased the number of bites required to obtain defeat. Naloxone, which blocked subsequent defeat-induced analgesia, decreased the number of bites required to obtain defeat. These results all support the suggestion that the number of bites to defeat is dependent on pain perception mechanisms. ICI-154,129 and the low dose of U-50,488 (1.0 mg/kg), which had only slight analgesic effects, had no effect on the number of bites required to obtain defeat. These latter results further suggest that only drugs with high analgesic properties or antagonistic properties for analgesia have a significant effect on the number of bites required to obtain defeat. It is, however, important to note that although the time to defeat was found to vary in proportion with the number of bites to defeat, the actual display of defeat was still independent of the total number of bites received.

The number of aggressive bouts was also altered by administration of the opiate agonists and antagonists. This raises the possibility that the effects of the opiate agonists and antagonists on the agonistic interactions may, in part, be mediated through changes in communication and/or interaction between the intruder and resident. It should, however, also be noted here that the intensity of aggression, as measured by the number of bites per unit time, did not vary after treatment with any of the opiate agonists or antagonists.

Opiate Agonists, Antagonists and the Behavioral Consequences of Agonistic Encounters

The opiate agonists and antagonists also selectively affected the analgesic, ingestive and locomotory consequences of social conflict and defeat. These results provide additional support for a differential activation of endogenous opioid systems during and/or following aggressive encounters and defeat. These findings also raised the possibility that the stress of defeat may lead to a global opioid activation. They also serve to further illustrate the utility of the "resident-intruder" paradigm and defeat in mice for analyzing stress-induced activation of opioid systems.

The demonstration of a significant analgesic response following the agonistic encounters confirms previous findings of the existence of defeat-induced analgesia [35,52]. It also confirms and extends previous observations that the agonistic encounter itself has a significant analgesic effect in the intruder animal [34, 36, 52, 54]. In addition, as previously reported, the level of defeat-induced analgesia was significantly greater than the antinociceptive response observed following just the aggressive interaction.

The analgesic response, which could be blocked by naloxone and was insensitive to ICI-154,129, may be interpreted as being mediated by mu opiate receptors. However, the reported nonspecific effects of naloxone on other classes of opiate receptors [49], as well as the possible involvement of nonopiate mechanisms [48], precludes a definitive conclusion. The kappa agonist U-50,488 enhanced the analgesic responses raising the possibility of a kappa opiate receptor or kappa opioid component being associated with the expression of the defeat-induced analgesia. Investigations with specific kappa opiate directed antagonists are necessary to address this possibility. In addition, the sigma opiate agonist (±) SKF-10,047 blocked the expression of defeated induced analgesia, suggesting a possible role for sigma opiates. However, the psychomimetic and debilitating effects associated with (±) SKF-10,047 limit any conclusions regarding the possible roles of sigma opiates in the mediation of the analgesic consequences of aggression and defeat.

The present results also show that the locomotory activity levels of the intruder mice increased after the aggressive encounters and, moreover, that the occurrence of defeat has no additional effect on this heightened activity. The increased level of activity recorded after the aggressive encounter was inhibited by ICI-154,129 and was insensitive to naloxone. These differential effects are 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 associated with an increased activity of delta opioids, (probably Met- and Leu-enkephalin) and were insensitive to antagonism by naloxone [37]. However, as indicated for the defeat-induced analgesia, the possibility of other classes of opiates being involved in the expression of

the aggression-induced increases in locomotory responses cannot be completely excluded.

The occurrence of increased ingestive responses following social conflict and defeat also supports the contention that a direct and global activation of endogenous opioid systems is associated with the agonistic encounters. The pattern and time course of the ingestive responses were analogous to the feeding behaviors observed in male CF-1 mice after either central injections of β-endorphin (considered to be a putative epsilon receptor directed opioid) and morphine, or peripheral administrations of morphine and levorphanol [52]. Moreover, the β-endorphin, morphine, levorphanol and aggression-induced ingestive responses were all similarly blocked by naloxone. These observations further suggest that a concerted, global activation of endogenous opioid systems is associated with the aggressive encounters and subsequent defeat.

The relative roles of the mu opiate receptor(s), and by inference mu opioids, in the regulation of food intake are somewhat controversial [37]. It has been suggested that the ingestive effects of prototypical mu opiate agonists, such as morphine, may arise through effects on other classes of opiate receptors, including those associated with β-endorphin [17,37]. Evidence has also been presented to suggest that mu opiates may be normally associated with the acquisition of food, while other opioid systems are involved in the actual ingestion process [27]. Recently, it has, however, been reported that mu opioid receptors in the amygdala can directly contribute to feeding [15]. Interestingly, the amygdala is a region that is also implicated in the modulation of aggression and its behavioral correlates [7].

There is also evidence that delta opiates are involved in the mediation of food ingestion. It has been shown that a number of peptides analogs with delta activity can enhance feeding after central administrations [37,50]. In the present study defeat-induced feeding was inhibited by peripheral administrations of the relatively specific delta opiate antagonists, ICI-154,129. In a parallel manner, it has been observed that central administrations of the other ICI delta antagonists decreases feeding induced by the specific delta agonists, D-Ala^2-D-Leu^5-enkephalin and, in certain cases, spontaneous feeding [21,22]. These observations thus support a role of delta opioid and delta opiate receptors in the mediation of normal and aggression- and defeat-induced feeding.

There is substantial evidence that kappa opiates and the endogenous kappa opioid, dynorphin, are major regulators of feeding [37]. A variety of kappa agonists, including the U-50,488 compound used in the present study, have been shown to enhance food intake [17,26]. In addition, dynorphin, when administered intracerebroventricularly, is a highly potent enhancer of feeding [17, 18, 37]. More localized injections have indicated that the site of action of dynorphin is in the ventro-medial hypothalamus [14,18]. All of these data indicate an important role for the kappa opioids in the regulation of normal, and most likely, aggression-induced feeding. It should be noted that the lack of effect of U-50,488 on defeat-induced feeding that was observed in the present study probably reflects a "ceiling" effect, in that the animals were already at their maximum level of food intake. Investigations with specific kappa opiate antagonists are required to determine the extent of kappa opioid involvement in the mediation of aggression and defeat-induced feeding.

In regards to the role of sigma opiates, in the present study it was observed that (±) SKF-10,047 blocked the defeated-induced feeding. Whether this results from either inhibitory effects mediated through opiate receptors, psychotomimetic actions, or debilitating effects remains to be determined. It should be mentioned that although (±) SKF-10,047 (10.0 and 30.0 mg/kg) had a nonsignificant effect on food intake over 3 hours, the food that was ingested was consumed primarily in the third hour. In addition, lower doses of (±) SKF-10,047 have been reported to have significant ingestive effects in rodents [16,24].

CONCLUSIONS

In summary, acute administrations of exogenous opioid agonists and antagonists were found to affect the agonistic encounters between male mice in a manner which is compatible and consistent with changes in activity and "pain" perception (analgesia) in the subordinate individual. The intensity of the agonistic encounters were not consistently directly affected by opioid administrations. However, the behavioral and physiological consequences of defeat and aggression were directly affected by the opiate agonists and antagonists and presumably dependent on opioid mechanisms. The display of the defeat posture in a submissive mouse caused a variety of physiological and behavioral responses, including the induction of analgesia, increased activity, and feeding, which are suggested to be mediated through the multiple opioid systems.

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