Dose- and Time-Dependent Bimodal Effects of \( \kappa \)-Opoid Agonists on Locomotor Activity in Mice

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ABSTRACT

The \( \kappa \)-opoid agonists U50488H, bremazocine, and BRL52537, and the \( \mu \)-opoid agonist morphine were compared in their ability to modify spontaneous motor activity in male NMRI mice. Higher, analgesic doses of the \( \kappa \)-agonists reduced rearing, motility, and locomotion in nonhabituated mice. These effects, as well as the analgesic action of U50488H, were blocked by the selective \( \kappa \)-opioid antagonists nor-binaltorphimine and DIPPA. In contrast, lower, subanalgesic doses (1.25 and 2.5 mg/kg for U50488H; 0.15 and 0.075 mg/kg for bremazocine, and 0.1 mg/kg for BRL52537) time dependently increased motor activity. The stimulatory effects of U50488H and bremazocine were not observed in habituated animals and were reduced by dopamine depletion. Surprisingly, the stimulatory effects of U50488H and bremazocine were not blocked by nor-binaltorphimine and DIPPA but they were completely eliminated by naloxone (0.1 mg/kg). The effects of morphine were dose-dependent; an initial limited suppression was followed by increased motility and locomotion (but not rearing) with a peak effect at 20 mg/kg both in habituated and nonhabituated mice. The selective \( \mu \)-opioid antagonist \( \beta \)-funaltrexamine blocked morphine-induced motor stimulation and analgesia but failed to affect the analgesic and motor stimulatory effects of U50488H. The results indicate that \( \kappa \)-opioid agonists interact with different functional subtypes of opioid receptors. A stimulatory, naloxone-sensitive but nor-binaltorphimine- and DIPPA-insensitive subtype of opioid receptor appears to operate only when the dopamine system is tonically active in nonhabituated animals. At higher doses, \( \kappa \)-agonists produce analgesia and motor suppression, effects mediated by a “classical” (inhibitory) \( \kappa \)-opioid receptor.

The pharmacology of opiates is complex in a number of aspects. Species differences are very obvious. In mice, morphine induces excitation and the typical Straub tail elevation, behaviors that are not observed in the rat, which mainly reacts by inhibition. Another aspect of complexity is the functional multiplicity and heterogeneity of opioid receptors elaborated by Martin (1984). The distinction of the morphine-type (\( \mu \)-) and \( \kappa \)-receptor could be confirmed by cloning (Dhawan et al., 1996). A third aspect of complexity becomes apparent in the study of the electrophysiological actions of opiates. Depending on the type of cell used and dose applied, both \( \mu \)- and \( \kappa \)-opioids can induce hyperpolarization or depolarization (Smart and Lambert, 1996) and either inhibit or stimulate neuronal cells.

In rodents it is generally observed that \( \mu \)- and \( \delta \)-agonists increase locomotion, whereas \( \kappa \)-agonists decrease locomotion (Mansour et al., 1995) in addition to inducing ataxia and sedation (Jackson and Cooper, 1988). However, in preweaning (Duke et al., 1997) and monoamine-depleted rats (Hughes et al., 1998) \( \kappa \)-opioid agonists have been reported to markedly increase locomotor activity. In Syrian hamsters \( \kappa \)-agonists elicit biphasic effects inducing hyperactivity at lower and hypoactivity at higher doses (Schurr and Walker, 1990). Opoid-induced effects on motor activity have been mainly related to interactions with mesolimbic and nigrostriatal dopamine (DA)-ergic neurotransmission, although some authors also claim the importance of substantia nigra and its non-DA projections in the motor effects of \( \mu \)- and \( \kappa \)-opioid agonists (Matsumoto et al., 1988). \( \mu \)- and \( \delta \)-Opioid receptor agonists have been shown to increase extracellular dopamine levels in the nucleus accumbens and striatum (Spanagel, 1995), whereas \( \kappa \)-opioid agonists exhibit the opposite action (Pan, 1998), effects that are blocked by the unselective opioid antagonist naxalone. Conversely, the selective \( \kappa \)-opioid antagonist nor-binaltorphimine (nor-BNI) dose dependently increased DA release (Spanagel, 1995). However, there are also conflicting results, showing that acute administration (Spanagel, 1995) or repeated injections of the \( \kappa \)-opioid agonists

ABBREVIATIONS: DA, dopamine; nor-BNI, nor-binaltorphimine 2HCl [17,17‘-(dicyclopropylmethyl)-6,6‘-imino-7,7’-bimorphinan-3,4’,14,14‘-tetrol]; \( \beta \)-FNA, \( \beta \)-funaltrexamine HCI [([E]-4-[[5x,6]-17-cyclopropyl]methyl-4,5-epoxy-3,14-dihydroxymorphinan-6-y]lamine]-4-oxo-2-butenonic acid methyl ester]; DIPPA, 2-(3,4-dichlorophenyl)-N-methyl-N’-[[(1S)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethy]lacetamide; U50488H, trans-(\( \ddagger \))-3,4-dichloro-N-methyl-N’-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide, methanesulfonate hydrate; BRL52537, (\( \ddagger \))-1-(3,4-dichlorophenyl)acet/or-2-(1-pyrrolidinyl) methylpipericine; H44-68, \( \alpha \)-methyl-p-tyrosine methyl ester.
U-69593 and U50488H (Heidbreder et al., 1998) fail to modify basal DA overflow in the nucleus accumbens and the caudate. It has also been reported that U50488H markedly increased DA release in the nucleus accumbens after local infusion (Donzanti et al., 1989) and potentiated both basal and depolarization-evoked dopamine release from a human neuroblastoma cell line (Keren et al., 1999). The reasons for these contradictory results have not been clarified.

The existence of multiple κ-opioid receptors has been proposed since the early studies of Attali et al. (1982). At least two κ-opioid receptor subtypes have been defined on the basis of in vitro binding studies, classified as κ1, a receptor subtype that preferentially binds arylnaloximides–κ-opioids such as U50488H (Lahtti et al., 1982) and κ2 (κ3), a subtype that binds benzomorphan–κ-opioids such as bremazocine (Clark et al., 1989; Horan et al., 1993). The acceptance of the existence of κ1 and κ2 subtypes has been limited due to the lack of functional evidence providing support for the data obtained in radioligand binding studies. Alternatively, the subtypes of the κ-receptor could, most probably, correspond to different affinity states of the same receptor, depending on its coupling with G proteins (Richardson et al., 1992). Cloning data have so far only provided support for the existence of a single κ-opioid receptor.

The aim of the present work was to study the influence of low (subanalgesic) and analgesic doses of the κ-opioid agonists U50488H, bremazocine, and BRL52537 on spontaneous motor activity in mice and to evaluate the sensitivity of these effects to opioid receptor blockade produced by the nonselective opioid antagonist naloxone, the selective μ-opioid antagonist β-funaltrexamine (β-FNA), and the κ-selective antagonists nor-BNI and DIPPA.

Materials and Methods

Animals. Experiments were carried out in male NMRI mice (18–22 g; B&K Universal, AB, Sollentuna, Sweden). Animals were kept under standard laboratory conditions with unlimited access to food and water. Animals were housed eight per cage in a light-controlled room (12-h light/dark cycle, lights on at 6:00 AM) at 21°C and 60% humidity. The experiments were approved by the local Ethics Board of Animal Experimentation.

Locomotor Activity System. Mice were individually tested in a dimly lit, sound-controlled area ventilated by fans. They were removed from their home cages and placed in the middle of an activity monitor (standard transparent A3 macrolon cage with 50 ml of wooden shavings on the floor) and the data-collecting system was immediately activated. In experiments with nonhabituated animals, mice were treated with the drug just before placement in the activity box and different parameters of motor activity were recorded during six 10-min intervals. In experiments with habituated animals, mice were habituated to the test cages for 30 min and thereafter the injection was made and the activity was recorded as described above.

Motor activity was measured in eight animals simultaneously by means of a multicycle red and infrared-sensitive motion detection system (Ogren et al., 1979). The system is fully computerized and uses beams of red and infrared lights in combination with vertical and horizontal photocell arrays (4-cm distance between cells) to detect movements of animals. Rearing was measured by counting the number of times an animal stands on its hind legs and interferes with any of the six invisible infrared beams passing horizontally through the cages. The height of these photocells was adapted to the size of the animal. Motility was measured as all movements of a distance of 4 cm or more detected by 48 vertical photocells, and represents a measurement of general activity. Locomotion was measured by counting the number of times an animal had covered eight horizontal photocells and moved from one side of the test cage to the other side (a distance of at least 32 cm).

Dose-dependent effects of drugs on rearing, motility and locomotion in mice were analyzed by two-way ANOVA for repeated measures, the factors being treatment (drug versus saline/control) and time (six 10-min recordings). This was followed by a post hoc Newman-Keuls test for every time point separately. The whole study was designed as a between-subjects (independent groups) experiment (i.e., each animal was used only once).

Analogesic Activity. Analogesic activity of U50488H and morphine was measured using the hot-plate (57°C) test. First, the basal nociceptive threshold was measured as the latency to paw-licking or lifting of the back limbs or jumping (whichever came first). Thereafter, the drug was injected and the nociceptive reaction was measured 30 min after treatment. The cut-off time for nonresponders was set at 30 s. The results (latencies and percentage of analgesia) were analyzed using ANOVA followed by a post hoc Newman-Keuls test.

Drugs. Naloxone HCl (Endo Laboratories, Wilmington, PA), U50488H [trans-],3,4-dichloro-N-methyl-N-2-(1-pyrrolidinyl)cyclohexyl)benzeneacetamide, methanesulfonate hydrate; Upjohn, Kalamaizou, MI, BRL52537 (t)-1-(3,4-dichlorophenyl)acetyl-2-(1-pyrrolidinyl) methylpiperidine; Tocris, Bristol, UK), morphine HCl (Sigma, St. Louis, MO), and bremazocine HCl (Research Biochemicals International, Natick, MA) were dissolved in saline and injected s.c. in a volume of 5 ml/kg just before the start of the experiment. The tyrosine hydroxylase inhibitor H44-68 (a-methyl-p-tyrosine methyl ester; AstraZeneca, Sodertalje, Sweden) was dissolved in saline and injected i.p. 2 h before an experiment. Nor-BNI 2HCl [17,17’-(dicyclopropylmethyl)-6,6’-7,7’-6,6’-imino-7,7’-bimorphinan-3,4’,14,14’-tetrol, β-FNA HCl [(E)-4-[[5,6-b]17-cycloproumethyl]-4,5-epoxy-3,14-dihydroxymorphinan-6-yl]amino]-4-oxo-2-butenic acid methyl ester, and DIPPA [2-(3,4-dichlorophenyl)-N-methyl-N-[(S)-1-(3-spirochyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide] (all from Tocris) were dissolved in water [with addition of 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals International) 1:1 w/w to dissolved compound] and injected s.c. 48 h before an experiment. All doses of drugs refer to the salts.

Results

Effects of Morphine, U50488H, Bremazocine, and BRL52537 in Nonhabituated Animals

Saline Control. In animals treated with saline (n = 16) there was a gradual decrease of motor activity over time and an almost complete elimination of rearing, motility, and locomotion after 1 h of habituation to the activity cages.

Morphine Effects (Fig. 1). There was a significant main effect of morphine treatment on motility (P < .01) and locomotion (P < .05); a significant time effect for rearing, motility, and locomotion (P < .0001, P < .001, and P < .001, respectively); and a significant dose × time interaction for all parameters [F(20,175) = 4.6, P < .001; 3.5, P < .001; and 2.4, P < .01, respectively]. The post hoc Newman-Keuls test, analyzed for each time point separately, revealed that there was a significant (P < .01) lower level of rearing 10 and 20 min after treatment with morphine at the doses of 20 and 40 mg/kg, and higher (with respect to saline-treated group) motility 30, 40, 50, and 60 min after treatment with 20 mg/kg, 40 to 60 min after treatment with 40 mg/kg, and 60 min after treatment with 10 mg/kg, and significantly higher locomotion 30 to 60 min after treatment with morphine at the dose of 20 mg/kg. Taken together, the data show that morphine in a time- and dose-dependent manner increases motility and locomotion but not rearing. The peak effect of morphine to
Enhance motor activity in mice was found at the 20-mg/kg dose.

**U50488H Effects (Fig. 1).** There was a significant effect of U50488H treatment on rearing ($P < .01$), motility ($P < .0001$), and locomotion ($P < .0001$); a significant time effect for rearing, motility, and locomotion ($P < .0001$, $P < .0001$, and $P < .0001$, respectively); and a significant dose × time interaction for all parameters ($F(20, 175) = 3.3, P < .001; 5.0, P < .0001$; and $4.6, P < .001$, respectively). The post hoc Newman-Keuls test, made for each time point separately, revealed that there was a significant ($P < .01$) increase (with respect to saline-treated group) in rearing and motility after 30 min of treatment with 1.25 mg/kg and a significant increase in rearing, motility, and locomotion after 40, 50, and 60 min of treatment with U50488H at the doses of 1.25 and 2.5 mg/kg. The lowest (1.25 mg/kg) dose of U50488H produced a monophasic stimulatory influence on motor activity, whereas the higher (2.5 mg/kg) dose exhibited a biphasic influence with inhibition of motor activity at 10 and 20 min and stimulation of activity at 40, 50, and 60 min post injection. Treatment with the 5-mg/kg dose tended to exhibit effects similar to those with 2.5 mg/kg, but the motor stimulation did not reach statistical significance. However, there was a significant inhibition of all parameters of motor activ-

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**Fig. 1.** Influence of morphine and U50488H on motor activity in nonhabituated mice. The agent was injected s.c. just before placement of the animals in the activity cages. The data represent the cumulative counts for rearing, motility, and locomotion in 10-min intervals (mean values, $n = 8$ group). For statistical significance see Results. The error bars are not shown to increase the clarity of the figure. The numbers in the legends present the doses of the drugs used (mg/kg).
ity after treatment with 5 or 10 mg/kg U50488H at 10 and 20 min after injection. In summary, U50488H displayed a dose-related influence on the locomotor activity of mice with inhibition at higher doses and stimulation at lower doses (preceded by slight initial suppression). The threshold dose range for the shift from stimulation to inhibition was between 2.5 and 5.0 mg/kg.

Bremazocine Effects (Fig. 2). There was a significant effect of bremazocine treatment on motility (\(P < .0001\)) and locomotion (\(P < .0001\)); a significant time effect for rearing, motility, and locomotion (all \(P < .0001\)); and a significant dose \times time interaction for all parameters \([F(20,175) = 4.9, P < .001; 12.5, P < .0001;\) and 11.6, \(P < .001\), respectively]. The post hoc test (made for each time point separately) revealed that there was a significant \((P < .01)\) increase (compared with saline) in motility and locomotion at 40 min and in all parameters at 50 and 60 min with 0.075 mg/kg, and a significant increase after treatment with 0.15 mg/kg in motility at 50 min and in all parameters at 50 and 60 min. The effect was biphasic with an inhibition of motor activity at 10 and 20 min and stimulation of activity at 40, 50, and 60 min. At higher (0.312 mg/kg and higher) doses there was a signifi-

![Graphs showing Bremazocine and BRL 52537 effects on motor activity in nonhabituated mice](image-url)
Significant inhibition of all parameters of motor activity at 10-, 20-, and 30-min time measurements. Experiments were also performed with 1.25-, 2.5-, and 5.0-mg/kg doses of bremazocine (data not shown). These doses produced a complete reduction of all parameters of motor activity at all time points of measurement. In summary, similar to the experiments with U50488H, bremazocine exhibited a biphasic influence on motor activity with an initial inhibition followed by a stimulation at lower doses and an inhibition at doses of 0.312 mg/kg and higher.

**BRL52537 Effects (Fig. 2).** There was a significant effect of BRL52537 treatment on rearing \( (P < .001) \), motility \( (P < .0001) \), and locomotion \( (P < .0001) \); a significant time effect for rearing, motility, and locomotion \( (all \ P < .0001) \); and a significant dose \( \times \) time interaction for all parameters \( [F(15,140) = 3.9, P < .001; 6.8, P < .0001; and 4.1, P < .001, respectively] \). The post hoc test (made for each time point separately) revealed that there was an increase (with respect to the saline-treated group) in rearing 40 to 60 min after treatment with 0.1 mg/kg and an increase in motility and locomotion at 30 to 60 min. Treatment with a dose of 0.01 mg/kg produced a significant increase in rearing at 30 min, in motility at 30 to 50 min, and in locomotion at 40 min. There was a significant \( (P < .01) \) decrease in all parameters (compared with saline control) at all time points after treatment with 1.0 mg/kg. Thus, the highest dose used (1.0 mg/kg) inhibited all parameters of motor activity, whereas lower doses had a stimulating effect, i.e., the same biphasic pattern that was observed with the other \( \kappa \)-agonists.

**Effects of Morphine, U50488H, and Bremazocine in Habituated Animals (Fig. 3)**

Animals habituated to the motor activity cages for 30 min were tested in analogy with the experiments using nonhabituated animals. ANOVA analysis indicated a significant main stimulatory effect of morphine (10 and 20 mg/kg) treatment on motility (both \( P < .01 \)) and locomotion (20 mg/kg, \( P < .05 \)) but failed to reveal a significant influence of the two lower doses of U50488H and bremazocine. The effects of higher doses of \( \kappa \)-agonists were inconclusive because the activity was strongly reduced by the habituation and could not be further inhibited (floor-effect).

**Fig. 3.** Influence of morphine, U50488H, and bremazocine on the motor activity in habituated mice. Either drug was injected s.c. after 30 min of habituation in the test cages (habituation scores are not shown). For further details see legend to Fig. 1. The high level of all parameters during the first 10-min interval represents the interaction with the counting system due to the handling and injection procedure. The data represent the cumulative counts for rearing, motility, and locomotion in 10-min intervals (mean values and standard errors, \( n = 8 \)/group).
Effect of Pretreatment with Dopamine Depletor H44-68 on the Motor Effects of U50488H (Fig. 4)

Mice were pretreated with H44-68 (100 mg/kg i.p.) 2 h before injection of U50488H (1.25 and 10 mg/kg) or saline. Locomotor activity was evaluated in nonhabituated animals. There was a significant treatment effect on motility and locomotion \( (P < .05\) and \( P < .01\), respectively) and a significant treatment \( \times \) time interaction for rearing \( [F(10,65) = 9.2, P < .01]\) and locomotion \( [F(10,65) = 2.3, P < .05]\). The time effect was also significant \( (P < .0001)\) for all parameters. The post hoc comparison revealed a significant \( (P < .01, \) against saline) decrease in rearing at 20 min in both U50488H-treated groups but no differences between high- and low-dose treatment groups. A significant decrease in locomotion was found in both U50488H-treated groups at 20 and 30 min. Again, there were no significant differences between the high- and low-dose U50488H groups.

Effects of Motor Stimulatory Doses of U50488H and Bremazocine in Nor-BNI-, DIPPA-, or Naloxone-Pretreated Nonhabituated Animals

Effects of Antagonists Alone. Nor-BNI (6 mg/kg) and DIPPA (4 mg/kg) were injected s.c. 48 h before an experiment. Naloxone (0.1 mg/kg) was injected s.c. just before an experiment. ANOVA analysis (data not shown) failed to reveal a significant influence of either compound on motor activity (compared with saline-treated animals) or a treatment \( \times \) time interaction.

U50488H and Bremazocine Effects in Nor-BNI-Pretreated Animals (Fig. 5, Left). There were significant treatment effects on rearing, motility, and locomotion \( (P < .05, P < .05, \) and \( P < .05, \) respectively) and significant treatment \( \times \) time interactions for all parameters \( [F(10,205) = 21.3, P < .0001; 86.2, P < .0001; \) and \( 129.6, P < .0001, \) respectively). The time effect was significant \( (P < .0001)\) for all parameters. The post hoc comparison revealed a significant \( (P < .01, \) comparison with saline-treated group) increase in rearing at 20, 30, 40, 50, and 60 min in the U50488H (1.25 mg/kg)-treated group and at 50 and 60 min in the bremazocine (0.075 mg/kg)-treated group. There was also a significant increase in motility and locomotion at 30, 40, 50, and 60 min in the U50488H (1.25 mg/kg)-treated group and at 40, 50, and 60 min in the bremazocine (0.075 mg/kg)-treated group. However, at 20 min bremazocine produced a significant inhibition of motility and locomotion. Taken together, the results show that nor-BNI treatment failed to influence the locomotor stimulation produced by low doses of U50488H and bremazocine.

U50488H Effects in DIPPA-Pretreated Animals (Fig. 5, Middle). There were significant pretreatment effects (DIPPA versus vehicle) effects on rearing and significant treatment \( (U50488H \) versus saline) effects on rearing, motility, and locomotion \( (P < .01, P < .001, \) and \( P < .001, \) respectively). The time effect was significant \( (P < .001)\) for all parameters. Treatment \( \times \) time interactions were significant for motility and locomotion \( [F(15,100) = 3.4, P < .01; \) and \( F(15,100) = 1.8, P < .05, \) respectively]. The post hoc comparison revealed a significant \( (P < .01, \) comparison with vehicle-saline-treated group) increase in motility and locomotion at 40, 50, and 60 min in the U50488H (1.25 mg/kg)-treated groups (both pretreated with vehicle and DIPPA) in comparison with the vehicle-saline and DIPPA-saline groups. Rearing also increased in the U50488H (1.25 mg/kg)-treated group, pretreated with vehicle, but not in the group pretreated with DIPPA (4 mg/kg). In summary, DIPPA pretreatment reduced the stimulation of rearing produced by the \( \kappa\)-agonist, but failed to influence stimulation of motility and locomotion.

U50488H and Bremazocine Effects in Naloxone-Pretreated Animals (Fig. 5, Right). ANOVA revealed a significant treatment effect only for locomotion \( (P < .05)\). However, time effect and treatment \( \times \) time interactions were significant \( [F(15,100) = 12.6, 8.9, \) and \( 11.7; \) all \( P < .0001)]\) for all parameters. Post hoc comparison revealed a significant \( (P < .01)\) inhibition of rearing, motility, and locomotion in bremazocine-treated animals at 20 min. However, there was
a significant increase in rearing and motility in bremazocine-treated mice at 60 min. With respect to the group Sal + U50488H (1.25 mg/kg), there was a significant inhibition ($P < .01$) of all parameters of activity starting from 30 min after $\kappa$-agonist injection in the group naloxone + U50488H (1.25 mg/kg). However, no differences in activity between groups “naloxone + vehicle” and “naloxone + U50488H” were found. It was concluded that naloxone (0.1 mg/kg) completely abolished the motor stimulation produced by the $\kappa$-agonists. Similar results were obtained with 1.0 mg/kg naloxone (data not shown). The lack of effect at 60 min might be explained by the short lasting effect of naloxone.

**Effects of a High (Motor-Inhibitory) Dose of U50488H in Nor-BNI- and DIPPA-Pretreated Animals**

**U50488H Effects in Nor-BNI-Treated Animals (Fig. 6).** There was a significant treatment effect on rearing, motility, and locomotion ($P < .01, P < .01$, and $P < .01$, respectively) and a significant treatment $\times$ time interaction for all parameters [$F(10,100) = 2.3, P < .05; 4.3, P < .01; and 4.4, P < .01$, respectively]. The time effect was significant ($P < .0001$) for all parameters. The post hoc comparison revealed a significant ($P < .01$, against saline) decrease in rearing at 20, 30, and 40 min in the U50488H treated group pretreated with vehicle, and a significant increase in rearing at 50 and 60 min in the nor-BNI-pretreated and U50488H (10 mg/kg)-treated group. The same differences (i.e., decrease in activity at 20, 30, and 40 min in the vehicle + U50488H and increase in activity at 50 and 60 min in the nor-BNI + U50488H group) were found for motility and locomotion. Taken together, the results show that nor-BNI pretreatment eliminated the motor inhibitory effect of U50488H and reverted the effect of the drug toward moderate motor stimulation.

**U50488H Effects in DIPPA-Treated Animals (Fig. 6).** There were significant treatment effects on rearing, motility, and locomotion ($P < .01, P < .001$, and $P < .001$, respectively) and significant treatment $\times$ time interaction for all parameters [$F(15,120) = 6.6, P < .01; 17.7, P < .001; and 17.1, P < .001$, respectively]. The time effect was significant ($P < .0001$) for all parameters. In the group pretreated with vehicle, U50488H significantly ($P < .01$, comparison with vehicle-saline-treated group) inhibited rearing, motility, and locomotion at 20, 30, and 40 min. The post hoc comparison revealed a significant increase in rearing, motility, and locomotion at
40, 50, and 60 min in the U50488H (10 mg/kg)-treated group, pretreated with DIPPA. In summary, DIPPA pretreatment changed the effect profile of U50488H with no action in the first 30 min after administration and reversal of the inhibitory to a stimulatory action, 40 to 60 min after injection.

**Effect of Nor-BNI Pretreatment on the Analgesic Activity of U50488H in the Hot-Plate Test (Table 1)**

Nor-BNI (6 mg/kg) was injected s.c. to a separate group of mice 48 h before the injection of U50488H similarly to that described for experiments on motor activity. An overall repeated measurement analysis of response latencies was performed with drug treatment (nor-BNI-treated versus vehicle-treated mice) and U50488H dose levels (four levels: 2.5, 5.0, 10, and 20 mg/kg) as between-group factors and time (basal versus 30-min values) as repeated measurement factor. ANOVA revealed that at 30 min the main effect of nor-BNI treatment ($P < .0001$), U50488H unit dose ($P < .01$), and dose × treatment interaction ($F(3, 24) = 6.20, P < .01$) were significant. The time effect was not significant, although the

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**Fig. 6.** Effects of U50488H (10 mg/kg s.c.) on motor activity of mice pretreated with nor-BNI (6 mg/kg s.c., 48 h before test) or DIPPA (4 mg/kg s.c., 48 h before test). For further details see legend to Fig. 1. The data represent the cumulative counts for rearing, motility, and locomotion in 10-min intervals (mean values and standard errors, $n = 8$ / group).
time × dose × treatment interaction was significant \((P < .05)\). In vehicle-pretreated animals 10 and 20 mg/kg U50488H produced a significant increase in nociceptive latencies at 30 min \((P < .01)\). In nor-BNI-pretreated animals neither dose of U50488H produced a significant analgesic effect.

**Effect of DIPPA Pretreatment on the Analgesic Activity of U50488H in the Hot-Plate Test (Table 2)**

DIPPA \((0.5, 1.0, 2.0, \text{and} 4.0 \text{ mg/kg})\) or its vehicle were injected s.c. 48 h before the experiment. After testing the basal nociceptive reaction in the hot-plate \((57°C)\) mice were treated with U50488H \((10 \text{ mg/kg})\) and nociceptive scores were measured 30 min after \(\kappa\)-agonist injection. Two-way ANOVA analysis of the nociceptive latencies (time factor: basal versus 30-min measurement; treatment factor: four doses of DIPPA and saline) revealed significant time effect \((P < .001)\), treatment effect \((P < .01)\), and time × treatment interaction \([F(3,24) = 8.8, P < .001]\). In vehicle-pretreated animals 10 mg/kg U50488H produced significant analgesia \((P < .01)\). Treatment with DIPPA at the dose of 4 mg/kg eliminated the analgesic effect of U50488H. In groups treated with DIPPA at the doses 0.5, 1.0, and 2.0 mg/kg, the analgesic effect of U50488H was less pronounced but still significant \((P < .05)\).

**Effect of \(\beta\)-FNA Pretreatment on Analgesic and Stimulatory Effects of U50488H and Morphine**

**Analgesia (Table 3).** Animals were pretreated with either \(\beta\)-FNA \((20 \text{ mg/kg s.c.)}\) or its vehicle and 48 h later the analgesic activity of morphine \((5 \text{ and } 10 \text{ mg/kg s.c.)}\) and U50488H \((5 \text{ and } 10 \text{ mg/kg s.c.)}\) was evaluated. The basal nociceptive reaction was measured before either U50488H or morphine treatment and nociceptive scores were remeasured 30 min after opioid agonist injection. An overall ANOVA analysis of the nociceptive latencies (time factor: basal versus 30-min measurement; treatment factor: saline, two doses of morphine and two doses of U50488H; pretreatment factor: \(\beta\)-FNA versus vehicle) revealed significant time effect \((P < .0001)\), treatment effect \((P < .01)\), and pretreatment effect \((P < .05)\) with significant \((P < .01)\) interactions between factors. In vehicle-pretreated animals both doses of morphine and U50488H produced significant analgesia \((P < .01)\). In \(\beta\)-FNA-treated groups only U50488H (both doses) produced significant increase in nociceptive latencies. In summary, pretreatment with \(\beta\)-FNA eliminated the analgesic effect of morphine but failed to affect analgesia produced by U50488H.

**Stimulatory Effect (Fig. 7).** Mice were pretreated with either vehicle or \(\beta\)-FNA and the effects of morphine \((20 \text{ mg/kg})\) or U50488H \((1.25 \text{ mg/kg})\) on locomotor activity were evaluated 48 h after pretreatment. In morphine-treated groups two-way repeated measurement ANOVA (group factor: \(\beta\)-FNA + morphine, vehicle + morphine, vehicle + saline; time factor: six 10-min intervals) revealed significant treatment effect on rearing and motility \((P < .05 \text{ and } P < .01, \text{ respectively})\) and significant treatment × time interaction for all parameters \((P < .001, P < .05, \text{ and } P < .01, \text{ respectively})\). The time effect was highly significant \((P < .0001)\) for all parameters. The post hoc comparison revealed a significant \((P < .01, \text{ compared with saline-treated group})\) decrease in rearing at 20 and 30 min in both morphine-treated groups with no differences between \(\beta\)-FNA- and vehicle-treated groups. A significant increase in motility at 30, 40, 50, and 60 min and a significant increase in locomotion at 50 and 60 min was found in the morphine-treated group pretreated with vehicle.

In U50488H-treated groups ANOVA revealed a significant treatment effect on rearing, motility, and locomotion \([F(2,17) = 6.0, P < .01; 13.2, P < .01; \text{ and } 31.5, P < .001, \text{ respectively})\) and no significant treatment × time interaction for all parameters. The time effect was significant \((P < .0001)\) for all parameters. An increase in rearing, motility, and locomotion was found in both U50488H-treated groups, how-

### Table 1

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>U50488H</th>
<th>Basal</th>
<th>T30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mg/kg</strong></td>
<td><strong>s</strong></td>
<td><strong>s</strong></td>
<td><strong>s</strong></td>
</tr>
<tr>
<td>Vehicle</td>
<td>20</td>
<td>11.52 ± 1.20</td>
<td>22.01 ± 1.58*</td>
</tr>
<tr>
<td>U50488H 10 mg/kg</td>
<td>9.52 ± 0.83</td>
<td>12.75 ± 1.46</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11.52 ± 1.72</td>
<td>13.08 ± 1.80</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>10.56 ± 1.39</td>
<td>10.32 ± 1.58</td>
<td></td>
</tr>
<tr>
<td>0 (saline)</td>
<td>10.15 ± 1.40</td>
<td>10.32 ± 2.11</td>
<td></td>
</tr>
<tr>
<td>Nor-BNI</td>
<td>20</td>
<td>10.94 ± 1.25</td>
<td>10.44 ± 1.73</td>
</tr>
<tr>
<td>U50488H 10 mg/kg</td>
<td>10.51 ± 0.53</td>
<td>10.76 ± 0.67</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>11.19 ± 1.70</td>
<td>10.97 ± 1.00</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>10.33 ± 1.37</td>
<td>10.30 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>0 (saline)</td>
<td>9.85 ± 2.11</td>
<td>10.30 ± 2.41</td>
<td></td>
</tr>
</tbody>
</table>

* Significant increase in nociceptive latencies \((P < .01)\) compared with the basal level of the nociceptive reaction.

### Table 2

<table>
<thead>
<tr>
<th>DIPPA</th>
<th>Basal</th>
<th>T30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mg/kg</strong></td>
<td><strong>s</strong></td>
<td><strong>s</strong></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0 (vehicle)</td>
<td>9.15 ± 0.83</td>
</tr>
<tr>
<td>DIPPA 0.5</td>
<td>9.08 ± 0.63</td>
<td>13.80 ± 0.98*</td>
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<tr>
<td>DIPPA 1</td>
<td>8.60 ± 0.43</td>
<td>12.68 ± 1.06*</td>
</tr>
<tr>
<td>DIPPA 2</td>
<td>8.95 ± 0.95</td>
<td>12.00 ± 0.16*</td>
</tr>
<tr>
<td>DIPPA 4</td>
<td>10.65 ± 0.63</td>
<td>10.95 ± 0.52</td>
</tr>
</tbody>
</table>

* Significant increase in nociceptive latencies \((** P < .01 \text{ and } * P < .05)\) compared with the basal level of nociceptive reaction.

### Table 3

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Basal</th>
<th>T30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-FNA</strong></td>
<td><strong>s</strong></td>
<td><strong>s</strong></td>
<td><strong>s</strong></td>
</tr>
<tr>
<td>Morphine 10 mg/kg</td>
<td>10.30 ± 0.83</td>
<td>12.75 ± 1.46</td>
<td></td>
</tr>
<tr>
<td>Morphine 5 mg/kg</td>
<td>10.12 ± 0.74</td>
<td>11.70 ± 0.56</td>
<td></td>
</tr>
<tr>
<td>U50488H 10 mg/kg</td>
<td>9.80 ± 0.56</td>
<td>20.13 ± 2.34*</td>
<td></td>
</tr>
<tr>
<td>U50488H 5 mg/kg</td>
<td>9.70 ± 0.59</td>
<td>16.85 ± 1.10*</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>9.85 ± 1.12</td>
<td>8.97 ± 2.11</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>9.63 ± 1.40</td>
<td>23.40 ± 2.07*</td>
<td></td>
</tr>
<tr>
<td>Morphine 5 mg/kg</td>
<td>9.78 ± 1.12</td>
<td>18.68 ± 2.23*</td>
<td></td>
</tr>
<tr>
<td>U50488H 10 mg/kg</td>
<td>10.95 ± 0.96</td>
<td>20.75 ± 2.22*</td>
<td></td>
</tr>
<tr>
<td>U50488H 5 mg/kg</td>
<td>9.52 ± 1.02</td>
<td>15.33 ± 0.75*</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>10.15 ± 1.26</td>
<td>10.34 ± 1.50</td>
<td></td>
</tr>
</tbody>
</table>

* Significant increase in nociceptive latencies \((P < .01)\) compared with the basal level of nociceptive reaction.
Moreover, without obvious differences between \(\beta\)-FNA- and vehicle-treated groups. In summary, treatment with \(\beta\)-FNA reduced the analgesic and motor stimulatory effects of morphine but not those of U50488H.

**Discussion**

With the development of transgenic technology, the mouse has become a species of interest for behavioral studies. Extrapolation from rat to mouse is, however, not trivial. For example, in the opioid field mice and rats differ in the amount and distribution of opioid receptor subtypes and in their way of responding to opioid drugs. Rats and mice respond to \(\mu\) and \(\delta\)-opioid agonists in an opposite way, giving sedation in rats but stimulation in mice. However, the present study indicates that \(\kappa\)-opioid agonists seem to give similar effects in rats and mice at higher doses and opposite effects at lower doses. It was unexpected that low subanalggesic doses of the \(\kappa\)-opioid agonists U50488H, BRL52537, and bremazone can stimulate spontaneous motor activity in NMRI male mice, whereas higher, analgesic doses of the drugs reduce locomotor activity. The stimulatory effect was present only in animals not habituated to the new test environment and it was not blocked by \(\kappa\)-opioid antagonists, but it was significantly reduced in naloxone-treated animals. On the contrary, both nor-BNI and DIPPA blocked the inhibitory effect of high doses of U50488H on locomotor activity and reversed it to moderate stimulation. Finally, it was found that H44-68, which has been shown to block \(\Delta\)-amphetamine-induced motor stimulation in mice via dopamine depletion (Ogren and Ross, 1977), totally eliminated the motor stimulatory effects of U50488H without altering motor inhibitory effects.

Binding studies have shown that U50488H is selective for \(\kappa\)-opioid receptors with little affinity for \(\mu\)- and \(\delta\)-receptors (Clark et al., 1983). Bremazone is less selective with affinity for \(\kappa\), \(\mu\), and \(\epsilon\)-receptors (Tseng and Collins, 1991). Nevertheless, both U50488H and bremazone are considered prototype \(\kappa\)-opioid agonists (Dhawan et al., 1996). U50488H has been shown to bind to the \(\kappa1\) subtype of receptors, whereas bremazone interacts with all subtypes of \(\kappa\)-opioid receptors (i.e., also binds to \(\kappa2/\kappa3\) receptors) (Nock et al., 1990, Horan et al., 1993). BRL52537 was shown to be a highly selective \(\kappa\)-opioid agonist without preferable binding to any subtype of \(\kappa\)-receptors (Vecchietti et al., 1991). Both nor-BNI and DIPPA are highly selective \(\kappa\)-opioid receptor antagonists in vivo and they exert long-lasting antagonistic effects, which may persist for at least 1 month (Horan et al., 1992; Jones and Holtzman, 1992; Broadber et al., 1994; Chang et al., 1994). However, their preferences for subtypes of \(\kappa\)-opioid receptors or species differences are not known.

The type of receptor activated by low doses of \(\kappa\)-agonists and resulting in motor stimulation is not easily defined. It could be argued that it is not a \(\kappa\)-receptor because nor-BNI is inactive. However, DIPPA is partially active, giving evidence for a limited \(\kappa\)-opioid receptor involvement. The observation that naloxone at a low (preferentially \(\mu\)-selective) dose blocks the stimulatory effects of U50488H and bremazone further supports an opioid receptor involvement. The failure of \(\beta\)-FNA to influence the stimulation produced by \(\kappa\)-agonists excludes the possibility of \(\mu\)-opioid receptor involvement. This paradoxical finding remains to be studied further.

The observation that \(\kappa\)-agonists stimulate motor activity only in nonhabituated animals with intact DA-ergic neurons indicates that \(\kappa\)-opioids elicit the stimulatory effects probably by lowering the threshold for activation of dopaminergic neurons by sensory stimulation. In fact, depletion of dopamine

**Fig. 7.** Locomotor effects of U50488H (1.25 mg/kg s.c.) and morphine (20 mg/kg s.c.) on motor activity in mice pretreated with \(\beta\)-FNA (20 mg/kg s.c., 48 h before agonist treatment). For further details see legend to Fig. 1. The data represent the cumulative counts for rearing, motility, and locomotion in 10-min intervals (mean values and standard errors, \(n = 8\)/group).
by H44-68 totally eliminated the stimulatory effects of US0488H, indicating the requirement of an intact dopaminergic system for the stimulatory effects of k-opioid agonists. Differences in the activation of DA systems may also give some explanation for the discrepancies in the data obtained in microdialysis studies (see the Introduction).

On the basis of electrophysiological studies it was previously suggested that k-opioid agonists elicit either stimulatory or inhibitory influence on neurons, depending on the dose applied (Crain and Shen, 1990; Keren et al., 1999). Studies in primary dorsal root ganglion cultures showed that neuronal stimulation occurred at nanomolar concentrations of k-opioids, whereas inhibition occurred at micromolar concentrations. This led to the proposal that there might be separate stimulatory and inhibitory k-opioid receptor subtypes linked to different effector systems (Crain and Shen, 1990). Stimulatory effects are blocked by choleratoxin treatment, whereas inhibitory effects are blocked by pertussis toxin treatment (Shen and Crain, 1990), indicating receptor coupling through Gs or Gi proteins, respectively. Because the natural concentration of the endogenous k-opioid ligand dynorphin is in the picomolar range (You et al., 1994) it is likely that the physiological effects of k-opioids are stimulatory. Although it is difficult to extrapolate electrophysiological data obtained in vitro to behavioral observations, the analogy with the present results is intriguing, and suggests that two functional subtypes of k-opioid receptors exist. Subtype A (probably inhibitory) has low affinity for agonists, mediates antinociceptive and motor inhibitory effects of k-opioids, and is blocked by the k-opioid antagonist nor-BNI. Subtype B (presumably stimulatory) has high affinity for agonists and contributes negligibly to the analgesic effects of k-opioids, but it mediates the motor stimulatory effects of k-opioids. This receptor is insensitive to antagonism by nor-BNI. Subtype A receptors may be linked to Gi or Go proteins, whereas subtype B receptors may be associated with Gs proteins. These two “subtypes” may in fact relate to two different conformations of a single receptor activating different second messenger pathways (Pauwels and Wurch, 1998). It is important to note that the proposed functional subtypes of receptors do not relate to the k1 and k2 terminology suggested on the basis of binding studies. Both US0488H (k1-selective) and bremazocine (k-unsselective) inhibited motor activity at higher doses while stimulating this activity at lower doses (although the time pattern of the stimulatory effects of the two drugs was slightly different). The inhibitory effects of k-agonists are readily inhibited at the “inhbitory” subtype of k-opioid receptors leaving effects at the stimulatory subtypes, which are less sensitive to antagonist blockade. In summary, k-opioid agonists US0488H, BRL52537, and bremazocine exhibit a bimodal dose-dependent effect on spontaneous motor activity of mice. It is not possible to conclude at this stage whether this effect can be generalized to other species than mice. The two opposite k-opioid receptor-mediated effects (i.e., stimulation at low doses and inhibition at higher) are most likely linked to different functional subtypes of k-opioid receptors or high- and low-affinity states of the same receptor. The stimulatory effect of k-opioids was observed only in nonhabituated animals and could be blocked by DA depletion, which probably indicates that tonic activity in DA-ergic neuronal systems is required for the stimulatory effect. The tonic activity of the endogenous k-opioid (=dynorphin) systems in systems related to reward, memory, neuronal survival, etc., is probably low. Consequently, the effects of low, subanalgescic doses of k-opioid agonists should be explored further.

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References


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