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European Journal of Pharmacology 276 (1995) 257–266

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μ- and κ-opioid receptor-mediated opioid effects on social play in juvenile rats

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Received 21 November 1994; revised 11 January 1995; accepted 13 January 1995

Abstract

Previously, morphine has been shown to influence social play behavior in rats on two levels. An increasing effect on social play was interpreted as an effect on the rewarding aspects of social play. A lower dose of morphine abolished the effects of an unfamiliar environment on social play, supposedly by affecting the integration of environmental stimuli. In the present study the effects of receptor-specific opioid drugs on social play and measures of social behavior unrelated to play were investigated. Fentanyl, a μ-opioid receptor agonist, seemingly mimicked both effects of morphine. The μ-opioid receptor antagonist, β-funaltrexamine, decreased social play, although a low dose of this drug increased it. BUBUC (Tyr-D-Cys(StBu)-Gly-Phe-Leu-Thr(OtBu)) and naltrindole, a δ-opioid receptor agonist and δ-opioid receptor antagonist, respectively, had no effects on social behavior. The κ-opioid receptor agonist, U50,488H (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide), dose dependently suppressed all measures of social behavior. The κ-opioid receptor antagonist, nor-binaltorphimine, abolished the effects of an unfamiliar environment on social play. These studies suggest that the opioidergic effect on social play is mediated through μ- and κ-opioid receptor systems.

Keywords: Opioid; Social behavior; Play behavior; μ-Opioid receptor; δ-Opioid receptor; κ-Opioid receptor

1. Introduction

Opioid systems have been shown to be involved in motivational processes and rewarded behaviors (Van Ree, 1987; Bozarth, 1988; Wise, 1989). Social play behavior is known to be a form of behavior with rewarding properties (Humphreys and Einon, 1981; Normansell and Panksepp, 1990; Calcagnetti and Schechter, 1992). Accordingly, a number of studies suggest that brain opioid systems are involved in the regulation of social play behavior. Treatment with morphine enhances (Panksepp et al., 1985; Niesink and Van Ree, 1989; Vanderschuren et al., 1995a) and treatment with the opioid receptor antagonists, naloxone or naltrexone, depresses social play (Beatty and Costello, 1982; Panksepp et al., 1985; Siegel and Jensen, 1986; Jalowiec et al., 1989; Niesink and Van Ree, 1989). In vivo autoradiography experiments have shown social play to displace [^{3}H]diprenorphine binding in a variety of brain areas, suggesting that release of opioid peptides takes place during social play (Panksepp and Bishop, 1981; Vanderschuren et al., submitted).

In juvenile rats, a distinction can be made between social behaviors related to play (social play; pinning, boxing/wrestling and following/chasing) and social behaviors unrelated to play (social exploration and contact behavior). These forms of social behavior differ regarding their ontogenetic pattern, in that social play mainly occurs between weaning and puberty (Bolles and Woods, 1964; Baenninger, 1967; Panksepp, 1981; Hole, 1988), whereas social behaviors unrelated to play occur during the entire lifespan of rats. In bouts of social play a strong correlation has been found between pinning, boxing and chasing (Poole and Fish, 1975; Panksepp and Beatty, 1980), while social exploration and contact behavior are not associated with social play to such an extent. Furthermore, social be-

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SSDI 0014-2999(95)00040-2

haviors related and unrelated to play may be mediated by different neuronal systems (Beatty et al., 1982; Beatty, 1983; Holloway and Thor, 1983; Thor and Holloway, 1983; Pellis et al., 1993; Vanderschuren et al., 1995a).

A previous study showed two different effects of morphine on social play behavior (Vanderschuren et al., 1995a). Morphine treatment powerfully increased pinning, boxing/wrestling and following/chasing, which was interpreted as an enhancement of the rewarding properties of social play. When rats are tested for social play in an unfamiliar environment, pinning and boxing/wrestling are initially suppressed, while total levels of play are not affected (Vanderschuren et al., 1995b). A dose of morphine 10 times lower than the dose that maximally increased social play, was capable of abolishing this initial suppression of social play. A morphine-induced shift in selective attention due to altered integration of sensory stimuli (Arnsten et al., 1981) could underlie this effect. Under normal circumstances, rats explore an unfamiliar environment before engaging in social play, but morphine-treated animals displayed a time course of social play under unfamiliar conditions as if they were tested in a familiar environment. Morphine treatment hardly affected social behaviors not related to play. The finding that morphine exerted these two effects at different doses suggested that opioid systems are involved in the regulation of social play behavior on two levels (reward and integration of environmental stimuli).

Three main classes (μ, δ and κ) of opioid receptors have been described. These three classes have been shown to differ regarding distribution in the brain (Mansour et al., 1987, 1988; Tempel and Zukin, 1987), as well as pharmacological properties (Gillan and Kosterlitz, 1982; Goldstein and Naidu, 1989; Fowler and Fraser, 1994). Recently, cloning of these receptors revealed that they are different proteins, although with marked similarities in their primary structures (Reisine and Bell, 1993; Uhl et al., 1994). The existence of subtypes of μ-, δ- and κ-opioid receptors has been suggested (Traynor and Elliott, 1993; Fowler and Fraser, 1994), but as yet there is no consensus regarding their pharmacological or functional specificity (Schoffelmeer and Mulder, 1994; Traynor, 1994). Also, brain μ- and δ-opioid receptors are thought to occur as separate molecules as well as in a μ-δ-opioid receptor complex (Rothman et al., 1993).

Involvement in reward-related processes has been shown for all types of opioid receptors. The rewarding effects of opioid drugs are generally thought to be mediated through μ-opioid receptors (Jaffe and Martin, 1990), but δ-opioid receptor systems have also been implicated in reward processes (Shippenberg et al., 1987; Bals-Kubik et al., 1990). κ-Opioid receptor stimulation results in dysphoric effects (Mucha and Herz, 1985). Although morphine primarily binds to μ-opioid receptors, it also has affinity for δ- and κ-opioid receptors (Goldstein and Naidu, 1989). Therefore, to investigate which opioid receptor system is involved in the regulation of social play and through which receptor system opioids influence social play, the present study examined the effects of μ-, δ-, and κ-opioid receptor-selective agonists and antagonists on social play behavior.

2. Materials and methods

2.1. Animals and housing conditions

Male Wistar rats bred from our own stock were used. Two weeks prior to experimentation 7-day-old rat pups were housed in litters of 8 with their mothers in a dimly lighted animal room (20–40 lx, lights on 6.00 a.m., lights off 8.00 p.m.) in Macrolon home cages measuring $40 \times 26 \times 20$ cm ($l \times w \times h$). The animal rooms were temperature-controlled (22 ± 1°C) and standard food (Hope Farms) and tap water were available ad libitum.

2.2. Experimental situation

Testing was performed in a sound-attenuated inner chamber located in a dimly lighted outer room. Background noise was produced by a fan. The testing arena consisted of an acrylic plastic cage measuring $35 \times 35 \times 50$ cm ($l \times w \times h$) with approximately 2 cm of wood shavings covering the floor. The test cage was illuminated by a 25 W red light bulb (0.4–1 lx) mounted 60 cm above the test cage. The behaviors of the animals were recorded on video tape (Sony U-matic). During a test session, only the pair of rats tested was present in the inner chamber. Video equipment except for cameras was in the outer room.

2.3. Procedure

After weaning on day 19 of life, the litters were distributed over 2 cages of 4 rats per cage. Pairs of rats were randomly subjected to one of the treatment conditions. To allow their adjustment to transportation and injections, all animals were brought to the outer room on the 2 days preceding the experiment where they received a subcutaneous injection of 0.1 ml saline.

On the day of the experiment, which was on day 21 of life, the animals were socially isolated in Macrolon cages measuring $22 \times 13 \times 20$ cm ($l \times w \times h$) for 3.5 h prior to the experiment. This isolation period has been shown to produce a half-maximal increase in the amount of social play (Niesink and Van Ree, 1989). All testing was done under dim light/unfamiliar test con-

ditions, since both effects of morphine on social play were observed in this situation (Vanderschuren et al., 1995a). The animals were treated subcutaneously with drug or placebo in a volume of approximately 0.1 ml. The drugs used were the μ-opioid receptor agonist, fentanyl (Jaffe and Martin, 1990) (3.0–30.0 μg/kg), the irreversible μ-opioid receptor antagonist, β-funaltrexamine (Portoghese et al., 1980) (0.3–3.0 mg/kg), the δ-opioid receptor agonist, BUBUC (Tyr-D-Cys(StBu)-Gly-Phe-Leu-Thr(OtBu)) (Gacel et al., 1990) (0.1–1.0 mg/kg), the δ-opioid receptor antagonist, naltrindole (Portoghese et al., 1988) (0.3–3.0 mg/kg), the κ-opioid receptor agonist, U50,488H(*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide) (Von Voigtlander et al., 1983) (0.1–3.0 mg/kg), and the κ-opioid receptor antagonist, nor-binaltorphimine (Takemori et al., 1988) (0.1–3.0 mg/kg). All drugs were administered 1 h before testing, except for nor-binaltorphimine and its respective placebo, which were administered 3 h before testing, because of reported initial μ-opioid receptor-antagonistic effects (Endoh et al., 1994). The test consisted of placing two similarly treated animals into the test cage for 15 min. Testing took place between 11.00 a.m. and 2.00 p.m., in the light part of the day/night cycle. The animals of a pair were not littermates and did not differ by more than 10 g in body weight. Animals were used only once.

Analysis from the video tape recordings was performed afterwards without knowledge of the treatment of the rats. Behavior was assessed per pair of animals, which means that the behavior of the individual rats was not analyzed. Frequencies of the following parameters were scored per 5 min for 15 min: *pinning*, which is defined as one of the animals lying with its dorsal surface on the floor of the test cage with the other animal standing over it; *boxing / wrestling*, a group of behaviors including boxing, wrestling and pouncing; *following / chasing*, moving in the direction of or pursuing the test partner, who moves away; *social exploration*, sniffing any part of the body of the test partner, including the anogenital area; *contact behavior*, crawling over and under the test partner and social grooming.

2.4. Statistical analyses

The data were analyzed per 15 min, except where indicated. Group medians (for pinning) or group means ± S.E.M. (other variables) were calculated. Since pinning levels were not normally distributed, pinning data were analyzed using a Kruskal-Wallis test followed by Mann-Whitney non-parametric tests. The other behaviors were analyzed using the analysis of variance (ANOVA) followed by Student-Newman-Keuls parametric tests.

2.5. Drugs

Fentanyl was purchased from Janssen (Beerse, Belgium), BUBUC from Bachem (Bubendorf, Switzer-

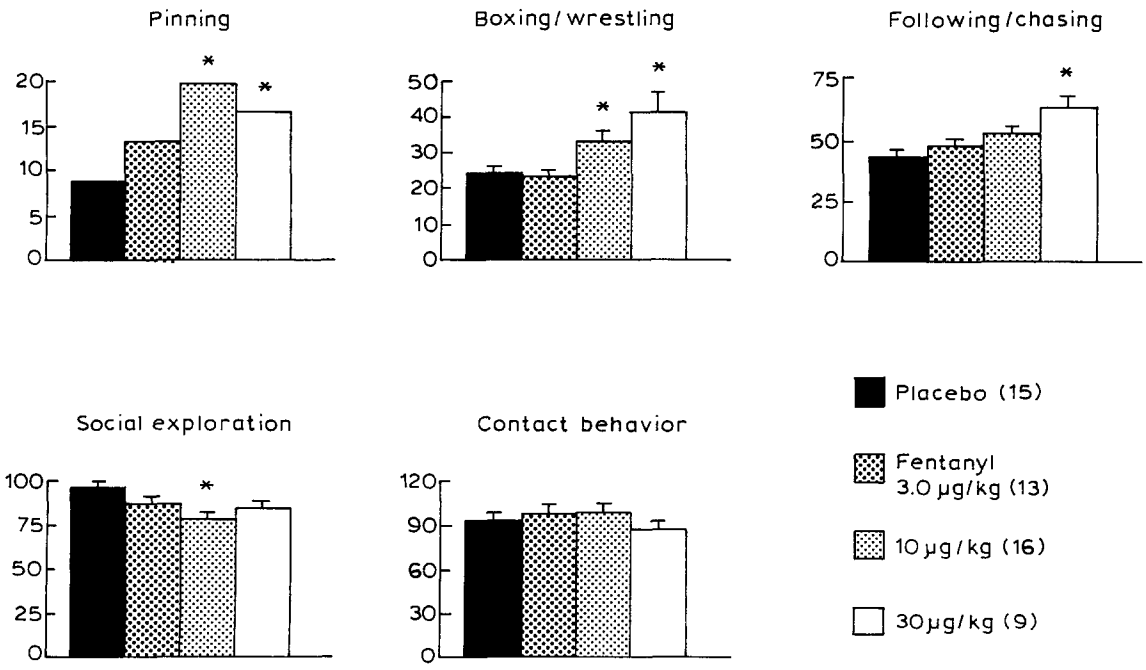

Fig. 1. Effects of graded doses of the μ-opioid receptor agonist, fentanyl, on the frequencies per 15 min of social behaviors in pairs of juvenile rats. The animals were socially isolated for 3.5 h prior to testing and were tested under dim light/unfamiliar conditions. The data are presented as medians (pinning) or means ± S.E.M. (other behaviors). Numbers in parentheses represent numbers of pairs tested. *Significantly different from saline ($P \leq 0.05$, Mann-Whitney (pinning), Newman-Keuls (other behaviors)).

land), β-funaltrexamine and nor-binaltorphimine from RBI (Natick, MA, USA) and naltrindole and U50,488H from Sigma (Axel, Netherlands). Fentanyl, naltrindole, U50,488H and nor-binaltorphimine were dissolved in saline. BUBUC was dissolved in a small volume of 50% acetic acid, diluted further with saline and brought to physiological pH with NaOH, and β-funaltrexamine was dissolved in a small volume of methanol and further diluted in distilled water. Control animals received injections of the corresponding vehicle. Solutions were freshly prepared in plastic containers on the day of the experiment.

3. Results

3.1. μ-Opioid receptor drugs

Treatment with fentanyl increased pinning ($\chi^2 = 7.54$, $P \leq 0.05$), boxing/wrestling ($F(3,49) = 6.59$, $P \leq 0.001$) and following/chasing ($F(3,49) = 5.04$, $P \leq 0.01$). Social exploration was decreased ($F(3,49) = 3.65$, $P \leq 0.01$) and contact behavior was not affected ($F(3,49) = 0.62$, n.s.) (Fig. 1). In the first 5 min of the test period, when pinning is supposed to be suppressed as a result of unfamiliarity to the test cage (Vanderschuren et al., 1995a), fentanyl increased pinning ($\chi^2 = 14.01$, $P \leq 0.01$) (Fig. 2). Treatment with fentanyl also increased following/chasing ($F(3,49) = 3.48$, $P \leq 0.05$), boxing/wrestling ($F(3,49) = 8.85$, $P \leq 0.001$) and contact behavior ($F(3,49) = 3.43$, $P \leq 0.05$), but not social exploration ($F(3,49) = 0.45$, n.s.) in the first 5 min of the test (not shown).

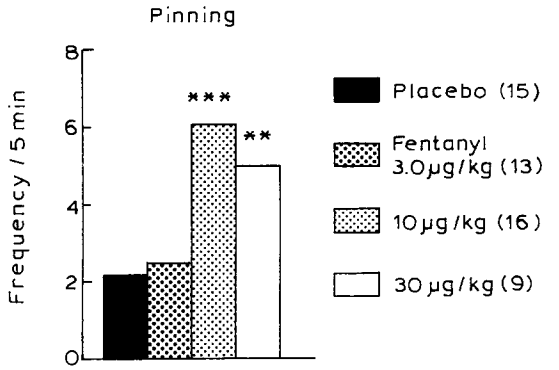

Fig. 2. Effects of graded doses of the μ-opioid receptor agonist, fentanyl, on the frequency of pinning in the first 5 min of the test period in pairs of juvenile rats. The animals were socially isolated for 3.5 h prior to testing and were tested under dim light/unfamiliar conditions. The data are presented as medians. Numbers in parentheses represent numbers of pairs tested. * Significantly different from saline ($P \leq 0.01$, Mann-Whitney), *** significantly different from saline ($P \leq 0.001$, Mann-Whitney).

Treatment with the μ-opioid receptor antagonist, β-funaltrexamine, affected social behaviors in a biphasic manner (pinning: $\chi^2 = 16.14$, $P \leq 0.001$; boxing/wrestling: $F(3,51) = 6.58$, $P \leq 0.001$; following/chas-

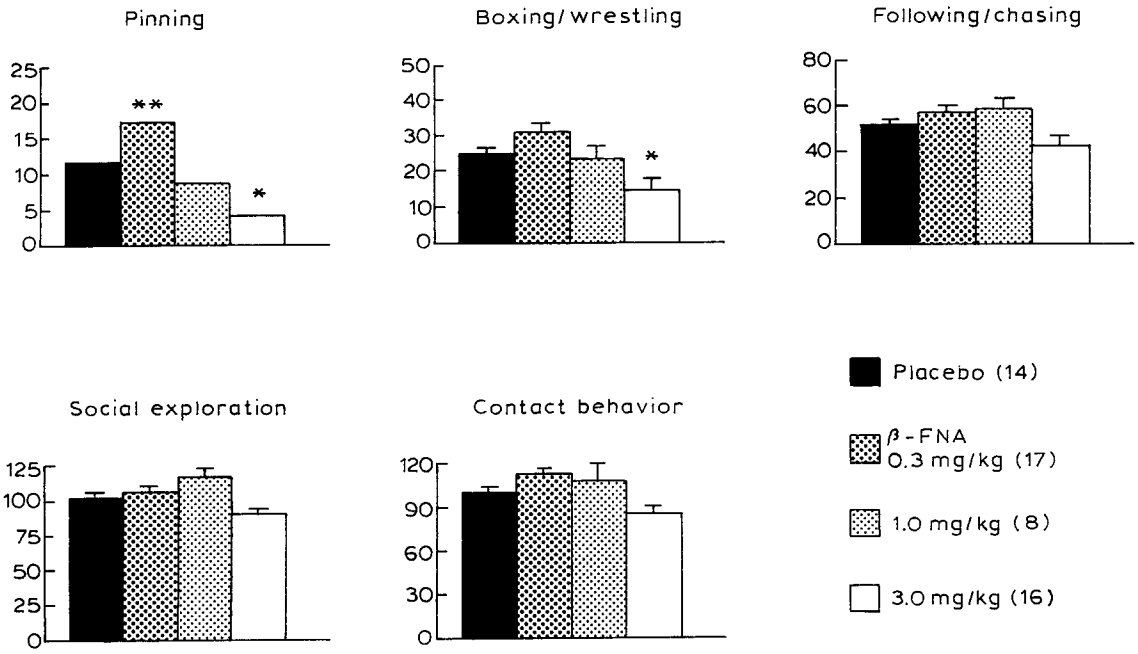

Fig. 3. Effects of graded doses of the μ-opioid receptor antagonist, β-funaltrexamine (β-FNA), on the frequencies per 15 min of social behaviors in pairs of juvenile rats. The animals were socially isolated for 3.5 h prior to testing and were tested under dim light/unfamiliar conditions. The data are presented as medians (pinning) or means ± S.E.M. (other behaviors). Numbers in parentheses represent numbers of pairs tested. * Significantly different from saline ($P \leq 0.05$, Mann-Whitney (pinning), Newman-Keuls (other behaviors)), ** significantly different from saline ($P \leq 0.01$, Mann-Whitney).

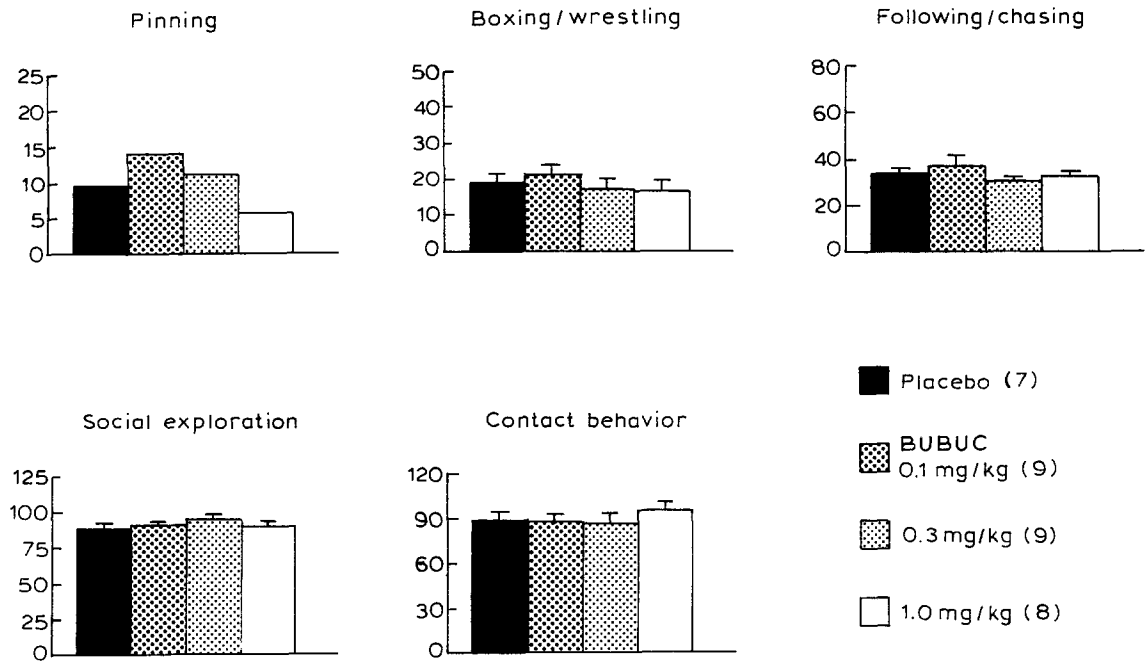

Fig. 4. Effects of graded doses of the δ-opioid receptor agonist, BUBUC, on the frequencies per 15 min of social behaviors in pairs of juvenile rats. The animals were socially isolated for 3.5 h prior to testing and were tested under dim light/unfamiliar conditions. The data are presented as medians (pinning) or means ± S.E.M. (other behaviors). Numbers in parentheses represent numbers of pairs tested.

ing: $F(3,51) = 3.81$, $P \leq 0.01$; social exploration: $F(3,51) = 4.86$, $P \leq 0.01$; contact behavior: $F(3,51) = 5.37$, $P \leq 0.01$). A dose of 0.3 mg/kg significantly increased pinning ($U = 58.0$, $P \leq 0.01$), and slightly increased boxing/wrestling and contact behavior (Fig. 3). In the first 5 min of the test, this dose of β-funaltrexamine increased pinning ($U = 68.0$, $P \leq 0.05$), but not the other behaviors (not shown). A dose of 3.0

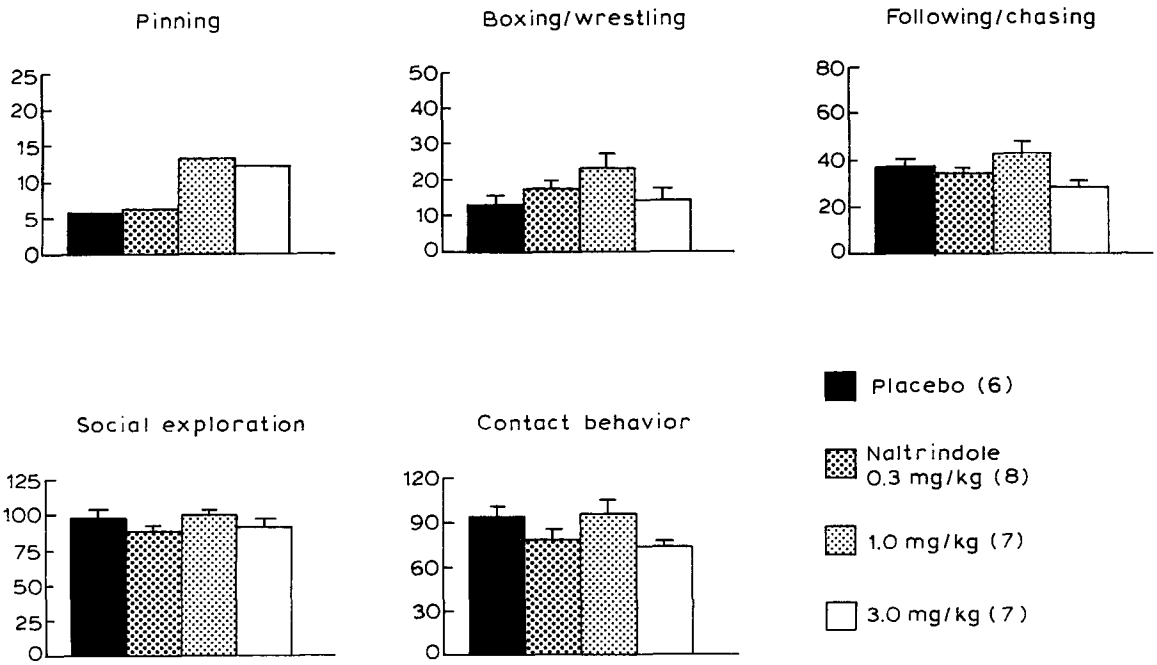

Fig. 5. Effects of graded doses of the δ-opioid receptor antagonist, naltrindole, on the frequencies per 15 min of social behaviors in pairs of juvenile rats. The animals were socially isolated for 3.5 h prior to testing and were tested under dim light/unfamiliar conditions. The data are presented as medians (pinning) or means ± S.E.M. (other behaviors). Numbers in parentheses represent numbers of pairs tested.

mg/kg suppressed pinning ($U = 61.0$, $P \leq 0.05$) and boxing/wrestling (Fig. 3), but did not influence social behavior in the first 5 min of the test (not shown).

3.2. δ-Opioid receptor drugs

The δ-opioid receptor agonist, BUBUC, did not affect any measure of social behavior when analyzed over 15 min (pinning: $\chi^2 = 3.45$, n.s.; boxing/wrestling: $F(3,29) = 0.54$, n.s.; following/chasing: $F(3,29) = 0.83$, n.s.; social exploration: $F(3,29) = 0.50$, n.s.; contact behavior: $F(3,29) = 0.41$, n.s.) (Fig. 4), or in the first 5 min of the test (not shown).

There was a slight tendency of naltrindole, the δ-opioid receptor antagonist, to increase pinning. This effect appeared not to be significant ($\chi^2 = 4.34$, n.s.); other behaviors were not affected by naltrindole treatment (boxing/wrestling: $F(3,24) = 2.02$, n.s.; following/chasing: $F(3,24) = 2.78$, n.s.; social exploration: $F(3,24) = 1.22$, n.s.; contact behavior: $F(3,24) = 2.20$, n.s.) (Fig. 5). When behaviors were analyzed per 5 min, no effect of naltrindole was found (not shown).

3.3. κ-Opioid receptor drugs

Treatment with the κ-opioid receptor agonist, U50,488H, had a profound dose-dependent suppressing effect on all measures of social behavior (pinning: $\chi^2 = 28.26$, $P \leq 0.001$; boxing/wrestling: $F(4,34) = 15.35$, $P \leq 0.001$; following/chasing: $F(4,34) = 13.17$, $P \leq 0.001$; social exploration: $F(4,34) = 38.93$, $P \leq 0.001$; contact behavior: $F(4,34) = 27.88$, $P \leq 0.001$) (Fig. 6). The suppressive effects of U50,488H were also to be seen in the first 5 min of the test (pinning: $\chi^2 = 15.22$, $P \leq 0.01$; boxing/wrestling: $F(4,34) = 4.77$, $P \leq 0.01$; following/chasing: $F(4,34) = 7.75$, $P \leq 0.001$; social exploration: $F(4,34) = 8.96$, $P \leq 0.001$; contact behavior: $F(4,34) = 14.08$, $P \leq 0.001$) (not shown).

Treatment with the κ-opioid receptor antagonist, nor-binaltorphimine, did not influence the total levels of any behavior (pinning: $\chi^2 = 1.89$, n.s.; boxing/wrestling: $F(4,29) = 0.43$, n.s.; following/chasing: $F(4,29) = 0.66$, n.s.; social exploration: $F(4,29) = 0.44$, n.s.; contact behavior: $F(4,29) = 0.99$, n.s.) (Fig. 7). In the first 5 min of the test, the highest dose of nor-binaltorphimine increased pinning ($U = 9.5$, $P \leq 0.05$) (Fig. 8), without significantly influencing the other behaviors (boxing/wrestling: $F(4,29) = 0.66$, n.s.; following/chasing: $F(4,29) = 0.68$, n.s.; social exploration: $F(4,29) = 0.03$, n.s.; contact behavior: $F(4,29) = 1.31$, n.s.) (not shown).

4. Discussion

In the present study, the effects of opioid receptor-specific drugs on social play behavior of juvenile rats

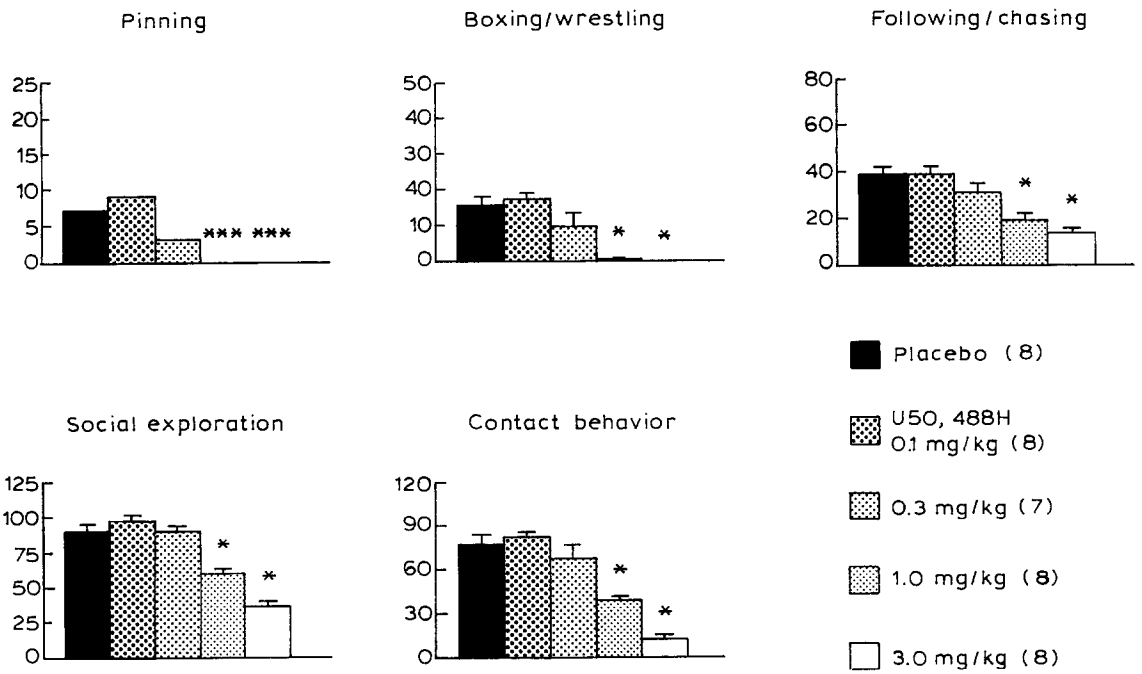

Fig. 6. Effects of graded doses of the κ-opioid receptor agonist, U50,488H, on the frequencies per 15 min of social behaviors in pairs of juvenile rats. The animals were socially isolated for 3.5 h prior to testing and were tested under dim light/unfamiliar conditions. The data are presented as medians (pinning) or means ± S.E.M. (other behaviors). Numbers in parentheses represent numbers of pairs tested. * Significantly different from saline ($P \leq 0.05$, Newman-Keuls), *** significantly different from saline ($P \leq 0.001$, Mann-Whitney).

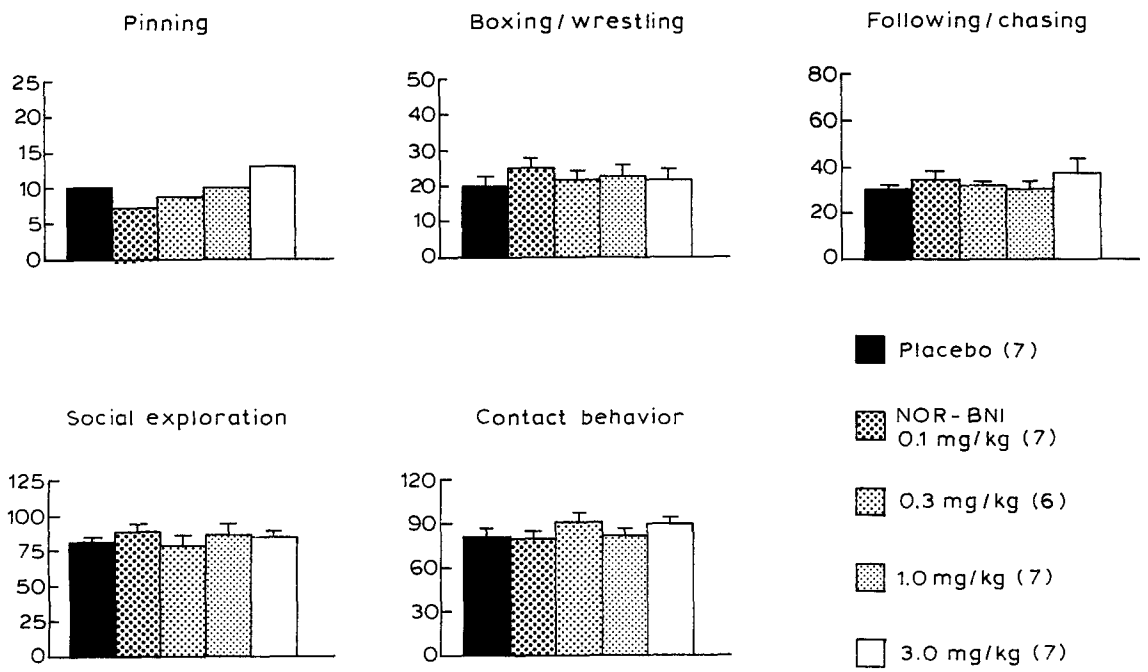

Fig. 7. Effects of graded doses of the κ-opioid receptor antagonist, nor-binaltorphimine (NOR-BNI), on the frequencies per 15 min of social behaviors in pairs of juvenile rats. The animals were socially isolated for 3.5 h prior to testing and were tested under dim light/unfamiliar conditions. The data are presented as medians (pinning) or means ± S.E.M. (other behaviors). Numbers in parentheses represent numbers of pairs tested.

were investigated. A previous study using morphine suggested that opioids are involved on two levels in the regulation of social play behavior; the effects of morphine were interpreted as effects on both the rewarding aspects of social play and the integration of environmental stimuli related to social play (Vanderschuren et al., 1995a). Treatment with the μ-opioid receptor agonist, fentanyl, seemed to mimic both effects of morphine. Measures of social play behavior, i.e. pinning, boxing/wrestling and following/chasing were increased, probably by enhancement of the rewarding value of play, whereas social exploration, a measure of social behavior not related to play was decreased. In addition, fentanyl increased pinning in the first 5 min of the test, when pinning was supposedly suppressed by unfamiliarity with the test cage. Hypothetically, this effect reflects an altered integration of environmental stimuli. However, morphine exerted the two effects at doses differing by one order of magnitude, suggesting that these two effects were distinct, but fentanyl exerted its effects at the same dosages. Differing affinities for opioid receptor types of these two drugs might underlie this phenomenon. Alternatively, fentanyl effects on social play might reflect only the reward-enhancing effect on play, since the dosage of morphine that increased play over the whole period also increased play in the first 5 min of the test (Vanderschuren et al., 1995a). The μ-opioid receptor antagonist, β-funaltrexamine, significantly suppressed pinning and boxing/wrestling. This effect, which is consistent with the social play-enhancing effects of fentanyl, may be the result of a decreased rewarding value of play. Surprisingly, a lower dose of β-funaltrexamine increased pinning. The nature of this phenomenon is unknown. This effect seemed not specific for social play, since both boxing/wrestling and contact behavior were slightly increased as well.

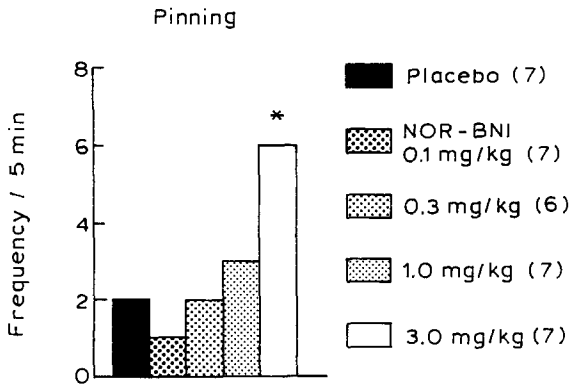

Fig. 8. Effects of graded doses of the κ-opioid receptor antagonist, nor-binaltorphimine (NOR-BNI), on the frequency of pinning in the first 5 min of the test in pairs of juvenile rats. The animals were socially isolated for 3.5 h prior to testing and were tested under dim light/unfamiliar conditions. The data are presented as medians. Numbers in parentheses represent numbers of pairs tested. * Significantly different from saline ($P \leq 0.05$, Mann-Whitney).

U50,488H, the κ-opioid receptor agonist, profoundly suppressed all social behaviors measured. This effect seems in agreement with reports that μ- and

κ-opioid receptor stimulation has antagonistic effects. μ-Opioid- and κ-opioid receptor agonists have opposite effects on agonistic behavior in mice (Benton, 1985). In a conditioning test, μ-opioid receptor agonists induce place preference, whereas κ-opioid receptor agonists induce place aversion (Mucha and Herz, 1985) and abolish morphine-induced place preference (Funada et al., 1993). However, unlike the effects of the μ-opioid receptor drugs, U50,488H effects were not restricted to social play: social exploration and contact behavior were suppressed as well. While μ- and κ-opioid receptor systems may act as functional antagonists in the regulation of the rewarding aspects of social play behavior, the effects of U50,488H do not seem to be specific for social play. This does not necessarily distinguish social play from other rewarded behaviors, since it is not clear whether the effects of U50,488H on rewarded behaviors represent behavior-specific or general dysphoric effects. Treatment with nor-binaltorphimine did not affect total levels of social play, which suggests that in the physiological situation, κ-opioid receptor systems are not involved in the regulation of the rewarding properties of social play. This is in accordance with the findings that nor-binaltorphimine has no reward-enhancing effects (Beczkowska et al., 1993; Carr et al., 1993; Negus et al., 1993). The highest dose of nor-binaltorphimine increased pinning in the first 5 min of the test, indicating that, regarding integration of environmental stimuli, a κ-opioid receptor system may also be implicated in the regulation of social play behavior.

Neither BUBUC, the δ-opioid receptor agonist, nor the δ-opioid receptor antagonist naltrindole affected any measure of social behavior. δ-Opioid receptor systems are suggested to be involved in reward processes (Shippenberg et al., 1987; Bals-Kubik et al., 1990) and in adult rats, δ-opioid receptor stimulation has been reported to increase social behavior (Negri et al., 1991). It seems, however, that in juvenile rats, δ-opioid receptor systems are not involved in the regulation of social behavior.

δ-Opioid receptors appear relatively late during development of the rat brain as compared to μ- and κ-opioid receptors (Kornblum et al., 1987; Leslie and Loughlin, 1993), but this is not likely to underlie the lack of effects of BUBUC and naltrindole on social play behavior. Although the patterns of opioid peptide and opioid receptor presence in the rat brain have not yet reached their adult pattern, 21-day-old animals show strong resemblances with adult animals (Kornblum et al., 1987; Leslie and Loughlin, 1993). Furthermore, all three types of opioid receptors are functional with respect to opioid effects on neurotransmitter release and adenylate cyclase activity in 21-day-old rats (De Vries et al., 1990).

Although the existence of opioid receptor subtypes has been suggested, it is unlikely that the present results involve activation of opioid receptor subtypes, since none of the drugs employed in the present study is known to specifically activate a subpopulation of μ-, δ-, or κ-opioid receptors. The finding that μ-opioid receptor drugs affect social play behavior, while δ-opioid receptor drugs do not, seems to exclude the involvement of a μ-δ-opioid receptor complex in the regulation of social play behavior. More detailed experiments will however be necessary to verify this latter suggestion.

It is unlikely that opioid effects on social play behavior are secondary to changes in motor activity. Amphetamine (Beatty et al., 1982) and caffeine (Holloway and Thor, 1983), drugs known to increase motor activity, markedly depress social play, while scopolamine profoundly depresses social play without affecting motor activity (Thor and Holloway, 1983; Beatty, 1983). Treatment with a dose of morphine higher than the one that enhanced play, resulted in increased motor activity, but not increased play (Vanderschuren et al., 1995a) and naloxone has been found to depress play more profoundly than general activity, suggesting that naloxone's effect on play was not secondary to changes in motor activity (Siegel and Jensen, 1986). When opioid receptor involvement in motor activity and in social play is compared, it appears that they are not parallel: motor activity can be increased with specific μ- and δ-opioid receptor agonists, whereas κ-opioid receptor agonists have no effect (Michael-Titus et al., 1989; Meyer and Meyer, 1993). It has also been suggested that the development of neuronal mechanisms mediating opioid-induced hyperactivity is not yet completed in 21-day-old rats (Caza and Spear, 1980; Spear et al., 1982). Treatment with μ-opioid receptor agonists in rats 20 days of age or less generally results in behavioral depression, while κ-opioid receptor agonists induce hyperactivity, and δ-opioid receptor agonists have no effect (Jackson and Kitchen, 1989). Again, these receptor-specific effects do not parallel the findings for social play behavior: social play can only be increased through μ-opioid receptor stimulation, while κ-opioid receptor stimulation depresses play, perhaps in an a-specific manner.

In summary, the present results suggest that, during social play behavior, μ-opioid receptor activation modulates the reward phenomena associated with social play. Treatment with the μ-opioid receptor agonist, fentanyl, increases social play, whereas the μ-opioid receptor antagonist, β-funaltrexamine, suppresses play. κ-Opioid receptor stimulation might work in an opposite way to the effects of μ-opioid receptor activation. However, under physiological circumstances, κ-opioid receptors seem not to be involved in the regulation of the rewarding value of play, since the κ-opioid receptor antagonist, nor-binaltorphimine, did not affect total

levels of social play. Both fentanyl and nor-binaltorphimine were capable of increasing pinning in the first 5 min of the test, suggesting that, in brain areas where opioids influence the integration of environmental stimuli associated with social play, μ- and κ-opioid receptor systems might also interact. δ-Opioid receptors are probably not involved in the regulation of social play.

Acknowledgements

This study was supported by a grant from the Korczak Foundation for autism and related disorders.

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