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Disruption of morphine conditioned place preference by delta-2-opioid receptor antagonist: Study of mu- and delta-opioid receptor expression at the synapse

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Abstract

The addictive properties of morphine limit its clinical use. Learned associations that develop between the abused opiate and the environment in which it is consumed are engendered through Pavlovian conditioning processes. Disruption of the learned associations between the opiate and environmental cues may be a therapeutic approach to prevent morphine dependence. Although a role for the delta-opioid receptor in the regulation of the rewarding properties of morphine has already been shown, in this study we further characterize the role of the delta-opioid receptor in morphine-induced conditioned responses by examining the effect of a selective delta-2-opioid receptor antagonist (naltriben) using a conditioned place preference paradigm in rats. Additionally, we used a subcellular fractionation technique to analyze the synaptic localization of mu- and delta-opioid receptors in the hippocampus in order to examine the molecular mechanisms that may underlie this morphine-induced conditioned behavior. Our data show that the administration of 1 mg/kg naltriben (but not 0.1 mg/kg) prior to morphine was able to block morphine-induced conditioned place preference. Interestingly, this naltriben-induced disruption of morphine conditioned place preference was associated with a significant increase in the expression of the delta-opioid receptor dimer at the postsynaptic density. In addition, we also observed that morphine conditioned place preference was associated with an increase in the expression of the mu-opioid receptor in the total homogenate. Overall, these results suggest that the modulation of the delta-opioid receptor expression and its synaptic localization may constitute a viable therapeutic approach to disrupt morphine-induced conditioned responses.

Keywords

morphine; CPP; delta-opioid receptor; rat; hippocampus

Introduction

Opioid drugs are widely used clinically for the treatment of moderate-to-severe pain. However, repeated opiate administration can lead to the development of physical dependence. The endogenous opioid system has been implicated with the processes of reward and reinforcement (Shippenberg et al., 2008). Morphine's effects have been mainly attributed to the mu-opioid receptor (MOPr) (Matthes et al., 1996). However, studies using a

non-selective delta opioid receptor (DOPr) agonist have demonstrated the involvement of this receptor in drug self-administration (Devine and Wise, 1994), suggesting a role for the DOPr in the modulation of the rewarding properties of morphine. Although two DOR subtypes (DOR1 and DOR2) have been identified (Jiang et al., 1991; Mattia et al., 1991; Sofuoglu et al., 1991), it has been reported that blockade of DOPr2 rather than DOPr1 may play an important role in the modulation of drug-induced behavior. For example, the use of selective DOPr2 antagonists prevents the attenuation of the discriminative stimulus properties of cocaine (Suzuki et al., 1994), suppresses morphine-induced hyperlocomotion in mice and attenuates the increase in dopamine turnover (Narita et al., 2001) that has been associated with the locomotor stimulant effects of opiates. More recently it has been shown that naltriben (NTB), a selective DOPr2 antagonist, prevents the sensitization that develops to the rewarding effects of morphine (Moron et al., 2009; Shippenberg et al., 2009). Therefore, in the present study we examined the effect of a selective DOPr2 antagonist in the rewarding properties of morphine using the conditioned place preference (CPP) paradigm.

The hippocampus plays a key role in the development of context-dependent associations (Di Chiara and Imperato, 1988; Everitt and Wolf, 2002; Koob et al., 1998; Parker et al., 2006; Wise, 1998). Moreover, the hippocampus has been implicated in the regulation of morphine-dependent conditioned behavior (Corrigall and Linseman, 1988; Ferbinteanu and McDonald, 2001). In addition, it has been shown that both DOPr and MOPr are present in the hippocampus, and interestingly it has been reported that the DOPr is located at the postsynaptic terminal and more specifically at the postsynaptic density (PSD) (Commons and Milner, 1997). Therefore, we hypothesize that the selective modulation of the DOPr2 may regulate morphine-induced conditioned responses and that this regulation can be associated with changes in the expression level and synaptic localization of the DOPr in the hippocampus. To test this hypothesis the expression and synaptic localization of MOPr and DOPr in the hippocampus were analyzed in rats that were conditioned to morphine, and in rats in which this conditioned behavior was altered by the administration of the DOPr2 antagonist NTB.

We found that administration of the selective DOPr2 antagonist, NTB, disrupted the conditioned response to an opiate-paired environment and that this effect was associated with an increase in the expression of the DOPr dimer at the PSD. Moreover, we found that morphine-CPP was associated with an increase in the expression of the MOPr in the hippocampus. Thus, these findings provide new evidence for the key role of DOPr in the modulation of the rewarding properties of morphine.

Materials and Methods

Animals and drugs

A total of 56 male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Houston, TX, USA) were used. Animals weighted 175-199 g at the beginning of the experiments and were housed 2 per cage with food and water *ad libitum*. Rats were allowed to acclimate for 10 days in a colony room at a constant temperature (21–23 °C) and humidity (45–50%) on a 12 h light–dark cycle (light 07:00–19:00 h). They were handled daily during the first week after arrival. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas Medical Branch and carried out in accordance with the guidelines laid down by the National Institutes of Health. Morphine sulfate and NTB (Sigma-Aldrich, St. Louis, MO) were diluted in sterile saline. All vehicle injections consisted of sterile saline. Morphine was used at a dose of 10 mg/kg (s.c.) as described (Billa et al., 2009). NTB was administered (i.p.) at a dose of 0.1 mg/kg (Shippenberg et al., 2009) and 1 mg/kg (Suzuki et al., 1994).

Apparatus

A three-compartment place preference apparatus made of Plexiglas (Med Associates, Inc., Georgia, VT), consisting of two main compartments (14.5 cm × 21.5 cm × 21 cm W x L x H), separated by a grey center compartment (30 cm × 21.5 cm × 21 cm high) was used. One of the main compartments was black with flooring consisting of 0.4 cm stainless steel rods spaced 1.5 cm apart. The other main compartment was white with steel mesh floor spaced 1.2 cm apart (0.1 cm diameter). The center zone was gray with a sheet-metal floor. The system was automated (Med Associates, Inc., Georgia, VT) and used 12 infrared photocell beams in the two main compartments and 6 in the grey one (spaced 5 cm apart and 3 cm above the chamber floor) to allow assessment of compartment preference. Chambers were illuminated with overhead fluorescent lights and lighting was adjusted to 7 lux in each conditioning environment and 30 lux in the grey chamber.

Place preference paradigm

Rats were divided into 4 treatment groups (n=8/group), see Table 1. Morphine CPP was performed essentially as previously described (Billa et al., 2009). Briefly, animals received a single preconditioning test (day 0) in which each rat was placed in the center box, and allowed to freely roam the entire CPP apparatus for 15 min in order to assess the unconditioned chamber preference. Rats showing a strong preference or aversion for one of the compartments were discarded (e.g. spent more than 90% of the total time in the same chamber). The next day, rats were assigned to receive saline or morphine paired with one of the two conditioning environments in a counterbalanced manner (unbiased procedure, (Bardo et al., 1995; Cunningham et al., 2003), such that half of the rats were conditioned in their preferred-chamber, and the other half were conditioned in their non-preferred chamber. During conditioning (days 1, 2, 3 and 4), twice-daily sessions were conducted, with animals receiving a 30 min conditioning session in one side of the CPP chamber in the morning and a second 30 min conditioning session in the opposite side of the CPP chamber 6 hours later. The morphine-CPP group received morphine (10 mg/kg, s.c.) in one side of the CPP chamber (morphine-paired) and saline in the other side (saline-paired) of the chamber. The saline-CPP group received saline in both sides of the CPP chamber. To study the role of DOPr2 antagonist on morphine CPP, animals received once-daily injections of NTB (0.1 mg/kg or 1 mg/kg; i.p) 15 min before the first saline or morphine injection as described (Moron et al., 2009).

On test day (day 5), each animal was placed into the central compartment with free access to all three compartments and their activities were monitored over a period of 15 min. A CPP is defined as an increase in the time spent in the chamber formerly paired with morphine (calculated as time spent in the morphine-paired compartment minus time spent in the saline-paired compartment) as compared to saline-conditioned animals (Billa et al., 2009). For subsequent biochemical analyses (see section below), all the animals were sacrificed immediately following the expression test and their hippocampi were dissected and stored at -80°C.

Subcellular fractionation and PSD isolation

Subcellular fractionation was performed essentially as described previously (Moron et al., 2007). Briefly, hippocampi from individual animals were homogenized in 1.5 ml of 0.32 M sucrose, 0.1mM CaCl₂ containing protease and phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO). The homogenate was brought to a final concentration of 1.25 M sucrose by adding 2 M sucrose and 0.1 mM CaCl₂. The homogenate was then placed in an ultracentrifuge tube and overlaid with 1 M sucrose and subjected to centrifugation at 100,000g for 3 h at 4°C. The synaptosomal fraction was collected at the 1.25 M/1 M sucrose interface. To obtain the synaptic junctions, the synaptosomal fraction was diluted with 20

mM Tris-Cl, pH 6, 0.1 mM CaCl₂, containing 1% Triton X-100 (TX-100) and mixed for 20 min at 4 °C, and centrifuged at 40,000g for 20 min at 4 °C. The pellet containing the isolated synaptic junctions was collected. To separate presynaptic proteins from the PSD, the pellet was resuspended in 20 mM Tris-Cl, pH 8, 1% TX-100, 0.1 mM CaCl₂. The mixture was again mixed for 20 min at 4 °C, and centrifuged at 40,000g for 20 min at 4 °C. The insoluble pellet containing the PSD fraction was collected and stored at –80 °C until use.

Immunoblotting

For immunoblotting, equal amounts (10–15 µg) of total protein from synaptic fractions obtained from individual animals were resolved in 7.5% SDS-PAGE gels and transferred to nitrocellulose membranes (Scheicher & Schuell, Bioscience, Keene, NH) by electroblotting. Membranes were incubated with selective antibodies to opioid receptors: anti-MOPr C-terminal (1:1000; Cat# AB5511; Chemicon, Temecula, CA), and anti-DOPr N-terminal (1:500; Cat# AB1560 Chemicon, Temecula, CA). After incubating with appropriate fluorescent secondary antibody membranes were scanned directly by the Odyssey Infrared Fluorescent Imaging System (Li-Cor Biosciences, Lincoln, NE). Blots were reprobed with an antibody to actin (1:10,000, Sigma-Aldrich, St. Louis, MO) to ensure equal loading and transfer. Band densities were analyzed using Odyssey Software (Li-Cor Biosciences, Lincoln, NE). Quantification was performed by measuring the intensity of the band with protein specific antibodies and comparing it to that of actin.

Data analysis

Morphine-CPP values are reported as mean time ± SEM difference in time (sec) spent in the morphine-paired chamber versus saline-paired chamber for the morphine treated animals and time spent in the black chamber versus the white chamber for the saline group. During pre-conditioning, the individual baseline preference for each of the compartments was analyzed using a two-tailed paired t-test. The CPP level was evaluated during the expression test using a two-way ANOVA, followed by a Newman-Keuls multiple comparison test. For the immunoblotting studies, measurement of MOPr and DOPr levels was normalized to actin levels in the lane, to control for variation in loading and transfer. Values were normalized to saline control values. A one-way ANOVA was used to determine any differences in opioid-receptor expression between groups, followed by a Newman-Keuls multiple comparison test. Significance was set at $p < 0.05$.

Results

Conditioned-place preference

Experiment 1: Effect of 0.1 mg/kg NTB—During preconditioning we found that animals generally spent more time in the black chamber compared to the white one (data not shown). Therefore, the apparatus and subject assignment procedure were randomly selected to generate an unbiased conditioning procedure (Bardo et al., 1995; Cunningham et al., 2003), such that animals were assigned to the drug-paired chamber in a counterbalanced manner (see Methods section). In control tests of preferences, animals which received saline during each of the conditioning sessions exhibited no significant preference for either of the place cues. The mean time (sec) spent in the black and white compartments respectively, were: 259.12 ± 25.25 sec and 321.07 ± 12.54 sec ($n = 8$ /group). Upon morphine conditioning, a two-way ANOVA showed a significant drug x CPP-chamber interaction ($F(2;36)=11.23$; $p<0.001$). As shown in Figure 1, Newman-Keuls test indicated that animals trained with morphine (10 mg/kg s.c., 4 conditioning sessions with the drug) presented a significant place preference for the morphine-paired compartment ($p < 0.01$). The mean time (sec) spent in the drug-paired and saline-paired compartments respectively, were: 375.1 ± 30.33 sec and 235.1 ± 27.61 sec ($n = 8$). This is in agreement with previous studies showing that morphine

(10 mg/kg) is effective as a conditioning stimulus when a total of four drug conditioning sessions are employed (Milekic et al., 2006; Ribeiro Do et al., 2005).

Next, in order to examine the role of the DOPr2 subtype in the regulation of morphine-induced conditioned behavior we analyzed the effects of the selective DOPr2 antagonist, NTB, on morphine-CPP. We first selected a dose of 0.1 mg/kg NTB as this dose has been shown to prevent sensitization to morphine-CPP (Shippenberg et al., 2009; Moron et al., 2009). However, Figure 1 shows that the administration of 0.1 mg/kg NTB prior to conditioning training did not have any effect on morphine CPP as animals pretreated with NTB exhibited a strong preference for the previously morphine-paired chamber ($p < 0.01$). Therefore, although a dose of 0.1 mg/kg NTB prevents sensitization to morphine CPP, this same dose does not affect the expression of morphine-induced place preference.

Experiment 2: Effect of 1 mg/kg NTB—Next, we analyzed the effects of a higher dose of NTB on morphine-CPP since previous studies have shown that 1 mg/kg NTB attenuated the rewarding properties of cocaine (Suzuki et al., 1994). In addition, a recent paper has shown that higher doses of NTB blocks ethanol-CPP and that this effect is associated with a selective blockade of DOPr2 (van Rijn and Whistler, 2009). Interestingly, we found that administration of 1 mg/kg NTB prior to conditioning training prevented the expression of morphine-CPP (see Figure 2). Indeed, a two-way ANOVA showed a significant drug $\times$ CPP-chamber interaction ($F(3;56)=8.61$; $p < 0.0001$). Newman-Keuls test showed that rats that received morphine (10 mg/kg) in one of the CPP chambers expressed a significant preference for this chamber ($p < 0.001$). To further confirm these data, we also made a comparison between the morphine group and the NTB plus morphine group and found out that morphine conditioned treated rats spent more time in the morphine-paired chamber than the rats that were pretreated with NTB prior to morphine conditioning ($p < 0.01$), verifying that this effect was specific to the antagonist. In contrast, no significant preference for any of the chambers was observed in animals that were pretreated with NTB prior to morphine or with NTB alone. The lack of effect of NTB on place preference also indicates that this compound does not elicit any place aversion, and therefore the disruption of morphine-CPP by NTB is not due to an aversive effect of the DOPr2 antagonist.

Morphine-CPP is associated with an increase in the expression of the MOPr in the hippocampus

In order to elucidate the molecular mechanisms underlying the disruption of morphine-CPP by 1 mg/kg NTB we next examined the expression and synaptic localization of MOPr and DOPr in the hippocampus. To this end, upon CPP testing animals were sacrificed, hippocampi dissected and subcellular fractionation performed as described in the Methods section. In a first set of experiments, we analyzed the effects on MOPr levels at hippocampal synaptic fractions. Figure 3 shows a significant increase in MOPr levels in the total homogenate upon morphine-CPP ($F(3;32)=5.52$; $p < 0.05$). Interestingly, this increase is not observed when animals are treated with NTB prior to conditioning training. In addition, although it appears to be an increase of MOPr levels at the PSD upon morphine-CPP, that parallels the increase observed in the total homogenate, this effect was not significant. These results show that morphine-CPP is associated with an increase in MOPr levels in the hippocampus and that this increase is abolished when morphine-CPP is prevented by NTB pre-treatment.

Disruption of morphine-CPP is associated with increased levels of the DOPr dimer at the PSD

In a next set of studies we examined how DOPr levels were modulated at hippocampal synapses upon morphine-CPP and when this conditioned response was prevented by the

selective DOPr2 antagonist NTB. It is known that DOPr may exist in either heteromeric or homomeric configuration (George et al., 2000). Interestingly, it has been recently reported that the DOPr2 are most likely DOPr homomers (van Rijn and Whistler, 2009). In order to study whether morphine-CPP or its prevention by the DOPr2 selective antagonist, NTB, had an effect on synaptic levels and configuration of DOPr we analyzed expression of DOPr by using an N-terminus (LVPSARAEQLQSSPLV) directed antibody that selectively recognizes the dimer and monomer configuration of DOPr. Previous work shows that this antibody recognizes a 72 kDa and a 36 kDa band that corresponds to the dimeric and monomeric forms of the DOPr (Persson et al., 2005). However, a recent study has reported that this antibody labels DOPr in the spinal cord of DOPr knockout mice just like their wild-type littermates (Scherrer et al., 2009). Therefore, we tested the specificity of the noted antibody in hippocampal tissue from a strain of DOPr knockout mice (Zhu et al., 1999) that was also used in the Scherrer and col. paper. When we examined DOPr expression in mouse hippocampal PSD fraction by western blot analysis we found that this antibody produces a number of non-specific bands in samples from mice (see Supporting information, Fig. S1) that are higher than those obtained with rat tissue. In addition, these non-specific bands appear in both genotypes; this could explain the similar immunoreactivity pattern in spinal cord sections from DOPr knockout and wild-type reported in the Scherrer paper. Interestingly, we observe that bands corresponding to the DOPr dimer (72kDa) and the DOPr monomer (36 kDa) are absent in hippocampal fractions from DOPr knockout mice (see Supporting information, Fig. S1). Therefore, our data indicate that although this antibody may not be used to perform immunohistochemical analyses because of the high number of non-specific bands detected, it can be used to perform western blot analyses by examining the expression of specific DOPr-associated bands.

Figure 4 shows that the analysis of the expression of the 72 kDa band, corresponding to the DOPr dimer, at the PSD showed a significant increase in levels in animals pretreated with NTB prior conditioning training ($F(3;32)=3.49$; $p<0.05$) when compared to the saline ($p<0.01$), morphine-CPP ($p<0.05$) or NTB alone ($p<0.05$) treatment groups. Interestingly, this increase in DOPr dimer expression at the PSD parallels the observed disruption of morphine-CPP in animals that were pretreated with NTB prior to conditioning training. However, this increase in DOPr dimer levels at the PSD was not observed in any other fraction. On the other hand, Figure 5 shows that expression of the 36 kDa band that corresponds to the DOPr monomer does not change across treatments in any synaptic fraction.

Discussion

The present study shows that the selective DOPr2 antagonist NTB prevents morphine-CPP when administered prior to conditioning training. In addition, our biochemical analyses indicate that morphine-CPP is associated with an increase in MOPr levels at the hippocampus and that this increase is blocked when morphine-CPP is disrupted by the DOPr2 antagonist NTB. More importantly, this NTB-induced prevention of morphine-CPP is associated with an increase in DOPr dimer expression at the hippocampal PSD.

In a first set of studies we found that a low concentration (0.1 mg/kg) of NTB did not have any effect on morphine-CPP which is in agreement with previous studies (Shippenberg et al., 2009). However, in this same study the authors showed that this dose of NTB was able to prevent sensitization to morphine-CPP. It has been reported that chronic administration of morphine induces trafficking of intracellular DOPr to the cell-surface (Cahill et al., 2001). Therefore, DOPr recruitment to the cell-surface by repeated morphine exposure prior to conditioning training might be one mechanism by which this lower dose of NTB prevents sensitization to morphine-CPP. However, during our paradigm of morphine-CPP, in which

animals only received morphine during conditioning training, recruitment of DOPr to the cell surface might not be that significant and therefore less DOPr would be available at the cell-surface to interact with this lower dose of NTB. This would be in agreement with our biochemical analyses that show no significant changes in DOPr expression after morphine-CPP.

On the other hand, we observed that a dose of 1 mg/kg NTB was able to disrupt morphine-CPP. Thus a higher dose of NTB would be able to block the lower number of DOPr that are recruited to the cell-surface during morphine conditioning. In addition, our data are also in agreement with previous reports that show that concentrations of 1 mg/kg NTB or higher attenuate the rewarding properties of abused substances. For example, it has been reported that 1 mg/kg NTB decreases the discriminative stimulus properties of cocaine (Suzuki et al., 1994). In addition, a recent paper has shown that higher doses of NTB (more than 1 mg/kg) are able to reduce ethanol consumption and ethanol-induced CPP and that these effects are associated with the selective blockade of the DOR2 subtype (van Rijn and Whistler, 2009).

Next, in order to explore the molecular mechanisms underlying the disruption of morphine-CPP by the DOPr2 antagonist NTB we analyzed the expression of MOPr and DOPr at hippocampal synaptic fractions. Our results show that morphine-CPP was associated with a significant increase in MOPr expression levels in the total homogenate. Interestingly, this increase in MOPr expression was abolished when morphine-CPP was disrupted by the administration of NTB. These results are in agreement with previous studies that show that repeated morphine exposure results in an increase in MOPr expression and in the number of binding sites in membrane fractions from whole rat brain (Fabian et al., 2002). In addition, it has also been reported that repeated morphine administration leads to long-lasting increases in MOPr transcripts in discrete brain areas (Byrnes, 2008), although other studies also no changes or even a down-regulation of MOPr upon morphine administration (Horner and Zadina, 2004).

Thus, the observed increase in MOPr expression after morphine-CPP might be partially contributing to the mechanisms leading to morphine dependence. Furthermore, our data also show that this increase in MOPr levels seem to be associated with an increase at the PSD (although this was not significant). We have previously shown that the PSD plays a key role in the mechanisms underlying morphine-induced neuroplasticity in the hippocampus (Moron et al., 2007) and that neuroadaptations at this synaptic fraction may also regulate morphine-induced conditioned behavior (Billa et al., 2009). Therefore, data from the present study will help to further understand how the modulation of opioid receptors at hippocampal synapses may play a role in the regulation of the rewarding properties of morphine.

It has been reported that whereas only one DOPr gene has been cloned (Kieffer et al., 1992; Evans et al., 1992), two DOR subtypes have been identified: DOPr1 and DOPr 2 (Zaki et al., 1996). These two isoforms can have opposing actions in the regulation of addictive behavior. For example, a recent study has shown that the two DOPr subtypes have opposing effects on ethanol consumption or ethanol-induced conditioned behavior (van Rijn and Whistler, 2009). In addition, the authors claim that this differential effect among DOPr subtypes is due to their molecular nature such that DOPr2 is most likely a DOPr homomer and DOPr1 is a heterodimer of MOPr and DOPr. As mentioned, DOPr is stored mainly in dense core vesicles (Ma et al., 2006). Its distribution to the plasma membrane is facilitated by several stimuli such as repeated morphine administration (Cahill et al., 2001). Since DOPr distribution is dynamically regulated, it is possible that different subtypes of DOPr could be selectively expressed by morphine exposure. On this regard, it has been shown that morphine could trigger the formation of DOPr1 receptors (such as MOPr and DOPr heterodimers) and that these complexes could be responsible for the behavioral

dysregulation produced by repeated morphine administration (Shippenberg et al., 2009; Moron et al., 2009). In our study we found that disruption of morphine-CPP by the selective DOPr2 antagonist NTB results in an increase in DOPr dimer levels at the PSD. Thus, our data suggest that selective blockade of DOPr2 leads to an increase in DOPr homomers by switching DOPr1 with DOPr2 receptors. One possible explanation is that the ratio of DOPr1 to DOPr2 is altered by the administration of a selective DOPr2 antagonist prior to morphine. In addition, our data also suggest that this increase in DOPr homomers (and therefore DOPr2 levels) might be partially responsible for this NTB-induced disruption of morphine-CPP. Further research needs to be performed to investigate this hypothesis.

Our results demonstrate that morphine-CPP and its prevention by NTB leads to changes in the expression of MOPr and DOPr at hippocampal synapses. These data further confirm the role of the hippocampus in the modulation of the neural mechanisms underlying morphine-induced conditioned responses. Indeed, we have recently reported the involvement of the hippocampus in the regulation of morphine-CPP and how the expression or disruption of this conditioned response is associated with the modulation of opioid-related signaling pathways in this brain area (Moron et al., 2009).

Overall, data in the present study show that alteration of the synaptic localization and composition of DOPr maybe a viable therapeutic approach to disrupt morphine-induced conditioned behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

MOPr	mu-opioid receptor
DOPr	delta-opioid receptor
PSD	postsynaptic density
NTB	naltriben
CPP	conditioned place preference

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Figure 1.

Effect of 0.1mg/kg NTB on conditioned place preference (CPP) to morphine. Rats were conditioned with morphine (10mg/kg; s.c.), saline, NTB plus morphine or NTB alone for 4 days (n=8 per group). Animals trained with morphine exhibited a significant place preference for the morphine-paired compartment. NTB (0.1mg/kg) injection 15 min prior to morphine conditioning did not have any effects on morphine-induced CPP. Data represent the time spent (sec) in the drug-paired chamber versus the saline-paired chamber, and for saline-treated animals data represent the time spent in the black chamber versus the white chamber (mean $\pm$ SEM). **p<0.01; two-way ANOVA followed by Newman-Keuls multiple comparison test. S, saline; M, morphine; NTB, naltriben. Treatment groups designations as described in Table 1: SS, SM, NTB+M NTB+S.

Figure 2.

Effect of 1mg/kg NTB on conditioned place preference (CPP) to morphine. Rats were conditioned with morphine (10 mg/kg s.c.) saline, NTB plus morphine or NTB alone for 4 days (n=8 per group). Animals trained with morphine exhibited a significant place preference for the morphine-paired compartment. NTB (1mg/kg) injection 15 min prior to morphine injection disrupted morphine-induced CPP. Data represent the time spent (sec) in the drug-paired chamber versus the saline-paired chamber, and for saline-treated animals data represent the time spent in the black chamber versus the white chamber (mean $\pm$ SEM).

***p<0.001; two-way ANOVA followed by a Newman-Keuls multiple comparison test. S, saline; M, morphine; NTB, naltriben. Treatment group designations as described in Table 1: SS, SM, NTB+M NTB+S.

Figure 3.

The expression of morphine-conditioned place preference (CPP) leads to an increase in the expression of the mu-opioid receptor (MOPr) in the hippocampus, an effect prevented by the administration of naltriben (NTB). Upon CPP testing, rats were killed, hippocampi dissected, and subcellular fractionation performed in individual samples ($n = 8$ per group). Fractions representing the homogenate, synaptosomes and the postsynaptic density (PSD) were subjected to Western Blot analysis using an antibody to MOPr. A representative western blot is shown for each synaptic fraction. Measurement of MOPr levels was normalized to actin levels in the lane. Data (mean $\pm$ SEM) are normalized to saline controls. * $p < 0.05$; One-way ANOVA followed by a Newman-Keuls multiple comparison. S, saline; M, morphine; NTB, naltriben. Treatment group designations as described in Table 1: SS, SM, NTB+M NTB+S.

Figure 4.

Prevention of morphine-conditioned place preference (CPP) by naltriben (NTB) leads to an increase in the expression of the delta-opioid receptor (DOPr) dimer at the hippocampal postsynaptic density (PSD). Upon CPP testing, rats were killed, hippocampi dissected, and subcellular fractionation performed in individual samples ($n=8$ per group). Fractions representing the homogenate, synaptosomes and the postsynaptic density (PSD) were subjected to Western Blot analysis using an antibody to DOPr that recognizes a 72 kDa band that corresponds to the DOPr dimer. A representative western blot is shown for each synaptic fraction. Measurement of DOPr dimer levels were normalized to actin levels in the lane. Data (mean $\pm$ SEM) are normalized to saline controls. * $p<0.05$; ** $p<0.01$, One-way ANOVA followed by a Newman-Keuls multiple comparison. S, saline; M, morphine; NTB, naltriben. Treatment group designations as described in Table 1: SS, SM, NTB+M NTB+S.

Figure 5.

Prevention of morphine-conditioned place preference (CPP) by naltriben (NTB) is not associated with a significant alteration of the delta-opioid receptor (DOPr) monomer. Upon CPP testing, rats were killed, hippocampi dissected, and subcellular fractionation performed in individual samples ($n = 8$ per group). Fractions representing the homogenate, synaptosomes and the postsynaptic density (PSD) were subjected to Western Blot analysis using an antibody to DOPr that recognizes a 36 kDa band that corresponds to the DOPr monomer. A representative western blot is shown for each synaptic fraction. Measurement of DOPr monomer levels were normalized to actin levels in the lane. Data (mean $\pm$ SEM) are normalized to saline controls. S, saline; M, morphine; NTB, naltriben. Treatment group designations as described in Table 1: SS, SM, NTB+M NTB+S.

Table

Treatment groups

	15 min before conditioning		During conditioning	
Saline (S)	saline		saline SS	
Morphine (M)	saline		Morphine SM	Saline SS
Naltriben+morphine (NTB+M)	NTB	saline	Morphine NTB+M	Saline NTB+S
Naltriben (NTB)	NTB	saline	Saline NTB+S or SS	