Behavioral evidence for μ-opioid and 5-HT$_{2A}$ receptor interactions

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Received 13 February 2003; received in revised form 3 June 2003; accepted 11 June 2003

Abstract

Electrophysiological studies have demonstrated a physiological interaction between 5-HT$_{2A}$ and μ-opioid receptors in the medial prefrontal cortex. Furthermore, behavioral studies have found that phenethylamine hallucinogens induce head shakes when directly administered into the medial prefrontal cortex. The receptor(s) by which morphine suppresses head shakes induced by serotonin agonists have not been characterized. We administered μ-opioid receptor agonists and antagonists to adult male Sprague–Dawley rats prior to treatment with the phenethylamine hallucinogen 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), which is known to induce head shakes via 5-HT$_{2A}$ receptors. The suppressant action of the moderately selective μ-opioid receptor agonist, buprenorphine ($ID_{50}$ 0.005 mg/kg, i.p.; a μ-opioid receptor partial agonist and δ-opioid receptor antagonist) was blocked by naloxone and pretreatment with the irreversible μ-opioid receptor antagonist clocinnamox. Another μ-opioid receptor agonist fentanyl also suppressed DOI-induced head shakes. In contrast, a δ-opioid receptor agonist was without effect on DOI-induced head shakes. Thus, activation of μ-opioid receptors can suppress head shakes induced by hallucinogenic drugs.

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Keywords: Phenethylamine; Hallucogen; 5-HT (5-hydroxytryptamine, serotonin); μ-Opioid receptor; Head shake; DOI; 5-HT$_{2A}$ receptor; Buprenorphine; Fentanyl

1. Introduction

A previous in vitro electrophysiological study suggested a functional relationship between the opioid and serotonergic systems in the rat whereby μ-opioid receptor agonists suppressed the excitatory postsynaptic currents (EPSCs) induced by activation of 5-HT$_{2A}$ receptors in the prefrontal cortex (Marek and Aghajanian, 1998a). A subsequent study that demonstrated a loss of μ-opioid receptor binding following fiber-sparing midline thalamic lesions, also found a decreased of frequency of 5-HT-induced EPSCs recorded in prefrontal layer V pyramidal cells (Marek et al., 2001). This result is consistent with previous suggestions for presynaptic effects of both μ-opioid and 5-HT$_{2A}$ receptor activation on glutamatergic nerve endings in the prefrontal cortex (Marek and Aghajanian, 1998b).

If these serotonergic-opioid interactions involving thalamocortical terminals play a major role in prefrontal cortical function, then one would expect behavioral effects mediated via activation of prefrontal cortical 5-HT$_{2A}$ receptors to be similarly suppressed by μ-opioid receptor agonists. Local infusion of 5-HT$_{2}$ agonists, (e.g., substituted amphetamines with hallucinogenic effects such as 1-(2,5-dimethoxyphenyl-4-iodo)-2-aminopropane (DOI)) into the medial prefrontal cortex induces head shakes (Granhoff et al., 1992; Willins and Meltzer, 1997). Pharmacological studies employing selective 5-HT$_{2A}$ or 5-HT$_{3C}$ receptor antagonists have shown that head shakes induced by DOI are mediated via activation of 5-HT$_{2A}$ receptors (Schreiber et al., 1995; Willins and Meltzer, 1997). Regarding potential serotonergic and opioid interactions in the prefrontal cortex, morphine is also known to suppress head shakes induced by non-selective 5-HT receptor agonists, which retrospectively can be seen to have 5-HT$_{2A}$ agonist activity (Corne et al., 1963; Vetulani et al., 1980).

However, the ability of morphine to suppress head shakes induced by a selective 5-HT$_{2}$ receptor agonist such as DOI has not been tested. Moreover, the opioid receptor(s) participating in this potential interaction with 5-HT$_{2A}$ recep-
tors has not been identified. In preliminary studies we determined that morphine suppressed DOI-induced head shakes (Alvaro et al., 1998). While morphine is ~20-fold and >100-fold more selective at binding to μ-compared to κ-opioid receptors and δ-opioid receptors (Pasternak, 1993), it is possible that an agonist action at other opioid receptors could modulate DOI-induced head shakes. Thus, it was of interest to test the effects of different opioid receptor agonists and antagonists on DOI-induced head shakes in order to determine if activation of μ-opioid receptors does suppress a behavioral response related to stimulation of prefrontal cortical with 5-HT2A receptors.

2. Materials and methods

2.1. Subjects

One-hundred ninety-two male Sprague–Dawley rats (180–280 g, Harlan, Indianapolis, IN) were housed in suspended stainless steel wire cages (18×36×20 cm) with two to four rats occupying each cage. The colony room was maintained at ~20 °C and relative humidity (60%). The room was illuminated 12 h/day (07:00–19:00). All rats had free access to laboratory chow (Teklad 4% Rat Diet) and water except during experimental sessions. The animals were used only once with the exception of the buprenorphine/14β-(p-chlorocinnamoylamino)-7,8-dihydro-N- cyclopropylmorphinone(clocinnamox) and the (+)-4-(a-R*)-a-(2S*,5R*)-4-allyl-2,5-dimethyl-1-piperazine) 3-hydroxybenzyl)-N,N-diethylbenzamide (BW 373U86)/naltrindole experiments. The principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were followed.

2.2. General procedure

All experiments were performed between 10:00 and 16:00. The animals were transferred to an individual clear polycarbonate cage (43×21.5×20 cm) with a sawdust covered floor. All animals were injected with either saline or an opioid receptor agonist (buprenorphine, i.p.; fentanyl, i.p.; BW373U86, s.c) after a 15–30 min habituation period. The animals were then injected with either saline or 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; i.p., 5 mg/kg) 15 min following the initial injection of either saline or an opioid receptor agonist. The μ-opioid receptor antagonist clocinnamox (1 mg/kg; s.c.) was administered 24 h prior to the μ-opioid receptor agonist buprenorphine since this agonist action at other opioid receptors is possible that an agonist action at other opioid receptors could modulate DOI-induced head shakes. Thus, it was of interest to test the effects of different opioid receptor agonists and antagonists on DOI-induced head shakes in order to determine if activation of μ-opioid receptors does suppress a behavioral response related to stimulation of prefrontal cortical with 5-HT2A receptors.

2.3. Behavioral observation

The animals were observed during consecutive 5 min periods for a total of 30 min following the DOI injection. In addition to counting each head shake response, forward locomotion was scored (movement from one end to the other end of the cage was scored as one cross). Backward locomotion, rearing, flat body posture, forepaw treading, skin jerks, chewing, and sniffing were observed and noted.

2.4. Statistical analysis

All data are expressed as the mean± S.E.M. The raw data were analyzed with a between group analysis of variance (ANOVA) and the Fisher Least Significance Difference (LSD) test. The buprenorphine/clocinnamox experiment was analyzed with t-tests (Bonferroni correction factor). Calculation of ID50 values were performed by non-linear curve fitting (Delta Graph) after expressing the data as percent suppression of the DOI-induced head shakes.

2.5. Drugs

The drugs used in this study were obtained from the following suppliers: Research Biochemicals (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl, DOI; buprenorphine HCl; naloxone HCl; naltrindole hydrochloride; BW373U86); Tocris (clocinnamox mesylate). Doses were calculated on the basis of the salt forms. The drugs were dissolved in saline (except clocinnamox, water) and injected i.p. (except clocinnamox, s.c. and BW373U86, s.c.) in a volume of 1 ml/kg body weight.

3. Results

3.1. Buprenorphine suppression of DOI-induced head shakes

The μ-opioid receptor partial agonist and κ-opioid receptor antagonist buprenorphine suppressed DOI (5 mg/kg)-induced head shakes in a dose-dependent manner with an ID50~0.0053 mg/kg; there was significant suppression at 0.01–1 mg/kg; and almost complete suppression at 1 mg/kg (Fig. 1). A two-factor ANOVA comparing buprenorphine against the opioid receptor antagonist naloxone (10 mg/kg, i.p.) found a significant effect of buprenorphine (Fig. 1; F(3,56)=8.67, P<0.001), a significant effect of the antagonist [F(1,56)=4.05, P<0.05], and a non-significant interaction between buprenorphine and the naloxone (F(3,56)=1.78, P>0.1). Following naloxone (10 mg/kg) pretreatment, buprenorphine was less potent in suppressing DOI-induced head shakes (ID50~0.134 mg/kg; significant suppression oc-
curred only at the 0.1 and 1 mg/kg doses). Post-hoc testing found that the suppressant effect of buprenorphine (0.01 mg/kg) was blocked by naloxone (P < 0.01). The lowest dose of buprenorphine (0.001 mg/kg) together with DOI (5 mg/kg) non-significantly increased forward locomotion after DOI to 173% of DOI alone, intermediate doses of buprenorphine (0.01 and 0.1 mg/kg) did not alter the DOI-induced locomotion (105% and 105%, respectively), while the highest dose of buprenorphine (1 mg/kg) with DOI decreased forward locomotion to 6.3 ± 3.4% of DOI alone (P < 0.05, Fisher LSD). Thus, buprenorphine did not suppress DOI-induced forward locomotion at two doses (0.01 and 0.1 mg/kg) which did suppress the frequency of DOI-induced head shakes. A two-factor ANOVA comparing buprenorphine against the opioid receptor antagonists (naloxone, 10 mg/kg, i.p.) found a non-significant effect of buprenorphine (Fig. 1; F(3,56)=1.75, P > 0.1), a non-significant effect of the antagonist (F(1,56)=0.05, P > 0.1). Post-hoc testing found that the suppressant effect of buprenorphine (1 mg/kg) was blocked by naloxone (P < 0.05).

3.2. Naloxone dose–response curve: block of buprenorphine suppression of DOI-induced head shakes

Having established a suppressant effect of 0.1 mg/kg buprenorphine on DOI-induced head shakes, the potency of naloxone (0.03–3 mg/kg, i.p.) in blocking this suppressant effect was tested using a between-subjects design. DOI (1.25 mg/kg, i.p.) induced 18.0 ± 5.1 (mean ± S.E.M., n=7) head shakes while virtually no head shakes (0 ± 0.1, n=7) were observed in the group treated with buprenorphine (0.1 mg/kg, i.p.) and DOI. Naloxone blocked the suppressant action of buprenorphine (0.1 mg/kg, i.p.) on DOI-induced head shakes by 2.9%, 33.6% and 100% at 0.03, 0.3 and 3.0 mg/kg, i.p., respectively (Fig. 2). The ID50 of naloxone for blocking the suppressant action of buprenorphine was 0.4 mg/kg, i.p.

3.3. Cloccinnamox block of buprenorphine suppression of DOI-induced head shakes

A sub-maximal dose of cloccinnamox for selectively blocking μ-opioid receptors was used in a within-subjects design experiment where animals were treated over successive 2-week intervals with (1) saline–saline; (2) saline–DOI (1.25 mg/kg, i.p.); (3) buprenorphine (0.1 mg/kg, i.p.)–DOI; (4) 24 h pretreatment with cloccinnamox (1 mg/kg, s.c.) and buprenorphine–DOI; (5) buprenorphine–DOI. Cloccinnamox pretreatment blocked the suppressant action of buprenorphine on DOI-induced head shakes (t(6)=4.41,
The effects of buprenorphine, clonidine, and DOI on cage crossings (Fig. 3) or rearing behavior (not shown) did not parallel the effects of these drugs on head shakes.

3.4. Fentanyl suppression of DOI-induced head shakes

The relatively selective µ-opioid receptor agonist fentanyl (0.01–0.3) was also tested for potency in suppressing DOI (1.25 mg/kg, i.p.)-induced head shakes. Fentanyl produced a dose-dependent suppression of DOI-induced head shakes ($F(3,35)=3.49, P<0.05$; Fig. 4). The suppressant effect of fentanyl was 78% at the highest dose tested ($P<0.05$). Non-significant decreases of 29% and 44% were observed at 0.01 and 0.03 mg/kg of fentanyl. Fentanyl also decreased DOI-induced cage crosses ($F(3,36)=2.94, P<0.05$). However, no significant change in cage crosses were noted until the highest fentanyl dose (~65% suppression, non-significant increases of 47% and 30% at 0.01 and 0.03 mg/kg of fentanyl, respectively).

3.5. Interactions between DOI, a δ-opioid receptor agonist, and a δ-opioid receptor antagonist

Since similar effects are often observed for µ- and δ-opioid receptor agonists, the effects of a δ-opioid receptor agonist ((±)-4-(a-R*)-a-(2S*,5R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-N,N-diethylbenzamide (BW 373U86); 2 mg/kg) and the δ-opioid receptor antagonist naltrindole (10 mg/kg) were also tested for interactions with DOI (1.25 mg/kg, i.p.). Preliminary work had suggested a dose-dependent increase in frequency of cage crosses by the δ-opioid agonist (1 and 2 mg/kg) following DOI administration, but without any change in the frequency of DOI-induced head shakes. A significant effect of naltrindole ($F(1,11)=12.16$, $P<0.01$), but not the δ-opioid receptor agonist ($F(1,11)=1.37, P>0.1$) was observed for forward locomotion (Fig. 4). A trend towards a significant interaction was observed ($F(1,11)=3.34, P<0.01$). Post-hoc testing found that the δ-opioid receptor agonist increased the frequency of cage crosses in DOI-treated rats compared to DOI alone ($P<0.05$). In addition, the δ-opioid receptor antagonist naltrindole completely blocked the effect of the δ-opioid receptor agonist ($P<0.01$). No significant effects of either the δ-opioid receptor agonist ($F(1,11)=0.04, P>0.1$), the antagonist naltrindole ($F(1,11)=0.27, P>0.1$) or the interaction of the agonist and antagonist ($F(1,11)=0.86, P>0.1$) were observed for the frequency of DOI-induced head shakes.

4. Discussion

The main finding from the present study was that activation of µ-opioid receptors suppressed DOI-induced head shakes, a 5-HT$_{2A}$ receptor mediated behavior. This conclusion is based on the following points. First, buprenorphine, a mixed µ-opioid receptor partial agonist and κ-opioid receptor antagonist (Cowan et al., 1977; Leander, 1987; Negus and Dykstra, 1988; Negus et al., 1990), potently suppressed DOI-induced head shakes with an ID$_{50}$ of ~0.005 mg/kg. The suppressant action by buprenorphine suggests that κ-opioid receptor agonism is not a common shared factor for both morphine (Alvaro et al., 1998) and buprenorphine with respect to the mechanism underlying suppression of DOI-induced head shakes. Second, the suppressant effect of the buprenorphine on DOI-induced head shakes was blocked by the classical opioid receptor antagonist naloxone. More specifically, the suppression by buprenorphine was blocked by a relatively low dose of the selective irreversible µ-opioid receptor antagonist, clonidine. Third, the relatively selective µ-opioid receptor agonist fentanyl also suppressed DOI-induced head shakes in a dose-dependent manner. Fourth, a δ-opioid receptor agonist had no effect on DOI-induced head shakes despite increasing locomotor activity of DOI-treated rats. This enhanced locomotor response was blocked by a δ-opioid receptor antagonist. The most parsimonious interpretation of these studies is that activation of µ-opioid receptors results in a suppression of DOI-induced head shakes.

4.1. µ-Opioid receptor agonist suppression of DOI-induced head shakes: behavioral competition

The effects of buprenorphine or fentanyl on DOI-induced head shakes could be due to a non-selective action of these drugs. Sedation or catalepsy are candidate behavioral states that a priori would appear incompatible with head shake behavior. Both head shakes and forward locomotor activity...
would be similarly affected by such non-selective drug effects. However, a simple relationship is not observed between head shakes and locomotor activity. First, the μ- opioid receptor agonists can suppress DOI-induced head shakes without simultaneously having effects on locomotor activity. For example, lower doses of buprenorphine (0.01 and 0.1 mg/kg) that did not suppress DOI-induced forward locomotion robustly suppressed DOI-induced head shakes. Second, other behavioral depressants such as subanesthetic doses of barbiturates did not have an effect on head shakes in mice induced by 5-hydroxytryptophan (Corne et al., 1963). Third, head shakes also occur during transitional behavioral states such as during induction or recovery from anesthesia. Therefore, the suppression by μ-opioid receptor agonists of DOI-induced head shakes may have occurred through a direct interaction on circuitry controlling this behavior, rather than simple incompatibility of two different behaviors (e.g., head shakes and catalepsy).

4.2. Relevant neurocircuitry

The locus of the 5-HT$_{2A}$ and μ-opioid receptor physiological interaction is not known. 5-HT$_2$ agonists appear to induce head shakes both at subcortical sites (Bedard and Pycock, 1977; Lucki and Minugh-Purvis, 1987) and in the medial prefrontal cortex (Granhoff et al., 1992; Willins and Meltzer, 1997). The 5-HT$_{2A}$ receptor has a restricted expression at subcortical sites, although it is expressed strongly in cranial and spinal motoneurons (Pompeiano et al., 1994; Wright et al., 1995). Along this vein, blockade of 5-HT$_{2A}$ receptors appears to decrease the amplitude of the nictitating membrane response of the rabbit (Welsh et al., 1998), a response mediated through the accessory abducens nucleus (Marek et al., 1983). Interestingly, hallucinogens such as LSD enhance the acquisition of conditioned reflexes for the rabbit nictitating membrane response while μ-opioid receptor agonists retard acquisition of conditioned reflexes (Schindler et al., 1985). However, the locus of these interactions is not known.

The medial prefrontal cortex is another potential site for physiological interactions between 5-HT$_{2A}$ and μ-opioid receptors. Direct infusion of substituted amphetamines with hallucinogenic effects into the medial prefrontal cortex induced head shakes in rats (Granhoff et al., 1992; Willins and Meltzer, 1997). Layer Va of the neocortex possesses an especially high density of both 5-HT$_{2A}$ and μ-opioid receptors (Tempel and Zukin, 1987; Blue et al., 1988). Electrophysiological studies found that μ-opioid receptor activation completely suppressed excitatory postsynaptic currents induced by 5-HT$_{2A}$ receptor activation in layer V pyramidal cells from the medial prefrontal cortex (Marek and Aghajanian, 1998a). Interestingly, μ-opioid receptor agonists were more potent and efficacious at suppressing 5-HT-induced EPSCs in the limbic-related medial prefrontal cortex than in the nearby fronto-parietal cortex. Others have previously noted that the medial prefrontal cortex has a higher density of μ-opioid receptors than other areas of the neocortex (Lewis et al., 1983; McLean et al., 1986; Tempel and Zukin, 1987). Finally, we recently found that medial thalamic lesions directed at the midline and intralaminar thalamic nuclei simultaneously decreased the density of μ-opioid receptors in the prefrontal cortex and also decreased the frequency of 5-HT-induced EPSCs by 60% (Marek et al., 2001). It remains to be determined whether the present behavioral results could be a consequence of such a physiological interaction between 5-HT$_{2A}$ and μ-opioid receptors in the medial prefrontal cortex.

4.3. Analogous action between 5-HT$_{2A}$ and mGlu2 receptors

Regarding the circuitry upon which DOI and these μ-opioid receptor agonists act, similar findings for interactions between metabotropic glutamate2 (mGlu2) and 5-HT$_{2A}$ receptors supports a primary prefrontal cortical site of action. Thus, electrophysiological studies suggested that mGlu2 receptors play a physiological role in modulating excitatory amino acid release in the medial prefrontal cortex (Marek et al., 2000; Kladzinska et al., 2002; Zhai et al., 2003). DOI-induced head shakes are suppressed by activation of mGlu2 receptors and enhanced by blockade of mGlu2 receptors (Gewirtz and Marek, 2000; Kladzinska et al., 2002). DOI-induced increases in the expression of BDNF or c-fos mRNA are similarly modulated by activation of mGlu2 receptors (Gewirtz et al., 2002; Zhai et al., 2003). mGlu2 receptors are decreased in the superficial and mid-cortical layers of the prefrontal cortex by thalamic lesions which also (1) decreased the density of μ-opioid receptors and (2) decreased the frequency of 5-HT-induced EPSCs by ~60% (Marek et al., 2001). Finally, preliminary studies have found that pretreatment with the mGlu2/3 agonist LY354740 (3 mg/kg, i.p.) suppressed the frequency of head shakes induced by local infusion of DOI into the medial prefrontal cortex (G. Marek, unpublished observations).

4.4. Clinical implications

Clinical implications of interactions between 5-HT$_{2A}$ and μ-opioid receptors (Marek and Aghajanian, 1998b) have been reviewed for the study of mood disorders. Anecdotal and historical data have suggested that activation of μ-opioid receptors is a common denominator among several treatments with putative antidepressant properties. Several open trials of the μ-opioid agonist buprenorphine suggested some utility in depressed patients (Emrich et al., 1982; Bodkin et al., 1995). Conversely, an anecdotal report for the non-selective opioid antagonist naloxone (2 mg, i.v.) described increased depression and anxiety in six patients, five of whom were diagnosed with unipolar depression (Cohen et al., 1984).

Experience in opiate detoxification settings has also suggested potential antidepressant effects of opioid receptor
agonists. For example, rapid opiate detoxification sometimes precipitates major depression which can be resolved either with tricyclic antidepressants or a resumption of methadone (Gold et al., 1979). Even slow detoxification from methadone has been reported to result in an organic mood syndrome (Kanof et al., 1993). In this vein, preclinical, clinical, and epidemiological evidence suggest that opiate abuse may occur in part, as a means of treating depressive symptoms (Markou et al., 1998).

Blockade or down-regulation of 5-HT$_{2A}$ receptors appears increasingly relevant to the antidepressant effects of most antidepressant drugs, excluding selective serotonin reuptake inhibitors (Marek et al., 2003). Even for selective serotonin reuptake inhibitors, recent clinical studies suggest that addition of medications with potent 5-HT$_{2A}$ blockade can lead to a therapeutic response in SSRI-refractory depressed patients (Maes et al., 1999; Ostrow and Nelson, 1999; Ferreri et al., 2001; Shelton et al., 2001; Carpenter et al., 2002). The thymoleptic effects of drugs with 5-HT$_{2A}$ receptor antagonist action coupled with the historical and anecdotal reports of thymoleptic properties of drugs with μ-opioid receptor agonist properties are intriguing in light of interactions between these two receptors in modulating glutamate release from thalamocortical afferents. Thus, use of model systems for the study of cortical 5-HT$_{2A}$ receptor function (whether with single-cell electrophysiology or behavior) may be of heuristic value for identifying novel treatments for mood disorders and other neuropsychiatric syndromes (Marek, 2000).

Acknowledgements

Supported by NIH K08 MH01551, RO1 MH62186, a NARSAD Young Investigator Award and the State of Connecticut. I especially thank Dr. George Aghajanian for support during the initial experiments. I thank Allyson Abo and Eric Ricchetti for technical assistance and Leslie Rosello for secretarial assistance.

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