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Modulation of DOI-induced increases in cortical BDNF expression by group II mGlu receptors

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Abstract

Previous studies have shown that 5-hydroxytryptamine$_{2A}$ (5-HT$_{2A}$) receptor activation induces changes in the pattern of brain-derived neurotrophic factor (BDNF) mRNA expression in the neocortex and hippocampus, and that 5-HT$_{2A}$ receptor blockade interferes with the induction of BDNF mRNA by stress. Recent studies have also shown that activation of metabotropic glutamate group II (mGlu2/3) receptors suppresses 5-HT$_{2A}$ receptor-stimulated excitatory postsynaptic potentials/currents (EPSP/Cs) in pyramidal neurons in medial prefrontal cortex. Conversely, blockade of mGlu2/3 receptors enhances 5-HT-induced EPSCs. The current study examined the effects of the highly selective mGlu2/3 agonist (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate (LY354740) and the mGlu2/3 antagonist 2*S*-2-amino-2-(1*S*,2*S*-2-carboxycycloprop-1-yl)-3(xanthy-9-yl)propanoic acid (LY341495) on BDNF mRNA expression in medial prefrontal cortex induced by the hallucinogen and 5-HT$_{2A/2B/2C}$ agonist 1-(2,5-dimethoxy-4-iodophenethyl)-2-aminopropane (DOI). LY354740 (0.1–10 mg/kg) dose-dependently suppressed DOI-induced BDNF mRNA levels in medial prefrontal cortex. In contrast, the mGlu2/3 antagonist LY341495 (1 mg/kg) enhanced DOI-induced BDNF mRNA levels. BDNF mRNA expression was not altered by administration of the mGlu agonist or the antagonist alone. These results are discussed with respect to a potential role for group II mGlu agonists in the treatment of depression and schizophrenia.

Keywords: Brain-derived neurotrophic factor; Hallucinogenic drugs; Phenethylamine hallucinogens; Serotonin; 5-Hydroxytryptamine$_{2A}$ receptor; 5-HT$_{2A}$ receptor; Medial prefrontal cortex; Glutamate; Metabotropic glutamate2/3 receptors; mGlu2 receptors

1. Introduction

The neurotrophin growth factors comprise a family of small, highly conserved proteins, including nerve growth factor (NGF), neurotrophin-3 (NT3), neurotrophin-4/5 (NT4/5) and brain-derived neurotrophic factor (BDNF). It is increasingly apparent that neurotrophins not only promote the survival and differentiation of neurons during development (McAllister et al., 1995; Nonner et al., 1996; Martinez et al., 1998), but also influence the function and plasticity of neuronal populations in the adult brain (Kang and Schuman, 1995; Levine et al., 1995; Thoenen, 1995; Korte et al., 1996; Patterson et al., 1996; Li et al., 1998; Pozzo-Miller et al., 1999). In this context, a number of striking relationships between BDNF and serotonergic neurotransmission (Mamounas et al., 1995; Celada et al., 1996; Siuciak et al., 1996; Dong-Ryulu et al., 1999; Lyons et al., 1999; Zetterstrom et al., 1999) are of interest in understanding the therapeutic action of the most commonly used drugs to treat mood disorders and schizophrenia.

One of the factors involved in regulating BDNF expression is stimulation of the 5-hydroxytryptamine$_{2A}$ (5-HT$_{2A}$) receptor. The phenethylamine hallucinogen 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT$_{2A/2B/2C}$ agonist, increases BDNF mRNA expression particularly in both the midlayer of the neocortex (layer IV and/or layer V) and the claustrum, while decreasing mRNA expression in the dentate gyrus of the hippocampus (Vaidya et al., 1997). These effects of DOI and the stress-induced reduction of BDNF in the hippocampus are completely blocked

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by co-administration of the highly selective 5-HT_{2A} antagonist M100907 (formerly MDL 100,907), but not a 5-$HT_{2B/2C}$ antagonist, indicating that BDNF mRNA expression is modulated by 5-HT_{2A} receptor stimulation (Vaidya et al., 1997, 1999).

The effects of 5-HT_{2A} receptor stimulation on BDNF expression are of considerable interest from a clinical perspective. BDNF, the most ubiquitous neurotrophin in the rodent brain (Ernfors et al., 1990; Hofer et al., 1990; Wetmore et al., 1990), has been implicated in a variety of functions such as memory and circadian regulation, and in the pathophysiology of brain disorders such as depression, drug addiction and epilepsy (Nibuya et al., 1995; Levine and Black, 1997; Horger et al., 1999; Liang et al., 2000; Binder et al., 2001). 5-HT_{2A} receptors may play a role in the pathophysiology and/or treatment of both psychosis and depression. Activation of 5-HT_{2A} receptors is thought to be responsible for the psychotomimetic properties of different classes of hallucinogens, namely, ergots (e.g., lysergic acid diethylamide, LSD), simple indoleamines (e.g., psilocybin), as well as the phenethylamines (Titeler et al., 1988; Glennon, 1990; Marek and Aghajanian, 1996; Vollenweider et al., 1998). Moreover, blockade of the 5-HT_{2A} receptor is thought to be critically involved in the action of ''atypical'' antipsychotic medications, such as clozapine, olanzapine, risperidone, seroquel and ziprasidone (Ceulemans et al., 1985; Altar et al., 1986; Rasmussen and Aghajanian, 1988; Meltzer et al., 1989; Roth et al., 1994). 5-HT_{2A} receptor blockade may also mediate the therapeutic effects of antidepressant medications that do not appreciably alter uptake of monoamines or monoamine oxidase activity (i.e., certain ''atypical'' antidepressants, such as mirtazapine, mianserin, nefazodone and trazodone) (Wander et al., 1986; Eison et al., 1990; Marek et al., 1992; de Boer, 1996).

Two competing, though not mutually exclusive, models to explain the positive regulation of neocortical BDNF by 5-HT_{2A} receptor activation exist. The first possibility is simply that the DOI-induced increase in BDNF mRNA expression might be due primarily to 5-HT_{2A} receptor activation directly on neocortical pyramidal cells with subsequent depolarization and elevation of intracellular calcium stores (Ashby et al., 1989; Araneda and Andrade, 1991; Tanaka and North, 1993; Aghajanian and Marek, 1997). An alternate possibility is that the DOI-induced increase in BDNF mRNA expression results primarily from glutamate release from thalamocortical afferents (Aghajanian and Marek, 1997; Marek and Aghajanian, 1998a; Marek et al., 2000, 2001). Our electrophysiological studies have suggested that metabotropic glutamate2 (mGlu2) receptors play an autoreceptor role in the thalamocortical terminals/axons from which 5-HT_{2A} receptor activation induces glutamate release (Marek et al., 2000, 2001). Thus, activation of mGlu2 receptors suppressed the frequency of 5-HT-induced excitatory postsynaptic currents (EPSCs), while blockade of mGlu2 receptors enhanced the frequency of 5-HT-induced EPSCs (Marek et al., 2000). The exact presynaptic compartment that is directly modulated by mGlu2 and 5-HT_{2A} receptors (terminal or preterminal) and the exact localization of these receptors (presynaptic or even postsynaptic with ''retrograde'' influence) remains to be determined. However, we have also recently found that DOI-induced head shakes, which appear to involve activation of prefrontal cortical 5-HT_{2A} receptors (Granhoff et al., 1992; Willins and Meltzer, 1997), are suppressed by a mGlu2/3 agonist (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate (LY354740) and enhanced by an mGlu2/3 antagonist 2*S*-2-amino-2-(1*S*,2*S*-2-carboxycycloprop-1-yl)-3(xanthy-9-yl)propanoic acid (LY341495), consistent with our electrophysiological findings (Gewirtz and Marek, 2000). Therefore, we sought to examine whether systemic administration of the mGlu2/3 agonist LY354740 and the mGlu2/3 antagonist LY341495 would suppress and enhance, respectively, the DOI-induced increase in BDNF mRNA expression in the mPFC.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (200–250 g, Harlan, Indianapolis, IN, USA) were group-housed and maintained on a 12-h light–dark cycle (lights on at 07:00 h, lights off at 19:00 h) with access to food and water ad libitum. All experiments were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), and were approved by the Yale University Animal Care and Use Committee.

2.1.1. Drugs

DOI was purchased from RBI (Natick, MA). LY354740 and LY341495 were kindly provided by Drs. James A. Monn and Darryle D. Schoepp of the Lilly Research Laboratories (Indianapolis, IN). Doses were calculated on the basis of the salt forms. The drugs were dissolved in saline, neutralized to a pH $\sim$ 7.4 and injected ip in a volume of 1 ml/kg body weight.

2.2. General procedures

All experiments were conducted between 10:00 and 17:00 h. To habituate rats to the stress associated with injection, the animals were injected with saline vehicle daily for 1 week prior to the experiment. On the experimental day, rats were injected with LY354740, LY341495 or saline (intraperitoneally) and, 15 min later, with DOI (5 mg/kg ip) or saline. Two hours after the second injection, the rats were decapitated. This latency has been found previously to allow for maximal BDNF mRNA expression in the neocortex (Vaidya et al., 1997). The brains were removed and frozen on dry ice, and stored at -80 °C.

Three experiments were conducted. The first experiment compared the effects of LY354740 and vehicle injection on DOI-induced BDNF mRNA transcription. The treatment groups were as follows: 10.0 mg/kg LY354740/DOI ($n=4$), saline/DOI ($n=4$), 10.0 mg/kg LY354740/saline ($n=4$), saline/saline ($n=4$). The second experiment compared the effects of different doses of LY354740 on DOI-induced BDNF expression. The treatment groups were as follows: 0.1 mg/kg LY354740/DOI ($n=4$), 1.0 mg/kg LY354740/DOI ($n=3$), 10.0 mg/kg LY354740/DOI ($n=3$), saline/DOI ($n=3$) and saline/saline ($n=3$). The third experiment examined the effect of LY341495 on DOI-induced BDNF transcription. The treatment groups were as follows: 1.0 mg/kg LY341495/DOI ($n=8$), 1.0 mg/kg LY341495/saline ($n=9$), saline/DOI ($n=8$) and saline/saline ($n=9$). This dose of LY341495 was chosen because it effectively blocks the suppressant action of the mGlu2/3 agonists LY354740 and LY379268 on the motoric disruption induced by phencyclidine (Cartmell et al., 1999).

2.3. In situ hybridization

In situ hybridization for BDNF mRNA was carried out as described previously (Nibuya et al., 1995). In brief, coronal sections of 14-μm thickness were cut on a cryostat and thaw-mounted onto RNase free Probe-on (+) slides (Fisher). Tissue sections were fixed in 4% formaldehyde, acetylated and dried. Levels of BDNF were examined by probing with ^{35}S-labeled antisense riboprobes. Rat BDNF cDNA clones were obtained from Regeneron (Tarrytown, NY). The sections were hybridized with 2×10^6 cpm/section for 18 h at 55 °C in hybridization buffer (50% formamide, 0.6 M NaCl, 10 mM Tris, 1 × Denhardt's solution, 2mM EDTA, 10 mM DTT, 10% dextran sulfate, 50 μg/ml salmon sperm DNA, 250 mg/ml tRNA). After hybridization, sections were washed in 2 × SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) at 25 °C and then treated with 20 μg/ml RNase A for 30 min in RNase buffer (0.5 M NaCl, 10 mM Tris, 1 mM EDTA). The sections were then washed for 10 min in 2 × SSC at room temperature and twice for 20 min on 0.2 × SSC at 55 °C. The sections were then rinsed in 0.2 × SSC, dried and exposed to BioMax Film (Kodak, Rochester, NY) for 5 days. Previous work in this laboratory using the same procedures did not yield any significant hybridization with ^{35}S-labeled sense BDNF riboprobes, indicating that the signal observed with the antisense riboprobe is specific.

2.4. Data analysis

Levels of BDNF were analyzed using the Macintosh-based National Institutes of Health Image program, version 1.57. A portion of the prelimbic area of medial prefrontal cortex (within an area 2.7–4.2 mm anterior to bregma) was analyzed for in situ hybridization. The region was analyzed by outlining the area of interest; an equivalent area was outlined for each sample. For each animal, the optical density measurements from both sides of four individual sections were analyzed, yielding eight measurements, from which the mean was calculated. To correct for nonlinearity, 14C step standards were used for calibration. Given our previous electrophysiological findings that 5-HT-induced synaptic currents originate from thalamocortical afferents (Marek et al., 2001), we also examined BDNF mRNA in situ hybridization from sections taken 3–3.5 mm posterior to bregma.

The results were then subjected to statistical analysis, using between-subjects analysis of variance (ANOVA) followed by the post-hoc Newman–Keuls test.

3. Results

3.1. A mGlu2/3 agonist blocks DOI-induced increases in cortical BDNF mRNA expression

DOI induced an increase in the expression of BDNF mRNA in the medial prefrontal cortex, an effect that was reversed by pretreatment with the mGlu2/3 agonist LY354740. This is illustrated in Fig. 1, which shows

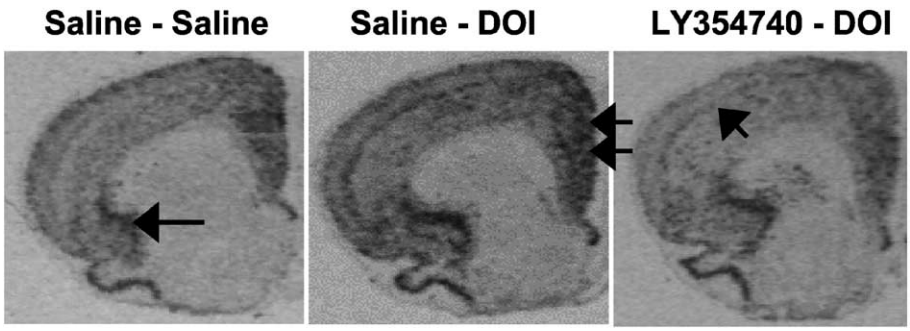

Fig. 1. Effect of the mGlu2/3 agonist LY354740 and DOI on BDNF mRNA in situ hybridization. Representative samples of BDNF mRNA in situ hybridization are shown for rats treated two injections of saline (left), saline followed by DOI (5 mg/kg ip, middle) or LY354740 (10 mg/kg ip) followed 15 min later by DOI (right). The arrow in the saline–saline section shows the claustrum. The arrows in the middle figure denote dorsal and ventral boundaries of the mPFC region, primarily comprising prelimbic cortex (Cg3), subjected to quantification. The arrow in the section from a LY354740–DOI-treated rat indicates the intense mid-neocortical layer corresponding either to layer IV or Va. The sections correspond to a level intermediate between Plates 10 and 11 or 2.2–1.7 mm anterior to bregma (Paxinos and Watson, 1986).

representative brain sections taken from subjects treated with two injections of saline, saline followed by DOI and LY354740 followed by DOI. DOI-induced increases in the expression of BDNF mRNA were greatest in mPFC, layer IV or Va of the neocortex (and deep layer III or Va of the mPFC) and the claustrum. The modulation of BDNF mRNA expression by DOI and the mGlu2/3 agonist appeared to be similar in the ventral anterior cingulate, the prelimbic region of the mPFC and extending inferiorly through the infralimbic region. LY354740 reduced the DOI-induced increase in BDNF mRNA levels for these areas. Fig. 2 summarizes the results of the quantification of BDNF mRNA expression in mPFC (largely the prelimbic region) in the different subject groups. Administration of DOI upregulated BDNF mRNA expresssion to 159% of control (saline/saline) values. This enhancement was completely blocked by 10 mg/kg LY354740. LY354740 administered on its own produced a small nonsignificant reduction in BDNF mRNA levels. These impressions were confirmed by an ANOVA, which revealed a significant effect of both DOI [$F(1,12)=17.4$, $P<.001$], LY354740 [F(1,12) = 14.4, $P<.002$] and a significant DOI × LY354740 interaction [$F(1,12)=8.2$, $P<.01$]. The post-hoc Newman–Keuls test revealed that the level of BDNF expression in the saline–DOI group was significantly greater than in the other three groups, including the LY354740–DOI group ($P<.001$). There were no significant differences between the other three groups ($P>.5$). Thus, LY354740 on its own did not significantly alter BDNF expression.

In the same sections from which the mPFC was examined, the effects of DOI and LY354740 was also determined in the frontoparietal (primary motor and somatosensory cortex; Fr3 and Par1) cortex (Fig. 1) and the claustrum. A significant effect of DOI [$F(1,10)=7.4$, $P<.05$] and LY354740 [$F(1,10)=11.1$, $P<.01$) on BDNF mRNA expression was

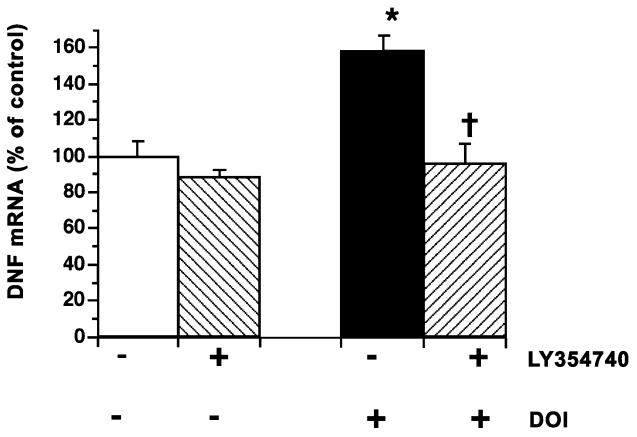

Fig. 2. Effects of the mGlu2/3 agonist LY354740 and DOI on BDNF mRNA in situ hybridization densitometry. Shown is a summary graph for the effects of LY354740 (10 mg/kg) and DOI (5 mg/kg) on BDNF mRNA levels in the prelimbic region of the mPFC corresponding to the experiment exemplified in Fig. 1. Significantly different from the saline–saline group, * * $P<.01$. Significantly different from the saline–DOI group, $^{\dagger\dagger}P<.01$.

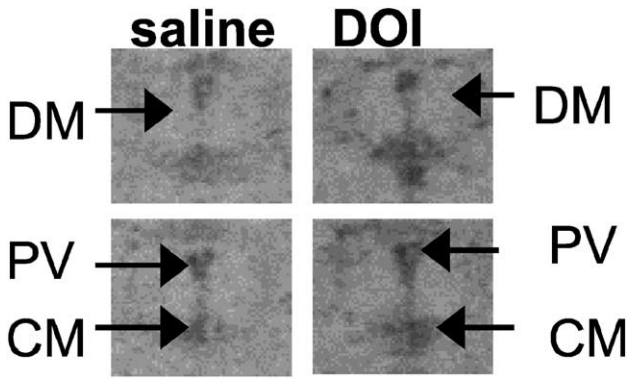

Fig. 3. Effect of saline compared to DOI injections on BDNF mRNA in situ hybridization in the thalamus. Representative samples of BDNF mRNA in situ hybridization are shown for two different rats treated with two injections of saline (left) or two different rats treated with saline followed by DOI (5 mg/kg ip, right). The arrows in the lower figures denote the paraventricular (PV) and central medial (CM) nuclei of the thalamus. These midline thalamic nuclei preferentially project to the prelimbic region of the mPFC (Berendse and Groenewegen, 1991). Note that the mediodorsal nucleus of the thalamus (arrows shown in top sections) is minimally affected compared to the midline thalamic nuclei. These sections correspond approximately to Plate 29 or 2.8 mm posterior to bregma (Paxinos and Watson, 1986).

observed in the frontoparietal cortex. There was not a significant interaction between these factors [$F(1,10)=0.5$, $P>.5$]. The only group that was significantly different from the saline/saline group was DOI/saline (153 ± 23% of the saline/saline, $P<.05$), while the DOI/LY354740 group (80 ± 7% of the saline/saline group, $P<.05$) was significantly lower than the DOI/saline group. The saline/LY354740 group was nonsignificantly decreased (59 ± 2%) compared to the saline/saline group.

In the claustrum (Fig. 1), a significant effect of DOI [$F(1,10)=13.4$, $P<.01$], but not of LY354740 [$F(1,10)=1.6$, $P>.2$] was present. However, a significant interaction occurred between these two drug treatments [$F(1,10)=5.7$, $P<.05$]. Again, the only group that was significantly different from the saline/saline group was the DOI/saline group (130 + 16% of the saline/saline group, $P<.05$), while the DOI/LY354740 group (80 ± 7% of the saline/saline group) was significantly lower than the DOI/saline group ($P<.05$). The BDNF mRNA levels for the saline/LY354740 group was nonsignificantly decreased (89 ± 8% of the saline/saline group).

DOI also induced an increase in BDNF mRNA expression in the intralaminar and midline thalamic nuclei (Fig. 3). The mGlu2/3 agonist LY354740, in preliminary experiments, did not appear to modulate this effect of DOI in the thalamus (not shown).

3.2. Blockade of DOI-induced BDNF mRNA levels in mPFC by LY354740 is dose-dependent

As in the first experiment, DOI produced a robust (54%) increase in BDNF mRNA. All three doses of LY354740

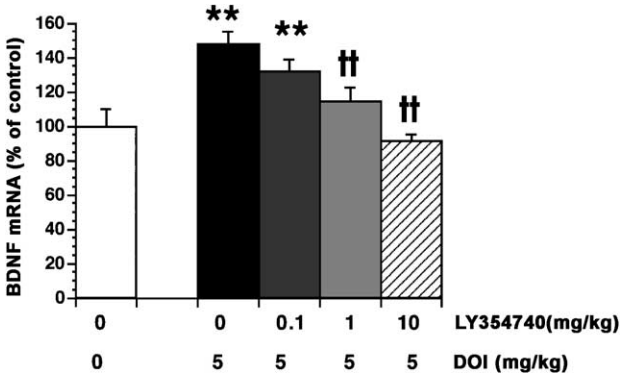

Fig. 4. Dose–response relationship for the potency of LY354740 (0.1–10 mg/kg ip) at suppressing the increase induced by DOI (5 mg/kg ip) in BDNF mRNA in the mPFC. Significantly different from the saline–saline group, $^{**}P<.01$. Significantly different from the saline–DOI group, $^{\dagger\dagger}P<.01$.

reduced upregulation of BDNF mRNA by DOI in mPFC. As shown in Fig. 4, the magnitude of the reduction was related linearly to dose, with 0.1 mg/kg LY354740 producing a small decrease and 10 mg/kg LY354740 producing a complete block of the DOI effect, consistent with the results of the previous experiment. These findings were supported by statistical analysis of the data. An ANOVA revealed a significant main effect of dose [$F(4,12)=12.8$, $P<.001$]. A comparison of the groups with the post-hoc Newman–Keuls test indicated that DOI induced a significant increase in BDNF transcription ($P=.001$), and that this increase was significantly reduced in the groups pretreated with 1 mg/kg LY354740 ($P<.01$) and 10 mg/kg LY354740 ($P<.01$). Furthermore, these groups did not differ significantly from the saline–saline controls ($P>.1$), indicating that LY354740 effectively blocked DOI-induced expression of BDNF mRNA at both these doses. The 0.1 mg/kg dose of LY354740 did not produce a significant reduction in BDNF transcription ($P=.1$) and, like the saline–DOI group, this group differed significantly from the saline–saline control group ($P=.001$). This suggests that a dose of 0.1 mg/kg LY354740 was not sufficient to produce a reliable reduction in DOI-induced expression of BDNF mRNA. Nonlinear curve fitting of the data in Fig. 3 (not shown) found an ED_{50} of 0.55 mg/kg for the suppressant action of LY354740 on the increased BDNF mRNA induced by DOI.

3.3. The mGlu 2/3 antagonist LY341495 enhances DOI-induced transcription of BDNF

DOI produced a robust increase in BDNF transcription in mPFC (182% of the saline/saline group), as in the previous experiments. Strikingly, BDNF mRNA expression was potentiated substantially in the group pretreated with the mGlu2/3 antagonist LY341495 (121% increase compared to the saline/saline group). These results are depicted in Fig. 5, which shows representative sections from subjects in each of the treatment groups, i.e., saline/saline, LY341495/saline, saline/DOI and LY341495/DOI. The interaction between LY341495 and DOI was shown clearly by the densitometry measurements of BDNF mRNA expression levels in mPFC, summarized in Fig. 6. An ANOVA indicated a significant effect of DOI [$F(1,30)=57.3$, $P<.001$]. The main effect of LY341495 approached significance [$F(1,30)=3.31$, $P=.08$] and the interaction was not significant [$F(1,30)=1.5$, $p=.2$]. The planned post-hoc Newman–Keuls comparisons revealed that BDNF mRNA levels in both the saline–DOI and LY341495–DOI groups were significantly higher than in the saline–saline and LY341495–saline groups ($P<.001$). Interestingly, however, BDNF mRNA expression in the

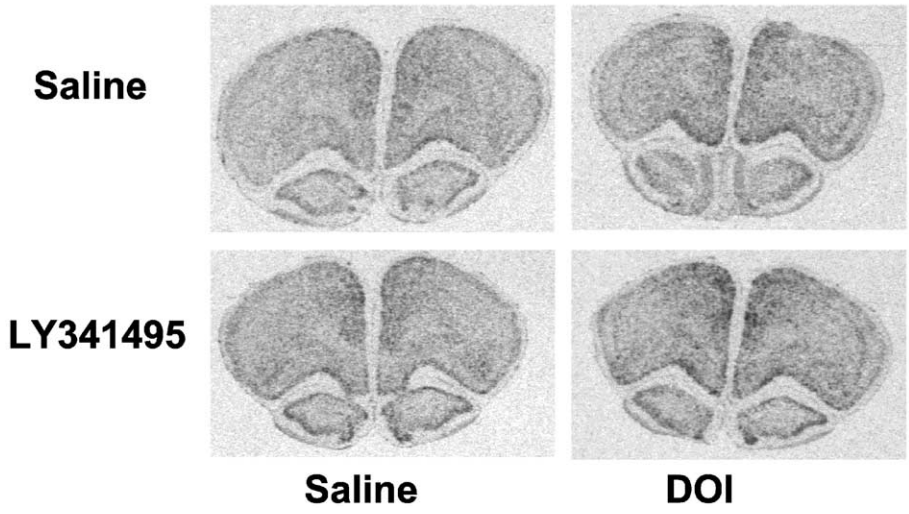

Fig. 5. Effect of the mGlu2/3 antagonist LY341495 and DOI on BDNF mRNA in situ hybridization. Shown are representative samples of BDNF mRNA in situ hybridization for rats treated two injections of saline (left upper), saline followed by DOI (5 mg/kg ip, left lower), LY341495 (1 mg/kg ip) followed 15 min later by saline (right upper) or LY341495 (1 mg/kg ip) followed 15 min later by DOI (right lower). The sections correspond to a level intermediate between Plates 6 and 7 or 4.2–3.7 mm anterior to bregma (Paxinos and Watson, 1986).

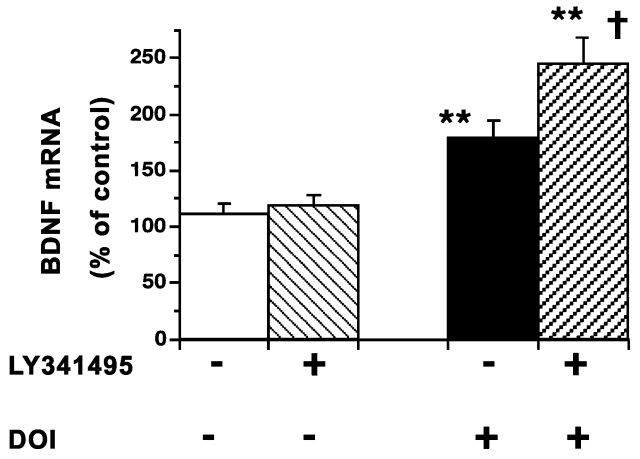

Fig. 6. Summary graph for the effects of the mGlu2/3 antagonist LY341495 and DOI on BDNF mRNA levels in the mPFC. Significantly different from the saline–saline group, $^{**}P<.01$. Significantly different from the saline–DOI group, $^{\dagger\dagger}P<.01$.

LY341495–DOI group was also significantly higher than in the saline–DOI group ($P<.05$), indicating that LY341495 significantly potentiated the effect of DOI. In contrast, LY341495 on its own did not enhance BDNF transcription ($P=.7$).

4. Discussion

This increased expression of BDNF mRNA induced by DOI in the neocortex and mPFC is known to be mediated by the activation of 5-HT_{2A} receptors (Vaidya et al., 1997). The results of the present study demonstrated that the mGlu 2/3 receptor agonist LY354740 suppressed this increase in a dose-dependent manner. In contrast, the mGlu antagonist LY341495 produced a frank enhancement of DOI-induced BDNF expression in mPFC. When administered in the absence of DOI, neither the mGlu 2/3 agonist nor the antagonist had a significant effect on BDNF mRNA expression.

These effects of LY354740 and LY341495 are likely mediated by interaction with mGlu2 and/or mGlu3 receptors, and not by interaction with other receptors. First, it is unlikely that the effects observed were mediated through ionotropic glutamate receptors in light of the high degree of specificity of this class of rigid glutamate analogs for metabotropic glutamate receptors (Monn et al., 1997; Schoepp et al., 1997; Kingston et al., 1998). Second, it is also unlikely that the drugs' effects were mediated by other metabotropic glutamate receptor subtypes. Although LY341495 has only a three- to seven-fold higher affinity for mGlu2/3 receptors than for the group III mGlu8 receptor (Kingston et al., 1998), LY354740 appears to have a 120–1200-fold higher affinity for mGlu2/3 receptors than for mGlu8 receptors (Monn et al., 1997; Schoepp et al., 1997). It should also be noted that a 1 mg/kg dose of the mGlu2/3 antagonist LY341495 probably not does not saturate mGlu2 receptors (Cartmell et al., 2000). Thus, the most parsimonious explanation of the present results is that activation/blockade of mGlu2/3 receptors mediates the effects of the mGlu agonist and antagonist tested, although we cannot rule out a contribution to the effect of LY341495 via blockade of mGlu8 receptors.

Previous electrophysiological studies provide a mechanism to explain these 5-HT/glutamate interactions at a cellular level. The present data on the regulation of BDNF by DOI and mGlu2/3 agonists/antagonists bear a striking resemblance to the interactions observed between mGlu2/3 and 5-HT_{2A} receptor activation for the in vitro mPFC slice preparation. 5-HT_{2A} receptor stimulation enhances excitatory postsynaptic currents (EPSCs) in layer V pyramidal neurons throughout the neocortex and mPFC in a focal fashion consistent with the peak densities of 5-HT_{2A} receptors in layers I and Va (Aghajanian and Marek, 1997; Marek and Aghajanian, 1999). Our electrophysiological studies suggested a focal action on axonal, rather than somatodendritic, compartments to induce excitatory amino acid release (Marek and Aghajanian, 1998b; Aghajanian and Marek, 1999). Whether 5-HT_{2A} receptors are exclusively present at postsynaptic sites in cortical soma and dendrites is in doubt. Although the majority of 5-HT_{2A} receptors in neocortex and mPFC are likely to be located postsynaptically on pyramidal cells, scattered presumed glutamatergic cortical terminals are labeled by a 5-HT_{2A} receptor antibody (Jakab and Goldman-Rakic, 1998). Further precedence for presynaptic localization of 5-HT_{2A} receptors is the observation by Cornea-Hebert et al. (1999) that 5-HT_{2A} receptor immunoreactive axons are found in every myelinated tract known to arise from immunoreactive cell bodies.

Whether 5-HT acts on postsynaptic 5-HT_{2A} receptors on pyramidal cells to influence a retrograde messenger or acts on presynaptic 5-HT_{2A} receptors in nerve endings terminating onto the apical dendrites of pyramidal cells, 5-HT appears to induce synaptic currents by releasing an excitatory amino acid, which then activates postsynaptic AMPA receptors on layer V pyramidal cells (Aghajanian and Marek, 1997; Zhang and Marek, 2000). Interestingly, cortical BDNF expression is dependent on glutamate release and specifically on glutamatergic stimulation of AMPA receptors (Zafra et al., 1990; Wetmore et al., 1994). Hence, the elevation in BDNF mRNA expression in response to DOI is entirely consistent with the increase in glutamate release and in AMPA receptor activation seen after 5-HT_{2A} receptor stimulation in the mPFC slice preparation. However, we cannot rule out a contribution of postsynaptic 5-HT_{2A} receptors on pyramidal cells to the DOI-induced increase in BDNF mRNA expression.

The interaction of mGlu receptors with the functional expression of cortical 5-HT_{2A} responses is suggested by our recent electrophysiological evidence that the mGlu2 receptor may function as an autoreceptor with respect to 5-HT-induced EPSCs. Namely, the mGlu2/3 agonist LY354740

suppressed, while the mGlu2/3 antagonist LY341495 enhanced, the frequency of 5-HT induced EPSCs (Marek et al., 2000). These findings were corroborated by the demonstration that chemical lesions which destroyed much of the midline and intralaminar thalamic nuclei reduced the frequency of 5-HT-induced EPSCs by ~60% and also decreased cortical binding of [^{3}H]LY354740 (Marek et al., 2001). As discussed previously, differential anatomical localization of mGlu2 and mGlu3 receptor mRNA in glutamatergic relay vs. GABAergic reticular cells in thalamus together with the apparent laminar overlap in the mPFC for the peak density of 5-HT$_{2A}$ and mGlu2 receptors suggest that these two receptors have important functional relationships (Blue et al., 1988; Ohishi et al., 1997; Marek et al., 2000, 2001). Thus, both our electrophysiological and the present studies suggest that 5-HT$_{2A}$ and mGlu2 receptors appear to exert opposing influences on release of glutamate from thalamocortical afferents.

The results of this study are also in complete accord with the effects of mGlu2/3 receptor activity on the behavioral effects of 5-HT$_{2A}$ receptor stimulation. The most commonly studied behavioral model for the activation of 5-HT$_{2A}$ receptors are head shakes/twitches induced either by direct or indirect 5-HT$_{2A}$ agonists. Studies with selective 5-HT$_{2A}$ antagonists in rats or mice with a targeted disruption of the 5-HT$_{2A}$ receptor have clearly shown the dependence of this behavior on 5-HT$_{2A}$ receptors (Schreiber et al., 1995; Willins and Meltzer, 1997; Gingrich et al., 1999). Moreover, head shakes can be induced by infusion of the 5-HT$_{2A}$ partial agonist DOI directly into the prefrontal cortex, suggesting that 5-HT$_{2A}$ receptors in this region are critically involved in generating hallucinogen-induced head shakes (Granhoff et al., 1992; Willins and Meltzer, 1997). We have found that LY354740 blocks head shakes induced by DOI in a dose-dependent manner with a similar potency as observed in the present study (Gewirtz and Marek, 2000). Moreover, the dose of LY341495 (1 mg/kg) that enhanced DOI-induced BDNF expression in this study significantly enhanced DOI-induced head shakes as well (Gewirtz and Marek, 2000). Thus, we have demonstrated parallel, opposing effects of the mGlu2/3 agonist LY354740 and the mGlu2/3 antagonist LY341795 on 5-HT$_{2A}$ receptor-stimulated changes in synaptic currents measured in layer V pyramidal neurons of the mPFC, on DOI-induced head shake behavior and on DOI-induced increases in BDNF mRNA expression in the mPFC.

In addition to the regulation of the neurotrophin BDNF, activation of neocortical 5-HT$_{2A}$ and/or 5-HT$_{2C}$ receptors appears to increase the expression of a number of immediate early genes including c-*fos*, *ngf1c* (induced by NGF), *tis1* (induced by the phorbol ester TPA) and *Arc* (an activity-regulated, cytoskeleton-associated protein) (Tilakaratne and Friedman, 1996; Pei et al., 2000). The activity-dependent expression of c-*fos* induced by DOI in the neocortex appears similar to the activity-dependent expression of BDNF and has been most extensively explored. For example, within the primary somatosensory cortex induction of c-*fos* occurs in cells located in layer Va, corresponding to the highest density of 5-HT$_{2A}$ receptors (Leslie et al., 1993; Scruggs et al., 2000). Scruggs and colleagues also described a lighter induction extending into the deep layer III. Selective 5-HT$_{2A}$ antagonists, but not a selective 5-HT$_{2B/2C}$ antagonist, blocked this action of DOI (Leslie et al., 1993; Scruggs et al., 2000). Furthermore, an AMPA/kainate antagonist also blocked the DOI-induced c-*fos* response, just as AMPA/kainate antagonists block 5-HT-induced EPSCs (Aghajanian and Marek, 1997; Scruggs et al., 2000; Zhang and Marek, 2000). Lesions of the ventral basal thalamus which included the posterior thalamic nucleus attenuated the DOI-induced c-*fos* response in the primary somatosensory cortex (Scruggs et al., 2000), just as thalamic lesions attenuated the induction of 5-HT-induced EPSCs recorded from cortical layer V pyramidal cells (Marek et al., 2001). Regarding modulation of this response by mGlu2/3 agonists, LY379268 also suppressed the DOI-induced c-*fos* response in the mPFC (George et al., 2000). Consistent with modulation by 5-HT$_{2A}$ receptors of thalamic afferent terminals, Scruggs and colleagues were unable to detect Fos-immunoreactivity in cortical cells labeled by their 5-HT$_{2A}$ receptor antibody despite the fact that 5-HT$_{2A}$ immunoreactivity is found in cortical pyramidal cells (Willins et al., 1997; Jakab and Goldman-Rakic, 1998; Cornea-Hebert et al., 1999).

The enhancement of BDNF mRNA expression in the neoocortex, mPFC and intralaminar/midline thalamic nuclei and transcription of immediate early genes in the neocortex by DOI is intriguing given that another class of psychotomimetic drugs, the noncompetitive NMDA antagonists, appear to have similar effects in the neocortex, cingulate cortex and the midline thalamic nuclei (Castren et al., 1993; Murray et al., 1996). While there are clearly differences between noncompetitive NMDA antagonists and hallucinogenic drugs with respect to activation of certain neural circuits involving the anterior thalamic nuclei, hippocampus and posterior cingulate, some of the similarities between NMDA antagonists and hallucinogenic drugs activating 5-HT$_{2A}$ receptors might relate to a shared activation of glutamatergic thalamocortical pathways (Scruggs et al., 2000; Marek et al., 2001; Sharp et al., 2001).

Hence, the present findings, together with electrophysiological and behavioral data, support the propositions that (1) a pathological increase in glutamate release in neocortex underlies psychotomimetic drug effects, which (2) can be normalized by mGlu2/3 receptor agonists. Given the time course of de novo BDNF protein expression, increases in neocortical BDNF clearly cannot underlie the acute behavioral effects of administration of psychotomimetic drugs. However, the involvement of BDNF in long-term plasticity and memory formation (Kang and Schuman, 1995; Kang and Schuman, 1996; Korte et al., 1996; Patterson et al., 1996; Mizuno et al., 2000) might suggest a role of BDNF in the long-term sequelae of hallucinogen use. For example, some users of hallucinogenic drugs develop long lasting

hallucinatory perceptual changes (e.g., hallucinogen-persisting perception disorder, Abraham, 1983). Finally, enduring psychoses induced in vulnerable individuals by psychedelic hallucinogens and noncompetitive NMDA antagonists could be related to long-lasting modulations of cortical circuitry by drug-induced alterations in gene expression.

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