Metabotropic glutamate mGlu2 receptor is necessary for the pharmacological and behavioral effects induced by hallucinogenic 5-HT2A receptor agonists

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Abstract

Hallucinogenic drugs, including mescaline, psilocybin and lysergic acid diethylamide (LSD), act at serotonin 5-HT2A receptors (5-HT2ARs). Metabotropic glutamate receptor 2/3 (mGluR2/3) ligands show efficacy in modulating the responses induced by activation of 5-HT2ARs. The formation of a 5-HT2AR-mGluR2 complex suggests a functional interaction that affects the hallucinogen-regulated cellular signaling pathways. Here, we tested the cellular and behavioral effects of hallucinogenic 5-HT2AR agonists in mGluR2 knockout (mGluR2-KO) mice. Mice were intraperitoneally injected with the hallucinogens DOI (2 mg/kg) and LSD (0.24 mg/kg), or vehicle. Head-twitch behavioral response, expression of c-fos, which is induced by all 5-HT2AR agonists, and expression of egr-2, which is hallucinogen-specific, were determined in wild type and mGluR2-KO mice. [^{3}H]Ketanserin binding displacement curves by DOI were performed in mouse frontal cortex membrane preparations. Head twitch behavior was abolished in mGluR2-KO mice. The high-affinity binding site of DOI was undetected in mGluR2-KO mice. The hallucinogen DOI induced *c-fos* in both wild type and mGluR2-KO mice. However, the induction of egr-2 by DOI was eliminated in mGlu2-KO mice. These findings suggest that the 5-HT2AR-mGluR2 complex is necessary for the neuropsychological responses induced by hallucinogens.

Keywords

Hallucinogenic drugs; LSD; Serotonin 5-HT2A receptor; Metabotropic glutamate mGlu2 receptor; G protein-coupled receptor (GPCR); Schizophrenia and psychosis

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Hallucinogenic drugs, such as mescaline, psilocybin and lysergic acid diethylamide (LSD) induce profound alterations of human consciousness, emotion and cognition [12,16,27].

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Inactivation of serotonin 5-HT2AR signaling by either genetic or pharmacological approaches results in markedly reduced behavioral responses to hallucinogenic drugs in both rodent models [10,18,34] and humans [33]. Thus, although hallucinogens bind other receptor subtypes [16], the 5-HT2A receptor is considered as necessary for the unique behavioral activity of these chemicals.

Metabotropic glutamate receptors mGlu2/3 have been the target of considerable attention regarding the molecular mechanism underlying psychosis [1,6,23,25]. We have recently reported that 5-HT2AR and mGluR2 are co-expressed in the same population of cortical neurons [14]. We found that 5-HT2AR and mGluR2 form a receptor complex in mouse and human brain, and activation of mGluR2 inhibits hallucinogen-specific neuronal signaling pathways [14]. Based on this and other findings [1,17,31], it has been proposed that mGluR2 agonists modulate, through a mechanism that requires the 5-HT2AR-mGluR2 complex, the signaling pathways induced by hallucinogenic 5-HT2AR agonists. Here we demonstrate that mice with disrupted mGluR2 signaling capacity (mGluR2-KO mice) are insensitive to the cellular and behavioral effects of hallucinogens. This observation suggests that the 5-HT2AR-mGluR2 complex, and not the 5-HT2AR alone, is the molecular target responsible for the actions of hallucinogenic drugs.

Experiments were performed on adult (10–14 weeks old) male 129S6/SvEv mice. 5-HT2A mice have been previously described [18,19]. mGluR2-KO mice were obtained from the RIKEN BioRe-source Center, Japan (see [26,36] for details). mGluR2-KO mice were backcrossed for at least ten generations onto a 129S6/SvEv background. All subjects were offspring of heterozygote breeding. Animals were housed at 12 h light/dark cycle at 23 °C with food and water ad libitum. The Institutional Animal Use and Care Committee approved all experimental procedures. 1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI; Sigma–Aldrich) was dissolved in saline and injected intraperitoneally (i.p.). Lysergic acid diethylamide (LSD; Sigma-Aldrich) was injected i.p. after suspension in a minimal amount of DMSO and made up to volume with normal saline.

Head-twitch behavior is known to be reliably and robustly elicited by hallucinogenic 5-HT2AR agonists in rodents [18,19]. We first assayed the head-twitch response induced by DOI and LSD in wild type and mGluR2-KO mice (Fig. 1). Two-way ANOVA indicated a statistical significance for the effects of the treatment [$F(2,19) = 31.05$; $p < 0.001$] and genotype [$F(1,19) = 74.10$; $p < 0.001$]. Significance was also found for the interaction between treatment and genotype [$F(2,19) = 20.05$; $p < 0.001$]. The post hoc analysis revealed that DOI and LSD activated a significant head-twitch response in wild type mice ($p < 0.001$). Notably, no significant head-twitch response was detected in mGluR2-KO mice for any of these two agonists ($p > 0.05$).

The decreased head-twitch response following administration of hallucinogens led us to examine the level of expression of 5-HT2AR in mGluR2-KO mice. Equilibrium binding saturation experiments were performed to determine the binding affinity (K_D) and receptor density (B_{max}) of 5-HT2ARs in wild type and mGluR2-KO mouse frontal cortex membrane preparations (Fig. 2; for experimental details, see [14]). Neither B_{max} nor K_D values of the binding of [^{3}H]ketanserin, a 5-HT2AR antagonist, were significantly changed in mGluR2-KO mice, which demonstrates that level of expression of 5-HT2AR is not affected in the absence of mGluR2 (B_{max}:wild type, 724.5±93 fmol/mg protein;mGluR2-KO, 701.5±80 fmol/mg protein. K_D: wild type, 2.27±0.8 nM;mGluR2-87 KO, 2.30±0.71 nM).

We next determined the affinity of the mGlu2/3 agonist LY379269 displacing [^{3}H]LY341495 in wild type and 5-HT2AR-KO mice (Fig. 3A), and that of the 5-HT2AR agonist DOI displacing [^{3}H]ketanserin binding in wild type and mGluR2-KO mice (Fig. 3B;

for experimental details, see [14]). Competition binding experiments of [3H]LY341495 were best described by a two-site model in wild type mouse frontal cortex membrane preparations [$F(2,28) = 4.71$; $p < 0.05$]. However, displacement of [3H]LY341495 binding by LY379268 was best described by a one-site model in 5-HT2AR-KO mice [$F(2,16) = 0.62$; $p = 0.55$]. The low affinity binding site for LY379268 did not differ between wild type and 5-HT2AR-KO mice (Fig. 3A). Similarly, competition binding experiments of [3H]ketanserin were best described by a two-site model in wild type mouse frontal cortex membrane preparations [$F(2,79) = 19.05$; $p < 0.001$]. Interestingly, displacement of [3H]ketanserin binding by DOI was best described by a one-site model in mGluR2-KO mice [$F(2,79) = 0.89$; $p = 0.41$]. The low affinity site for DOI did not differ between the wild type and mGluR2-KO mice (Fig. 3B). The changes in high-affinity binding suggested that the cellular responses induced hallucinogenic 5-HT2AR agonists may be altered in mGluR2-KO mice. This hypothesis was tested by measuring the gene response to hallucinogens in mouse frontal cortex.

We have previously demonstrated that hallucinogenic 5-HT2AR agonists induce a unique pattern of gene expression in mouse cerebral cortex that predicts the behavioral effect of the tested ligand [18,19]. Here we examined the role of mGluR2 in the cellular responses induced by hallucinogens in mouse frontal cortex, a region involved in acute psychotic episodes [17]. As previously shown [18,19], the hallucinogen DOI induced expression of *c-fos* and *egr-2* in wild type mice (Fig. 3C; for primer pair sequences, see [14]). In mGluR2-KO mice, we found that DOI also induces the expression of *c-fos*, whereas regulation of *egr-2* was abolished (Fig. 3C).

This study demonstrates that mGluR2 is necessary for at least some of the cellular and behavioral responses induced by hallucinogenic 5-HT2AR agonists. We found that the head-twitch response was not produced by the hallucinogens DOI and LSD in mGluR2-KO mice. We also demonstrated that the hallucinogenic gene response signature required the expression of mGluR2, and that the high-affinity binding sites for LY379268 and DOI were undetectable in the absence of 5-HT2AR or mGluR2, respectively, in mouse frontal cortex. Although mouse models of neuropsychiatric disturbances have limitations [3,12], these findings suggest that the 5-HT2AR requires the expression of mGluR2 to induce hallucinogenic-dependent psychoactive states.

5-HT2AR and mGluR2 are members of the G protein-coupled receptor (GPCR) superfamily, also referred to as seven transmembrane receptors [28,29]. The ternary complex model (receptor [R], agonist, and G protein [G]) has typically been the most commonly used pharmacological model to describe the mechanism of activation of GPCRs [16,21]. In this model, the receptor exists in two conformational states: an inactive state, R, and an active state R*G. Agonists show a higher affinity for R*G than for R, whereas neutral antagonists show roughly equal affinities for the two states [21]. According to the ternary complex model, agonists present a biphasic pattern displacing radiolabeled antagonists (see also Fig. 3A and B), and the fraction of high-affinity binding site is decreased when R*G complexes are uncoupled by the non-hydrolyzable analog of guanosine triphosphate GTPγS [14,15]. However, recent findings provide evidence indicating that GPCR dimers lead to positive and negative ligand-dependent cooperative binding [2,4,20,24,32], for which theoretical pharmacological models have proposed GPCRs as dimeric/oligomeric structures [8,11,13]. We have previously demonstrated that mGluR2 activation increases the affinity of 5-HT2AR agonists, while, by contrasts, 5HT2AR activation decreases the affinity of mGluR2 agonists in mouse brain cortex and tissue culture preparations ([14]; similar results were obtained in the present study–data not shown). In frontal cortex membrane preparations, hereweshow that the biphasic displacement curve of [3H]ketanserin by DOI became monophasic in mGluR2-KO mouse, and that the biphasic displacement curve of [3H]LY341495 by LY379268 became monophasic in 5-HT2AR-KO

mouse. Although further investigation is necessary, concurrently these binding data support the expression of 5-HT2AR and mGluR2 as a GPCR heterocomplex in mouse frontal cortex, and provide an explanation for the functional crosstalk observed between the components of the receptor complex.

The G protein subtypes activated by 5-HT2AR and mGluR2 are mainly $G_{q/11}$ and $G_{i/o}$, respectively [5,7]. However, some reports have implicated pertussis toxin-sensitive $G_{i/o}$ proteins in the cel1lular responses mediated by 5-HT2AR activation [22,30]. In mouse cortical primary neurons, we previously discovered that the signaling elicited by hallucinogen and non-hallucinogen 5-HT2AR agonists causes induction of *c-fos* and requires $G_{q/11}$-dependent signaling [18]. However, the signaling of hallucinogenic 5-HT2AR agonists also induces *egr-2*, which is $G_{i/o}$-dependent [18]. Our data now demonstrate that the induction of *c-fos* by DOI was not affected. In contrast, the induction of *egr-2* was abolished in mGluR2-KO mice, which provide the first demonstration that the hallucinogen-specific signaling signature is affected in the absence of mGluR2 in whole animal models, and suggest that the 5-HT2AR-mGluR2 complex is necessary for the responses induced by LSD-like drugs. It is important to note that the level of expression of 5-HT2AR as determined by [^{3}H]ketanserin binding saturation curves was unaffected in mGluR2-KO mouse frontal cortex, which further supports the hypothesis that expression of mGluR2 is necessary for the cellular and behavioral effects induced by hallucinogenic 5-HT2AR agonists. Our data do not exclude the possibility that the closely related mGluR3 also plays a role in the unique effects induced by LSD-like hallucinogens. Thus, althoughmGluR2, and not mGluR3, has been shown to be responsible for the antipsychotic like responses induced by the mGlu2/3 agonists LY379268 [35] and LY404039 [9], further investigation is necessary to determine the pharmacological and behavioral effects of hallucinogens in wild-type and mGluR3-KO mice.

In conclusion, we have shown that the behavioral responses to hallucinogenic 5-HT2A agonists are absent inmGlu2-KOmice. The cognitive and perceptual changes induced by hallucinogenic drugs exhibit similarities with the endogenous psychosis of schizophrenia [12,17]. The level of expression of 5-HT2A and mGlu2 has been found to be dysregulated in postmortem human brain of untreated schizophrenic subjects [14]. Deciphering the molecular mechanism of action of hallucinogens should provide new inquiries to understand the molecular and cellular mechanisms that underlie the complex clinical phenotype of schizophrenia.

Acknowledgments

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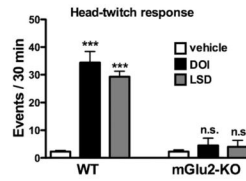
Fig. 1.
Behavioral response to hallucinogens DOI and LSD. Wild type and mGluR2-KO mice (n = 4–5 per treatment group) were injected with vehicle, DOI (2 mg/kg) or LSD (0.24 mg/kg), and the head-twitch response was scored 15 min after injection for 30 min. ***$p < 0.001$; Bonferroni's *post hoc* test of two-way ANOVA. Data are means ± S.E.M.

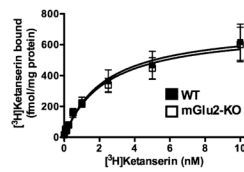**Fig. 2.**
Expression of 5-HT2AR in mGluR2-KO mice. [³H]Ketanserin binding saturation curves in wild type (black) and mGluR2-KO (white) mouse frontal cortex membrane preparations (*n* = 6 per group).
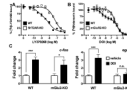
Fig. 3.
Cellular response to hallucinogenic 5-HT2AR agonist DOI. (A) LY379268 displacement of [^{3}H]LY341495 binding was performed in wild type (black) and 5-HT2AR-KO (white) mouse frontal cortex membrane preparations. Competition curves were analyzed by nonlinear regression to derive dissociation constants for the high and low affinity states of the receptor. One-site model or two-site model as a better description of the data was determined by *F* test. Two-site model, $p < 0.001$. A two-site model provided a better description of the data in wild type mice. Wild type mice: $K_{i\text{-high}}$ (log*M*), -9.51 ± 0.45; $K_{i\text{-low}}$ (log*M*), -7.95 ± 0.26; % high-affinity binding sites, 36.6 ± 1; and 5HT2AR-KO mice: $K_{i\text{-low}}$ (log*M*), -7.60 ± 0.07 ($n = 3$–5). (*B*) DOI displacement of [^{3}H]ketanserin binding was performed in wild type (black) and mGluR2-KO (white) mouse frontal cortex membrane preparations. A two-site model provided a better description of the data in wild type mice. Wild type mice: $K_{i\text{-high}}$ (log*M*), -8.56 ± 0.28; $K_{i\text{-low}}$ (log*M*), -6.31 ± 0.16; % high-affinity binding sites, 35.9 ± 4; and mGluR2-KO mice: $K_{i\text{-low}}$ (log*M*), -6.72 ± 0.08 ($n = 5$–6). (C) Cellular response in mouse frontal cortex assayed by qRT-PCR. Wild type or mGluR2-KO mice were injected with vehicle (white) or DOI (black; 2mg/kg). Changes in expression levels are reported as fold change over vehicle. *$p < 0.05$; ***$p < 0.001$; Bonferroni's *post-hoc* test of two-factor ANOVA ($n = 5$–6 per group). Data are means±S.E.M.