

The hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) increases cortical extracellular glutamate levels in rats

Jennifer L. Scruggs, Dennis Schmidt, Ariel Y. Deutch*

Neuroscience Program and Departments of Psychiatry and Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37212, USA

Received 7 March 2003; received in revised form 15 April 2003; accepted 16 April 2003

Abstract

Activation of the cerebral cortex is seen during hallucinations. The 5-HT_{2A/C} agonist 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) is a potent hallucinogen that has been proposed to act by targeting 5-HT_{2A} heteroreceptors on thalamocortical neurons and eliciting release of glutamate from these cells, which in turn drives cortical neurons. We used in vivo microdialysis to determine if DOI increases extracellular glutamate levels. Systemic administration of DOI significantly increased extracellular glutamate levels in the somatosensory cortex of the freely-moving rat. Similarly, intracortical administration of DOI by reverse dialysis increased cortical extracellular glutamate levels. No consistent changes in either extracellular GABA or glycine levels were observed in response to DOI. The increase in glutamate levels elicited by intracortical DOI was blocked by treatment with the selective 5-HT_{2A} antagonist MDL 100,907. These data are consistent with the hypothesis that 5-HT_{2A} receptor-mediated regulation of glutamate release is the mechanism through which hallucinogens activate the cerebral cortex.

© 2003 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: 5-HT_{2A} receptor; Cortex; Glutamate; Hallucinogen; In vivo microdialysis; Thalamus

Activation of the cerebral cortex occurs during hallucinations. The cortical activation observed during hallucinations in schizophrenia and other endogenous conditions is specific for the sensory modality of the hallucinatory content [11, 12], with a more extensive cortical activation seen in drug-induced hallucinations [14,15].

Hallucinogens such as lysergic acid diethylamide (LSD) and 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) are potent 5-HT_{2A/C} agonists whose effects appear to mainly reflect actions at 5-HT_{2A} receptors [9,13]. Although cortical layer V neurons express 5-HT_{2A} receptors [16], hallucinogens such as DOI do not appear to directly activate these cells [10]. Instead, hallucinogen-induced cortical activation has been proposed to occur indirectly, by activating 5-HT_{2A} heteroreceptors on thalamocortical neurons, thereby evoking glutamate release to activate cortical neurons [2,3,10]. Pharmacological and electrophysiological data are consistent with this hypothesis, but no studies have

directly determined if hallucinogens increase cortical glutamate release in vivo. We used in vivo microdialysis to determine if DOI increases extracellular glutamate levels in the somatosensory cortex (SSC), which receives a glutamatergic projection from the ventrobasal thalamus [8] and is strongly activated by DOI [10].

Adult male Sprague–Dawley rats (Harlan; Birmingham, AL) were group-housed on a 12 h light/dark cycle with food and water available ad libitum. All experiments were performed in accord with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*. Unilateral guide cannulas (Bioanalytical Systems, Inc. [BAS]; West Lafayette, IN) were implanted into the SSC at a 40° angle (AP: −0.4, ML: +3.2, DV: −2.5 relative to bregma). Five to seven days later, freely-moving animals were transferred to sound-attenuated dialysis chambers with food and water freely available; the light/dark cycle was the same as in the vivarium. Microdialysis probes (2.0 mm exchange length; BAS) were inserted through the guide cannulas and the probes perfused overnight at 0.2 µl/min with 1.25 mM CaCl₂, 0.83 mM MgCl₂, 120 mM NaCl, 20 mM NaHCO₃, 2.2 mM KCl, 0.5 mM Na₂SO₄, 0.5 KH₂PO₄, 4.9 mM D-glucose, and 0.2 mM ascorbic acid. The next

* Corresponding author. Psychiatric Hospital at Vanderbilt, Suite 313, 1601 23rd Avenue South, Nashville, TN 37212, USA. Tel.: +1-615-327-7080; fax: +1-615-322-1901.

E-mail address: ariel.deutch@mcmail.vanderbilt.edu (A.Y. Deutch).

morning the flow rate was increased to 2.0 $\mu\text{l}/\text{min}$ and 60 min later fractions were collected.

Four 30 min baseline samples were collected, after which 5 mg/kg (s.c.) DOI or vehicle was administered and additional fractions were collected. We also determined if intracortical DOI administration (100 μM) elicits glutamate release by administering the hallucinogen via reverse dialysis into the SSC for 30 min; the DOI concentration was based on previous work from our lab [1]. Four 30 min dialysate fractions were collected after local DOI administration, and the animals were then challenged with systemic DOI (5 mg/kg, s.c.); DOI was administered systemically after local DOI infusion to assess if glutamatergic afferents responded to the hallucinogen to the same degree after an earlier challenge. Finally, we determined if DOI-elicited changes in glutamate levels depend on 5-HT_{2A} receptor activation. The selective 5-HT_{2A} antagonist MDL 100,907 (1.0 μM) was delivered to the SSC by reverse dialysis in the fractions immediately before, during, and immediately after local DOI infusion. This MDL 100,907 concentration was based on pilot studies indicating that 1.0 μM did not alter but that 10 μM significantly increased extracellular glutamate levels.

At the end of the experiment rats were deeply anesthetized, perfused with 4% paraformaldehyde and serial coronal sections (50 μM) were cut through the forebrain and stained with toluidine blue. A person unaware of the treatment condition of the animals assessed probe placement. Animals in which at least 50% of the exchange length of the probe was in layers II–superficial V of the barrel cortex were considered to have appropriate probe placements.

Glutamate levels were measured by HPLC, as were levels of GABA and glycine. Briefly, samples were derivitized in *o*-phthalaldehyde (11 mg in 250 μl ethanol, 4.5 ml 0.1 M sodium tetraborate, 250 μl 1.0 M sodium sulfite) and the reaction was continued for 60 min before the sample was injected onto a 1.5 μm C18 column (Alltech Associates; Deerfield, IL). The mobile phase for glutamate and glycine was 100 mM potassium phosphate with 8% methanol (pH 3.5); for GABA analyses the pH was 3.8 and 12% methanol was used. The flow rate was 1.2 ml/min for both analyses, with the column temperature set at 40 °C. A Shimadzu RF-10Axi fluorescent detector was set at 340 Å excitation and 450 Å emission. An ESA 5100A electrochemical detector, which was placed in series with the fluorescent detector, was equipped with an 5011 analytical cell and operated at E1 = 0.250 V, E2 = 0.75 V. Data were analyzed by means of repeated measures ANOVA with subsequent post-hoc analyses when indicated.

Systemic DOI administration evoked a slow but sustained increase in extracellular glutamate levels in the SSC (see Fig. 1). ANOVA revealed a significant time $\times$ treatment interaction ($F(4, 48) = 2.78$, $P = 0.037$). Post-hoc analyses revealed that DOI significantly increased extracellular glutamate levels relative to vehicle-injected

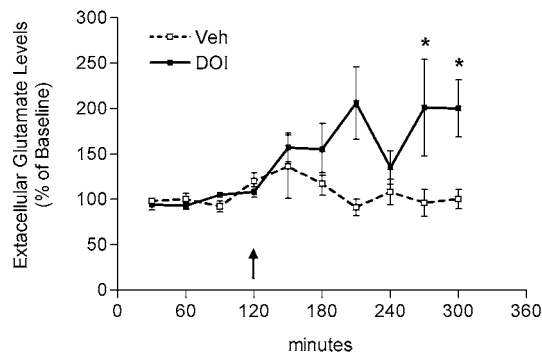


Fig. 1. The effects of systemic DOI administration (5.0 mg/kg, s.c.; $n = 9$) on extracellular glutamate levels in the SSC. The arrow indicates the point at which DOI was injected. A significant time $\times$ treatment interaction was uncovered, with subsequent analyses showing that glutamate levels in the last two fractions were significantly greater than those in corresponding samples from vehicle (Veh)-injected control animals ($n = 5$). $*P \leq 0.05$ relative to vehicle controls.

rats. Systemic DOI challenge also increased extracellular GABA levels in the SSC (data not shown), with ANOVA uncovering a significant time $\times$ treatment interaction ($F(4, 44) = 2.80$, $P = 0.037$). GABA levels were increased 2 h after DOI challenge ($P = 0.008$), and showed a non-significant trend toward an increase in the previous fraction ($P = 0.087$). Extracellular glycine levels were unchanged.

Intracortical DOI caused a temporally-specific increase in extracellular glutamate levels (Fig. 2). A significant time effect was uncovered ($F(12, 72) = 3.641$, $P = 0.0003$), and post-hoc tests found that glutamate levels were significantly increased in the fraction after DOI delivery but returned to normal by the next fraction. After the levels of glutamate returned to normal, the animals were injected with DOI systemically. This systemic challenge tended to increase glutamate levels but not to a statistically significant degree ($P = 0.084$). Local DOI administration did not increase extracellular GABA or glycine levels in the SSC (data not shown). The ability of local DOI to increase extracellular glutamate levels was blocked by treatment with the

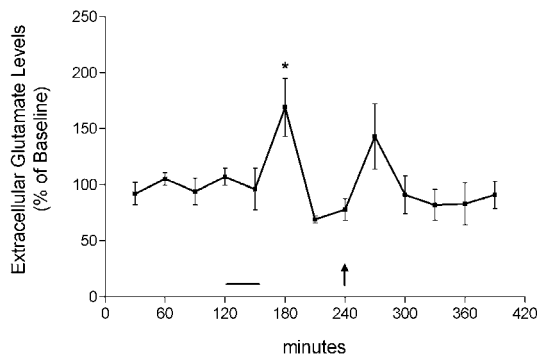


Fig. 2. The effects of intracortical DOI administration (100 μM) on extracellular glutamate levels in the SSC. DOI was delivered through the dialysis probe during the period indicated by the horizontal line, and the rats ($n = 7$) were subsequently treated with systemic DOI (5.0 mg/kg, s.c.) at the time point indicated by the arrow. $*P \leq 0.05$ relative to baseline values.

selective 5-HT_{2A} antagonist MDL 100,907 (Fig. 3), which had no effect of its own.

Our data indicate that both systemic and intracortical administration of hallucinogenic 5-HT_{2A/C} agonist DOI increased extracellular glutamate levels in the cortex, consistent with the hypothesis that hallucinogens activate the cortex by increasing glutamate release from thalamocortical neurons. The ability of DOI to increase glutamate levels was blocked by pretreatment with the selective 5-HT_{2A} antagonist MDL 100,907, indicating a 5-HT_{2A}-mediated effect. These findings agree well with previous data showing that DOI-induced Fos expression and the effects of serotonin on layer V pyramidal cells are 5-HT_{2A}-mediated [4,10]. Our data also agree with reports indicating that DOI-induced cortical Fos expression and DOI-elicited excitatory postsynaptic currents (EPSCs) in cortical layer V pyramidal cells are both blocked by pretreatment with AMPA/K_A glutamatergic antagonists [4,10]. Thus, our data are consistent with the hypothesis that activation of glutamatergic thalamocortical neurons subserves hallucinogen-induced cortical activation. It is also possible that co-localized transmitters are involved or that DOI drives afferents in addition to thalamocortical glutamatergic axons to activate the cortex.

Systemic administration of DOI increased extracellular glutamate levels in the SSC, as did intracortical DOI administration. However, when we measured glutamate levels in animals that received systemic DOI after local infusion of the hallucinogen, the systemic challenge failed to significantly increase glutamate levels, although a trend was noted. This may reflect rapid agonist-induced internalization of the 5-HT_{2A} receptor [5] after local DOI delivery, with a resultant decrease in available receptor sites when the animal was challenged systemically.

The source of the increased glutamate levels seen after DOI challenge is probably thalamocortical neurons. It is unlikely that metabolic intermediary or glial glutamate pools are responsible, because GABA and glycine did not change in parallel with glutamate. The suggestion that the

increase in glutamate is derived from thalamic neurons is consistent with ventrobasal thalamic neurons providing the major glutamatergic projection to the SSC [8] and expressing both 5-HT_{2A}-like immunoreactivity [6] and low levels of 5-HT_{2A} mRNA (Dr. L. Jennes, pers. commun.). In contrast, glutamatergic afferents from cortical areas originate in the supragranular layers, in which most neurons do not express the 5-HT_{2A} receptor.

We found that both intracortical and systemic administration of DOI increased glutamate levels, suggesting that DOI actions at 5-HT_{2A} heteroreceptors on thalamocortical axon terminals are sufficient to increase glutamate levels and thereby activate cortical neurons. Activation of 5-HT_{2A} receptors on somatodendritic parts of thalamocortical neurons may also increase cortical glutamate output; further work will be required to unravel the relative contribution of terminal and somatodendritic 5-HT_{2A} receptors.

Cortical GABA levels were increased in response to systemic but not local DOI challenge. In pilot studies we found that systemic DOI did not increase Fos expression in GABAergic cells of the barrel cortex (unpublished observations), consistent with an extracortical source of GABA being activated by systemic DOI challenge. This source may be basal forebrain GABAergic neurons, which innervate the SSC [7]. It is interesting to note that we previously found that DOI administered to the prefrontal cortex increased extracellular GABA levels, and that systemic DOI challenge increased the percentage of prefrontal cortical GABAergic neurons expressing Fos [1]. It appears that DOI-evoked changes in both GABA release and Fos expression may vary across cortical regions, consistent with recent data indicating differences in metabotropic glutamate receptor regulation of DOI-evoked Fos expression in the somatosensory and prefrontal cortices [17].

The distribution of neurons expressing the 5-HT_{2A} receptor is roughly comparable across cortical regions. Thus, any differences in the degree to which hallucinogens activate various cortices may be due to variations in the expression of the 5-HT_{2A} receptor across the different thalamic nuclei. This suggestion agrees with the observation that hallucinogens cause relatively few motor effects: the 5-HT_{2A} receptor is expressed predominantly in sensory and 'non-specific' thalamic nuclei rather than thalamic motor nuclei [6]. Our data suggest that hallucinogens alter thalamocortical glutamatergic drive and thereby disrupt the normal relationship between thalamus and cortex.

Acknowledgements

This study was supported by an NRSA grant (MH 63602) to J.L.S., grants MH 45124 and MH 57995 (A.Y.D.), and the National Parkinson Foundation Center of Excellence at Vanderbilt University. We thank Jim Fadel for advice on data analysis and Tamara Altman for assistance with figures.

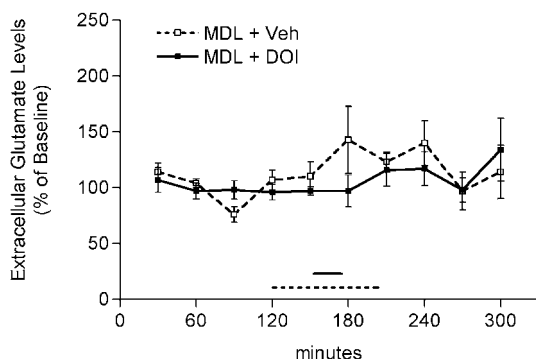


Fig. 3. Pretreatment with the 5-HT_{2A} antagonist MDL 100,907 (1.0 μ M; dashed horizontal line; $n = 3$) delivered through the dialysis probe for the fractions before, during, and after intracortical DOI administration (solid horizontal line) prevented the DOI-induced increase in extracellular glutamate levels but had no effect of its own ($n = 5$).

References

- [1] W.M. Abi-Saab, M. Bubser, R.H. Roth, A.Y. Deutch, 5-HT₂ receptor regulation of extracellular GABA levels in the prefrontal cortex, *Neuropsychopharmacology* 20 (1999) 92–96.
- [2] G.K. Aghajanian, G.J. Marek, Serotonin and hallucinogens, *Neuropsychopharmacology* 21 (1999) 16S–23S.
- [3] G.K. Aghajanian, G.J. Marek, Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells, *Neuropharmacology* 36 (1997) 589–599.
- [4] G.K. Aghajanian, G.J. Marek, Serotonin, via 5-HT_{2A} receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release, *Brain Res.* 825 (1999) 161–171.
- [5] S.A. Berry, M.C. Shah, N. Khan, B.L. Roth, Rapid agonist-induced internalization of the 5-hydroxytryptamine_{2A} receptor occurs via the endosome pathway in vitro, *Mol. Pharmacol.* 50 (1996) 306–313.
- [6] V. Cornea-Hebert, M. Riad, C. Wu, S.K. Singh, L. Descarries, Cellular and subcellular distribution of the serotonin 5-HT_{2A} receptor in the central nervous system of adult rat, *J. Comp. Neurol.* 409 (1999) 187–209.
- [7] I. Gritti, L. Mainville, M. Mancina, B.E. Jones, GABAergic and other noncholinergic basal forebrain neurons, together with cholinergic neurons, project to the mesocortex and isocortex in the rat, *J. Comp. Neurol.* 383 (1997) 163–177.
- [8] V.N. Kharazia, R.J. Weinberg, Glutamate in thalamic fibers terminating in layer IV of primary sensory cortex, *J. Neurosci.* 14 (1994) 6021–6032.
- [9] R.A. Newton, S.L. Phipps, T.P. Flanagan, N.R. Newberry, J.E. Carey, C. Kumar, B. McDonald, C. Chen, J.M. Elliott, Characterisation of human 5-hydroxytryptamine_{2A} and 5-hydroxytryptamine_{2C} receptors expressed in the human neuroblastoma cell line SH-SY5Y: comparative stimulation by hallucinogenic drugs, *J. Neurochem.* 67 (1996) 2521–2531.
- [10] J.L. Scruggs, S. Patel, M. Bubser, A.Y. Deutch, DOI-induced activation of the cortex: dependence on 5-HT_{2A} heteroreceptors on thalamocortical glutamatergic neurons, *J. Neurosci.* 20 (2000) 8846–8852.
- [11] S.S. Shergill, L.A. Cameron, M.J. Brammer, S.C. Williams, R.M. Murray, P.K. McGuire, Modality specific neural correlates of auditory and somatic hallucinations, *J. Neurol. Neurosurg. Psychiatry* 71 (2001) 688–690.
- [12] D.A. Silbersweig, E. Stern, C. Frith, C. Cahill, A. Holmes, S. Grooten, J. Seaward, P. McKenna, S.E. Chua, L. Schnorr, T. Jones, R.S.J. Frackowiak, A functional neuroanatomy of hallucinations in schizophrenia, *Nature* 378 (1995) 176–179.
- [13] R.L. Smith, R.J. Barrett, E. Sanders-Bush, Mechanism of tolerance development to 2,5-dimethoxy-4-iodoamphetamine in rats: down-regulation of the 5-HT_{2A}, but not 5-HT_{2C}, receptor, *Psychopharmacology* 144 (1999) 248–254.
- [14] F.X. Vollenweider, K.L. Leenders, C. Scharfetter, P. Maguire, O. Stadelmann, J. Angst, Positron emission tomography and fluorodeoxyglucose studies of metabolic hyperfrontality and psychopathology in the psilocybin model of psychosis, *Neuropsychopharmacology* 16 (1997) 357–372.
- [15] F.X. Vollenweider, M.F. Vollenweider-Scherpenhuyzen, A. Babler, H. Vogel, D. Hell, Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action, *NeuroReport* 9 (1998) 3897–3902.
- [16] D.L. Willins, A.Y. Deutch, B.L. Roth, Serotonin 5-HT_{2A} receptors are expressed on pyramidal cells and interneurons in the rat cortex, *Synapse* 27 (1997) 79–82.
- [17] M.S. Zhai, C.A. George, J. Zhai, E.S. Nisenbaum, M.P. Johnson, L.K. Nisenbaum, Group II metabotropic glutamate receptor modulation of DOI-induced *c-fos* mRNA and excitatory responses in the cerebral cortex, *Neuropsychopharmacology* 28 (2002) 45–52.