*Neuroscience* 290 (2015) 159–164

# STIMULATION OF GLUTAMATE RECEPTORS IN THE VENTRAL TEGMENTAL AREA IS NECESSARY FOR SEROTONIN-2 RECEPTOR-INDUCED INCREASES IN MESOCORTICAL DOPAMINE RELEASE

**E. A. PEHEK** [a,b,c*] **AND A. E. HERNAN** [a,d,e]

[a] *Louis Stokes Cleveland Department of Veterans Affairs, Cleveland, OH 44106, USA*

[b] *Case Western Reserve University, Department of Psychiatry, Cleveland, OH 44106, USA*

[c] *Case Western Reserve University, Department of Neurosciences, Cleveland, OH 44106, USA*

[d] *Case Western Reserve University, Department of Biology, Cleveland, OH 44106, USA*

[e] *University of Vermont College of Medicine, Burlington, VT 05405, USA*

**Abstract—Modulation of dopamine (DA) released by serotonin-2 (5-HT2) receptors has been implicated in the mechanism of action of antipsychotic drugs. The mesocortical DA system has been implicated particularly in the cognitive deficits observed in schizophrenia. Agonism at 5-HT2A receptors in the prefrontal cortex (PFC) is associated with increases in cortical DA release. Evidence indicates that 5-HT2A receptors in the cortex regulate mesocortical DA release through stimulation of a ''long-loop'' feedback system from the PFC to the ventral tegmental area (VTA) and back. However, a causal role for VTA glutamate in the 5-HT2-induced increases in PFC DA has not been established. The present study does so by measuring 5-HT2 agonist-induced DA release in the cortex after infusions of glutamate antagonists into the VTA of the rat. Infusions of a combination of a *N*-methyl-D-aspartic acid (NMDA) (AP-5: 2-amino-5-phosphopentanoic acid) and an AMPA/kainate (CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione) receptor antagonist into the VTA blocked the increases in cortical DA produced by administration of the 5-HT2 agonist DOI [(±)-2,5-dimethoxy-4-iodoamphetamine] (2.5 mg/kg s.c.). These results demonstrate that stimulation of glutamate receptors in the VTA is necessary for 5-HT2 agonist-induced increases in cortical DA. Published by Elsevier Ltd. on behalf of IBRO.**

**Key words: antipsychotic, prefrontal cortex, microdialysis, schizophrenia, AMPA, NMDA.**

## INTRODUCTION

Multiple studies have demonstrated that dopamine (DA) systems are regulated by serotonin (5-HT) receptors (Alex and Pehek, 2007). The serotonin 5-HT2A receptor is of particular interest due to its role in hallucinogenic drug action and its putative involvement in atypical antipsychotic drug mechanisms. A substantial body of literature has shown that 5-HT2A receptors in the prefrontal cortex (PFC) regulate mesocortical DA release (Gobert and Millan, 1999; Pehek et al., 2001, 2006; Bortolozzi et al., 2005). The mesocortical DA system has been implicated particularly in the cognitive deficits observed in disorders such as schizophrenia (Weinberger, 1987). 5-HT2A receptors are localized, in part, to the apical dendrites of pyramidal cells in the rat PFC (Willins et al., 1997; Jakab and Goldman-Rakic, 1998; Hamada et al., 1998; Cornea-Hébert et al., 1999; Weber and Andrade, 2010). More recent data have found that a large number of pyramidal cells projecting to the midbrain ventral tegmental area (VTA), the DA cell body site of origin of the mesocortical tract, contain 5-HT2A receptors (Vasquez-Borsetti et al., 2009). Furthermore, pyramidal neurons projecting from the PFC innervate DA cells in the VTA (Sesack and Pickel, 1992).

These data suggest that cortical 5-HT2A receptor regulation of mesocortical DA is mediated by actions on a ''long-loop'' neuronal circuit involving glutamatergic corticotegmental projections to DA cells in the VTA (Vasquez-Borsetti et al., 2009). We have previously published data to support such a circuit (Pehek et al., 2006). In addition to increasing DA in the PFC, injections of the 5-HT2 agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) also increased glutamate efflux in the VTA, which was blocked by intracortical infusions of the selective 5-HT2A antagonist M100907. These findings indicate that the effects of DOI are mediated by 5-HT2A receptors localized in the PFC. Furthermore, they demonstrate that 5-HT2A-induced increases in PFC DA are correlated with

*Correspondence to: E. A. Pehek, Research 151(W), Louis Stokes Cleveland Department of Veterans Affairs, Cleveland, OH 44106, USA. Tel: +1-216-791-3800x4237.
E-mail address: eap6@cwru.edu (E. A. Pehek).

*Abbreviations:* aCSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ANOVA, analysis of variance; AP-5, 2-amino-5-phosphopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DA, dopamine; DOI, (±)-2,5-dimethoxy-4-iodoamphetamine; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; 5-HT, Serotonin; 5-HT2, serotonin-2; NMDA, *N*-methyl-D-aspartic acid; PFC, prefrontal cortex; VTA, ventral tegmental area.

http://dx.doi.org/10.1016/j.neuroscience.2015.01.029
0306-4522/Published by Elsevier Ltd. on behalf of IBRO.

increases in VTA glutamate. However, they do not establish causality. A more direct test of the hypothesis is necessary and is the objective of the present work. This experiment determined if increases in glutamatergic tone in the VTA are necessary for 5-HT2A agonist-induced cortical DA release.

Dual probe *in vivo* microdialysis in conscious rats was employed followed by measurements of dialysate DA with high-performance liquid chromatography (HPLC) and electrochemical detection. We tested if concurrent blockade of both AMPA and *N*-methyl-D-aspartic acid (NMDA) receptors in the VTA would attenuate the increase in dialysate DA produced by administration of the 5-HT2 agonist DOI. A mixture of the NMDA antagonist 2-amino-5-phosphopentanoic acid (AP-5) and the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was infused by reverse dialysis into the VTA. It was hypothesized that such infusions would block DOI-induced increases in cortical DA.

## EXPERIMENTAL PROCEDURES

### Animals

Male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA), weighing between 200 and 350 g at the time of surgery, were used for all experiments. Rats were housed two per cage in a temperature controlled room with a 12-h/12-h light/dark cycle. Food and water were available *ad libitum*. All animal procedures were in strict accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the local animal care committee.

### Surgery

Rats were anesthetized with a mixture of xylazine and ketamine (6 and 70 mg/kg, respectively; administered i.m.) and mounted in a stereotaxic frame. Microdialysis probes were implanted into the PFC (+3.2 AP, ML 0.8, DV −5.5) and the VTA (−5.60 AP, ML 0.6, DV −8.4) (Paxinos and Watson, 1998; see Fig. 1). Placements were ipsilateral and approximately half of the placements were on the right and half on the left. The probes were then secured in place with three set screws covered with cranioplastic cement. Probe locations were verified histologically at the completion of the experiments. If improperly placed, animals were excluded from the experiments.

### Microdialysis

Microdialysis probes were of a concentric flow design (Yamamoto and Pehek, 1990). Average recovery for DA was 10–15%. PFC probes were constructed with a 5.0-mm active dialyzing surface membrane (Spectra/Por Hollow, MW cutoff = 13,000, diameter = 240 μm) to effectively dialyze from the dorsal anterior cingulate to the most ventral region of the infralimbic PFC. VTA probes were constructed with a 1.0-mm active dialyzing surface at the most ventral extension of the probe to effectively dialyze the mediolateral parabrachial and paranigral VTA (see Fig. 1). The tips of the probes (approximately 0.3 mm) were plugged with glue and thus did not recover the analyte. The afternoon prior to microdialysis experiments animals were placed in clear Plexiglas microdialysis chambers (Harvard Apparatus, Hollister, MA, USA) with food and water available *ad libitum*. Animals were tethered to counterbalance arms (Instech, Plymouth Meeting, PA, USA) that permitted free movement about the chamber. Eighteen to Twenty-four hours after probe insertion, a micro-infusion pump (PHD 2000™, Harvard Apparatus) and liquid swivel (Instech) were used to perfuse a modified Dulbecco's artificial cerebrospinal fluid (aCSF) buffer solution (137 mM NaCl, 3 mM KCl, 1.2 mM $MgSO_4$, 0.4 mM $KH_2PO_4$, with 1.2 mM $CaCl_2$ and 10 mM glucose; pH 7.4) through the probes. Samples were collected every 30 min after baseline levels of DA were stable (typically 2–3 h). After baseline collections, drugs dissolved in the aCSF were administered by reverse dialysis. Tubing connections were switched manually while maintaining a constant flow rate and collection volume. Samples were immediately analyzed for DA content by HPLC.

### Drugs

The 5-HT2 agonist (±)-DOI hydrochloride was obtained from Sigma–Aldrich (St. Louis, MO, USA). DOI was injected systemically (s.c.) and was dissolved in water (2.5-mg/kg/ml, dose refers to the salt). The AMPA/kainate antagonist CNQX disodium salt was obtained from Sigma–Aldrich. The NMDA antagonist (±)AP-5 was obtained from Tocris Bioscience (Ellisville, MO, USA). Since both AMPA and NMDA receptors have been implicated in the regulation of DA release, a combination of CNQX (50 μM) and AP-5 (200 μM) was dissolved in the aCSF and infused into the VTA by reverse dialysis. This drug combination, infused into the VTA at these concentrations, has been used previously by others (Taber et al., 1995; Karreman and Moghaddam, 1996). Empirical evidence indicates that the concentrations of drugs crossing the dialysate membrane during reverse dialysis are extremely small. For example, the amounts of various drugs (DA uptake blockers) that crossed the dialysate membrane *in vitro* ranged from 2.0% to 8.6% (Nomikos et al., 1990). However, *in vitro* studies of recovery fail to account for impediments to drug diffusion that normally occur *in vivo* in the brain tissue. One study that calculated the true *in vivo* diffusion of an antiviral nucleoside demonstrated that the recovery was one-third of that observed *in vitro* (Wang et al., 1993). Thus, what appear to be relatively high concentrations of drugs must be used in most microdialysis studies.

The antagonists were initially dissolved in water containing 1.5–5 μl of glacial acetic acid to make a 10 mM stock solution. They were then further diluted to the appropriate μM concentrations with aCSF. The pH of all aCSF solutions was adjusted to 7.4. Following the achievement of stable baseline dialysate DA, CNQX + AP-5 were infused for 1 h before the injection of DOI.