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PREFERENTIAL RELOCATION OF THE *N*-METHYL-D-ASPARTATE RECEPTOR NR1 SUBUNIT IN NUCLEUS ACCUMBENS NEURONS THAT CONTAIN DOPAMINE D1 RECEPTORS IN RATS SHOWING AN APOMORPHINE-INDUCED SENSORIMOTOR GATING DEFICIT

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Abstract—Sensorimotor gating as measured by prepulse inhibition (PPI) to startle-evoking auditory stimulation (AS) is disrupted in schizophrenia and in rodents receiving systemic administration of apomorphine, a dopamine D1/D2 receptor agonist, or MK-801, an *N*-methyl-D-aspartate (NMDA) receptor antagonist. The functional analogies and our prior results showing apomorphine- and AS-induced relocation of the dopamine D1 receptor (D1R) in the nucleus accumbens (Acb) shell suggest that apomorphine and AS may affect the subcellular distribution of the NMDA receptor NR1 subunit, a protein that forms protein–protein interactions with the D1R. We quantitatively compared the electron microscopic immunogold labeling for NR1 in dendritic profiles distinguished with respect to presence of D1R immunoreactivity and location in the Acb shell or core of rats receiving a single s.c. injection of vehicle (VEH) or apomorphine (APO) alone, or combined with AS (VEH+AS, APO+AS). The rats in the APO+AS group were previously shown to have PPI deficits, whereas the rats in the VEH+AS group had normal PPI. A significantly higher percentage of plasmalemmal and a lower percentage of cytoplasmic NR1 immunogold particles were seen in D1R-labeled dendritic spines in the Acb shell of the APO+AS group compared with all other groups. D1R-containing small dendrites in the Acb shell of the APO+AS group also showed a significantly higher density of plasmalemmal and a lower density of cytoplasmic NR1 immunogold particles compared with VEH or APO groups. In the Acb core, the APO+AS group had significantly fewer dendritic spines co-expressing NR1 and D1R compared with VEH or VEH+AS groups. These results, together with our earlier findings, suggest that NMDA receptors are preferentially mobilized in D1R-containing Acb neurons of rats showing apomorphine-induced disruption of PPI in a paradigm using acoustic stimulation. **© 2008 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: accumbens shell, dendritic spine, immunogold, prepulse inhibition, receptor trafficking, schizophrenia.

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Abbreviations: Acb, nucleus accumbens; ANOVA, analysis of variance; APO, apomorphine-injected; APO+AS, apomorphine injection followed by auditory stimulation; AS, auditory stimulation; BSA, bovine serum albumin; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; IgG, immunoglobulin G; NMDA, *N*-methyl-D-aspartate; NR1, NMDA receptor NR1 subunit; PB, phosphate buffer; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PPI, prepulse inhibition; TBS, Tris-buffered saline; VEH, vehicle-injected; VEH+AS, vehicle injection followed by auditory stimulation.

Prepulse inhibition (PPI) is an operational measure of sensorimotor gating, where the presence of a weak stimulus (prepulse) reduces the startle response to an immediately following intense auditory (AS) or other startling stimulus (Hoffman and Searle, 1965; Ison and Hammond, 1971). This PPI is disrupted in schizophrenia patients (Braff et al., 1978; Grillon et al., 1992), and also in rodents receiving systemic apomorphine, a dopamine D1/D2 receptor agonist (Swerdlow et al., 1986; Davis et al., 1990; Druhan et al., 1998; Geyer and Swerdlow, 1998; Kinney et al., 1999).

The PPI-disruptive effect of apomorphine is mediated in part by increased dopamine transmission in the nucleus accumbens (Acb; Swerdlow et al., 1986). The effective sites may include both the motivation-associated shell and the motor-associated core subregions of the Acb (Heimer et al., 1991; Zahm and Brog, 1992). These subregions are not only functionally distinct, but also differ in their major sources of dopamine and glutamate. The medial Acb shell receives exclusive dopaminergic input from the ventral tegmental area, whereas the core receives dopaminergic inputs from both the substantia nigra pars compacta and the ventral tegmental area (Brog et al., 1993; Groenewegen et al., 1999). The medial Acb shell also receives greater glutamatergic innervation from subcortical limbic regions compared with the core (Brog et al., 1993; Groenewegen et al., 1999). We have recently shown by electron microscopic immunogold labeling that these Acb subregions significantly differ in the extent to which apomorphine and AS affect dopamine D1 receptor (D1R) distribution in spiny dendrites receiving convergent dopaminergic and glutamatergic inputs (Hara and Pickel, 2007).

Glutamate *N*-methyl-D-aspartate (NMDA) receptors are also abundant in the Acb, and blockade of these receptors disrupts PPI in a manner similar to that produced by apomorphine (Reijmers et al., 1995). The essential NR1 and other subunits of the NMDA receptor, which were once thought to be stationary, are now known to undergo dynamically regulated surface expression, agonist-induced internalization, and rapid lateral movement to and from synaptic sites (Lan et al., 2001; Fong et al., 2002; Nong et al., 2003). In the dorsal striatum, activation of D1Rs induces trafficking of the NMDA receptor NR1 subunit between subcellular compartments (Dunah and Standaert, 2001) and conversely, NMDA receptor activation alters D1R subcellular distributions (Scott et al., 2002). Studies in cell lines and hippocampal neurons indicate that this interactive trafficking may be ascribed to physical protein–

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protein associations between the C-terminals of D1R and the NR1 subunit (Lee et al., 2002; Fiorentini et al., 2003; Pei et al., 2004).

Together, these observations suggest that neurons showing apomorphine and AS-induced trafficking of D1R in the Acb shell (Hara and Pickel, 2007) are likely to have NR1 distributions that are preferentially affected by these same experimental manipulations. To test this hypothesis, we examined the electron microscopic immunocytochemical labeling of the NR1 subunit in relation to the D1R in the Acb shell and core of rats receiving a single injection of vehicle (VEH) or apomorphine (APO) alone, or in combination with AS (VEH+AS, APO+AS). The rats in the APO+AS group used in this study were previously reported to have a significant disruption of PPI, whereas rats in the VEH+AS group had normal PPI (Lessard and Pickel, 2005; Hara and Pickel, 2007). The results from the current study provide the first ultrastructural evidence that redistribution of NMDA receptors in D1R-containing neurons occurs following apomorphine-induced disruption of PPI.

EXPERIMENTAL PROCEDURES

Animal preparation

The animal protocols in this study strictly adhered to NIH guidelines concerning the Care and Use of Laboratory Animals in Research, and were approved by the Animal Care Committee at Weill Medical College of Cornell University. Every effort was made to minimize the number of animals used and their suffering. Adult male Sprague–Dawley rats (280–350 g; Taconic Farms, Germantown, NY, USA) arrived 14 days prior to their experimental use. All rats were housed two per cage under a 12-h light/dark cycle. The level of background noise in the home cages was 68–70 dB. Food and water were available *ad libitum*.

Apomorphine (R-(−)-apomorphine-HCL, ICN Biomedicals Inc., Aurora, OH, USA) was dissolved in the vehicle solution (0.9% saline and 0.1 mg/ml ascorbic acid) using a sonicator. The apomorphine solution was injected s.c. at 1 mg/kg, a dose previously shown to disrupt PPI to acoustic startle (Mansbach et al., 1988). The vehicle solution alone was used as a control.

Four groups of three rats/group were examined in this study: a single s.c. injection of 1) vehicle without AS (VEH), 2) apomorphine without AS (APO), 3) vehicle followed by AS used for testing PPI (VEH+AS), and 4) apomorphine followed by the same AS (APO+AS). Rats in VEH+AS and APO+AS groups received the vehicle or apomorphine injection 5 min before introduction to the acoustic startle chamber and exactly an hour before aortic arch perfusion. The rats in VEH and APO groups also received the injection an hour before perfusion.

Acoustic startle testing

All test sessions were performed in a one chamber SR-LAB startle apparatus with digitalized signal output (San Diego Instruments, San Diego, CA, USA). The startle apparatus consisted of 1) a transparent acrylic cylinder 8.2 cm in diameter to hold the rat, 2) Radioshack Supertweeter (Radioshack Corporation, Fort Worth, TX, USA) mounted inside the isolation cabinet to generate the background noise and acoustic stimuli (prepulse and pulse), and 3) microcomputer and interface assembly (San Diego Instruments) to measure and record the startle magnitude as transduced by cylinder movement via a piezoelectric device mounted below a Plexiglas stand. The acoustic startle amplitude was defined as the peak of an average of 250 readings collected every 1 ms beginning at the onset of the acoustic stimulus. Sound levels were measured and calibrated with a Radioshack digital sound level meter with a resolution of 1 dB and accuracy of ±2 dB. (Radioshack Corporation).

To reduce variation in behavioral results arising from handling stress, all rats were handled for 10 min daily starting on the day of shipment arrival until the day of acoustic startle testing 2 weeks later. All rats that underwent the acoustic startle evaluation were tested in the light cycle (Weiss et al., 1999). Seven days prior to acoustic startle testing, the rats were exposed to a brief matching startle session, a procedure that is commonly used to reduce group variability in responses (Geyer and Swerdlow, 1998; Pothuizen et al., 2005). For this, rats were placed in the startle chamber and exposed to 65 dB(A) background noise for 5 min, followed by 11 pulses, each consisting of 40 ms bursts of 120 dB(A) noise, with inter-trial intervals averaging 15 s (range 8–23 s). The peak and average responses from each rat on each of the 11 trials were collected, and the initial response value was recorded separately from the remaining 10 responses for each rat, the latter of which were averaged together. Data from these sessions were used to divide the rats into two groups (VEH+AS and APO+AS) with similar mean and variance of basal startle amplitudes.

On the day of the acoustic startle and PPI evaluation, each rat received a s.c. vehicle or apomorphine injection. The rats in VEH+AS and APO+AS groups were introduced into the acrylic cylinder 5 min after the injection. The acoustic startle testing started with 5 min of 65 dB background noise to allow the rats to acclimate to the environment. The first six trials were pulse alone trials, which consisted of a 40 ms burst of 120 dB(A) noise. Then a pseudorandom sequence of pulse alone trials and prepulse+pulse trials was presented at inter-trial intervals averaging 15 s (range 8–23 s). The pulse alone trial was presented 10 times, and the three different prepulse+pulse trial types were each presented five times. These consisted of a 20 ms prepulse stimuli (3, 6 or 12 dB above the 65 dB(A) background noise), which do not elicit startle responses, preceding the 120 dB(A) pulse by 100 ms (onset-to-onset). The session concluded with an additional five pulse alone trials. The acoustic startle testing lasted approximately 15 min. The VEH and APO rats were returned to their home cages for 1 h after the apomorphine or vehicle injection. The VEH+AS and APO+AS rats were returned to their home cages for approximately 40 min after the end of the acoustic startle testing.

The data showing apomorphine-induced disruption of PPI for the animals used in these ultrastructural studies were previously reported (Lessard and Pickel, 2005; Hara and Pickel, 2007).

Tissue preparation

All rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) an hour after vehicle or apomorphine injection. We chose this time point as one that would enable internalization, surface expression, and lateral movement of NR1 (Lan et al., 2001; Fong et al., 2002; Nong et al., 2003). A longer time point would be more likely to evoke changes in gene expression. The rats were perfused via the ascending aorta first with 10 ml of heparin (1000 U/ml), then with 60 ml of 3.75% acrolein and 2% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB; pH 7.4), followed by 200 ml of 2% PFA in 0.1 M PB. The brains were removed and postfixed with 2% PFA in 0.1 M PB for 30 min at 4 °C, and then cut coronally into 40 μm sections on a Vibratome (Leica, Deerfield, IL, USA). Holes were punched at different locations within the cortex to distinguish between rats of different experimental groups. All Acb sections used for this study were collected at 1.60 mm anterior to Bregma (Paxinos and Watson, 1986) and contained both the shell and the core. All the sections from each of the four experimental groups were co-processed, and special care was taken to use identical sampling methods. These sections were placed in 1% sodium borohydride in 0.1 M

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