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DENDRITIC DISTRIBUTIONS OF DOPAMINE D1 RECEPTORS IN THE RAT NUCLEUS ACCUMBENS ARE SYNERGISTICALLY AFFECTED BY STARTLE-EVOKING AUDITORY STIMULATION AND APOMORPHINE

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Abstract—Prepulse inhibition of the startle response to auditory stimulation (AS) is a measure of sensorimotor gating that is disrupted by the dopamine D1/D2 receptor agonist, apomorphine. The apomorphine effect on prepulse inhibition is ascribed in part to altered synaptic transmission in the limbic-associated shell and motor-associated core subregions of the nucleus accumbens (Acb). We used electron microscopic immunolabeling of dopamine D1 receptors (D1Rs) in the Acb shell and core to test the hypothesis that region-specific redistribution of D1Rs is a short-term consequence of AS and/or apomorphine administration. Thus, comparisons were made in the Acb of rats killed 1 h after receiving a single s.c. injection of vehicle (VEH) or apomorphine (APO) alone or in combination with startle-evoking AS (VEH+AS, APO+AS). In both regions of all animals, the D1R immunoreactivity was present in somata and large, as well as small, presumably more distal dendrites and dendritic spines. In the Acb shell, compared with the VEH+AS group, the APO+AS group had more spines containing D1R immunogold particles, and these particles were more prevalent on the plasma membranes. This suggests movement of D1Rs from distal dendrites to the plasma membrane of dendritic spines. Small- and medium-sized dendrites also showed a higher plasmalemmal density of D1R in the Acb shell of the APO+AS group compared with the APO group. In the Acb core, the APO+AS group had a higher plasmalemmal density of D1R in medium-sized dendrites compared with the APO or VEH+AS group. Also in the Acb core, D1R-labeled dendrites were significantly smaller in the VEH+AS group compared with all other groups. These results suggest that alerting stimuli and apomorphine synergistically affect distributions of D1R in Acb shell and core. Thus adaptations in D1R distribution may contribute to sensorimotor gating deficits that can be induced acutely by apomorphine or develop over time in schizophrenia.

Key words: accumbens shell, immunogold, receptor trafficking, schizophrenia, sensorimotor gating, spine.

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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; NMDA, *N*-methyl-D-aspartate; 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 there is a reduction in startle response when auditory (AS) or other startling stimulus (pulse) is immediately preceded by a weak, non-startling stimulus (prepulse) (Hoffman and Searle, 1965; Ison and Hammond, 1971). This PPI is disrupted in schizophrenic patients (Braff et al., 1992; Grillon et al., 1992), and also in rodents when apomorphine, a dopamine D1/D2 receptor agonist, is systemically administered 5 to 10 min prior to testing (Davis et al., 1990; Druhan et al., 1998; Geyer and Swerdlow, 1998; Kinney et al., 1999). Thus, the acute administration of apomorphine may elicit adaptation within the sensorimotor systems that mimic those pathologically occurring in schizophrenia.

The PPI-disruptive effect of apomorphine is mediated in part by increased dopamine in the nucleus accumbens (Acb) (Swerdlow et al., 1986, 1990, 1992). The relevant functional sites likely include both the shell and core subregions of the Acb, but they may be differentially affected (Wan et al., 1994; Wan and Swerdlow, 1996). This is suggested by the known anatomical connectivity of each subregion (Voorn et al., 1989, 1994; Heimer et al., 1991). The Acb shell receives substantial mesolimbic dopaminergic innervation, while the core is functionally similar to the motor-associated dorsal striatum and receives dopaminergic input from the substantia nigra pars compacta (Groenewegen et al., 1999). Experience-dependent limbic inputs also terminate in the Acb shell, while the sensory cortical inputs target the core region that is involved in appetitive instrumental learning and locomotor activity (Heimer et al., 1991; Zahm and Brog, 1992; Kelley et al., 1997; Groenewegen et al., 1999; Smith-Roe and Kelley, 2000; Di Ciano et al., 2001).

Although dopamine D2 receptors (D2Rs) in the Acb play an important role in the regulation of PPI in rats, there are considerable synergistic interactions between dopamine D1 receptors (D1R) and D2R in the sensorimotor gating system (Wan and Swerdlow, 1993; Wan et al., 1996). D1Rs in the Acb are also critically involved in regulating locomotor activity and attentional processes mediated by mesoaccumbal dopamine release (Carey et al., 1998; Schwienbacher et al., 2002; Misener et al., 2004). Furthermore, D1R activation in the striatum facilitates sensorimotor cortical functions (Steiner and Kitai, 2000). Unlike D2Rs, D1Rs are predominantly located in distal dendrites and dendritic spines, which are major sites for synaptic plasticity (Delle Donne et al., 2004; Hara and Pickel, 2005). The surface expression of D1R is not stationary, but dynamically changed by synaptic activity and the availabil-

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ity of D1R agonists (Dumartin et al., 1998; Lamey et al., 2002). AS, such as that used in acoustic startle testing, transiently decreases extracellular dopamine in the Acb (Humby et al., 1996). Moreover, glutamate activation of *N*-methyl-D-aspartate (NMDA) receptors that are co-expressed with D1R in many Acb dendrites, can increase D1R plasma membrane insertion, as well as recruitment of D1Rs to dendritic spines (Scott et al., 2002; Pei et al., 2004; Hara and Pickel, 2005). Thus, either apomorphine binding to D1/D2 receptors or AS resulting in decreased dopamine and/or altered activation of glutamatergic inputs to Acb may affect the expression of D1Rs in selective neuronal compartments within the Acb shell and core neurons. To test this hypothesis, we examined the electron microscopic immunocytochemical labeling of D1Rs in the Acb shell and core of normal rats and rats exposed to AS in measures of PPI in the presence or not of apomorphine. The results provide the first ultrastructural evidence that apomorphine and AS synergistically affect the dendritic plasmalemmal distribution of D1Rs preferentially in the Acb shell.

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. 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. This dose was chosen based on previous studies showing that apomorphine injected at the same range of doses disrupts 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: single s.c. injection of 1) vehicle without AS (VEH), 2) apomorphine without AS (APO), 3) vehicle followed by AS from acoustic startle testing (VEH+AS), and 4) apomorphine followed by AS from acoustic startle testing (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 (Fig. 1). 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, San Diego, CA, USA) to measure and record the startle magnitude as transduced by cylinder movement via a piezoelectric device mounted below a Plexiglas stand. The acoustic startle

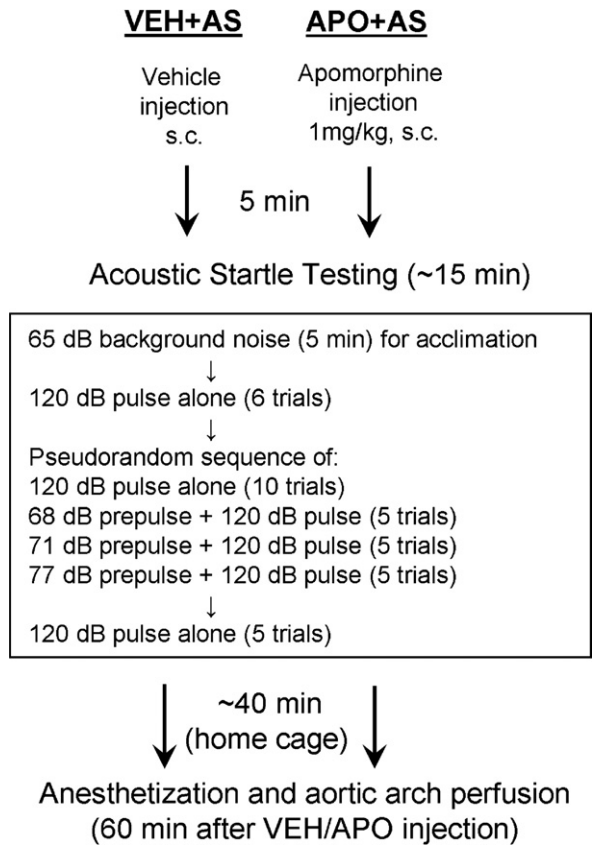

Fig. 1. A schematic diagram of the experimental paradigm for VEH+AS and APO+AS groups. Five minutes after the rats received either a vehicle or apomorphine s.c. injection, they were introduced to the acoustic startle apparatus. The startle testing started with 5 min of 65 dB background noise to allow the rats to acclimate to the environment. Then they were given six 120 dB pulse alone trials followed by a pseudorandom sequence of pulse alone trials and prepulse+pulse trials. The session concluded with an additional five pulse alone trials. After the completion of acoustic startle testing, the VEH+AS and APO+AS rats were returned to their home cage for approximately 40 min until the perfusion (60 min after the vehicle/apomorphine injection). The VEH and APO groups also received a single s.c. injection of vehicle and apomorphine, respectively, but did not undergo acoustic startle testing and remained in their home cage for 60 min until the perfusion (not shown).

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 (Radioshack Corporation).

To reduce variation in behavioral results arising from 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 background noise for 5 min, followed by 11 pulses, each consisting of 40 ms bursts of 120 dB 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