Strain Differences in the Gating-Disruptive Effects of Apomorphine: Relationship to Gene Expression in Nucleus Accumbens Signaling Pathways

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Background: Prepulse inhibition (PPI) of startle is a measure of sensorimotor gating that is deficient in certain psychiatric disorders, including schizophrenia. Sprague Dawley (SD) rats are more sensitive to PPI-disruptive effects of apomorphine (APO) at long interstimulus intervals (ISIs) (60–120 msec) and less sensitive to PPI-enhancing effects of APO at short ISIs (10–30 msec) compared with Long Evans (LE) rats.

Methods: Prepulse inhibition was tested in SD and LE rats after APO (.5 mg/kg) or vehicle in a within- subject design and sacrificed 14 days later. Total RNA was extracted from the nucleus accumbens (NAC). Approximately 700 dopamine-relevant transcripts on the Affymetrix 230 2.0 microarray were analyzed.

Results: As previously reported, SD rats exhibited greater APO-induced PPI deficits at long intervals and less APO-induced PPI enhancement at short intervals compared with LE rats. One hundred four genes exhibited significantly different NAC expression levels in these two strains. Pathway analysis revealed that many of these genes contribute to dopamine receptor signaling, synaptic long-term potentiation, or inositol phosphate metabolism. The expression of some genes significantly correlated with measures of APO-induced PPI sensitivity in either SD or LE rats. The expression of select genes was validated by real-time reverse transcription polymerase chain reaction (RT-PCR).

Conclusions: Differences in PPI APO sensitivity in SD versus LE rats are robust and reproducible and may be related to strain differences in the expression of genes that regulate signal transduction in the NAC. These genes could facilitate the identification of targets for ameliorating heritable gating deficits in brain disorders such as schizophrenia.

Key Words: Apomorphine, DNA microarray, dopamine, gene expression, prepulse inhibition, schizophrenia

Identifying neural and genetic mechanisms underlying many psychiatric disorders has proven difficult. In part, this difficulty reflects a reliance on complex, descriptive, and often variable clinical phenotypes as the fulcrum for parsing biological substrates. An alternative strategy is to study the neural and genetic bases of physiological abnormalities that accompany the clinical disorders, which may be closer to the disease genes, compared with the clinical symptoms (1–3).

Prepulse inhibition (PPI) of the acoustic startle reflex is the reduction in startle magnitude when the startle-eliciting stimulus (pulse) is preceded 30 to 500 msec by a weak stimulus (prepulse) (4). Prepulse inhibition is an operational measure of sensorimotor gating that is heritable (5,6) and regulated by forebrain circuitry, including portions of limbic-associated cortex and subcortical structures (7,8). Prepulse inhibition is deficient in several neuropsychiatric disorders, including schizophrenia (9–11), and PPI deficits occur in rats after administration of dopamine agonists, including the indirect dopamine agonist amphetamine and the direct dopamine agonist apomorphine (APO) (12,13).

Baseline and drug-induced changes in PPI exhibit robust differences across rat strains. For example, Sprague Dawley (SD) rats exhibit significantly greater sensitivity to the PPI-disruptive effects of both amphetamine and APO (14–23) compared with Long Evans (LE) rats. This strain difference is specific to dopamine (DA) agonists, as these two strains do not exhibit differential sensitivity to the PPI-disruptive effects of serotonin agonists and *N*-methyl-D-aspartate (NMDA) receptor antagonists (14,19). This strain difference in PPI sensitivity to DA agonists is heritable (17,18), independent of fostering conditions or differences in maternal-pup interactions (17), and stable across testing and breeding facilities (15) and is first observed before postnatal day 18 (18).

Several lines of evidence link SD versus LE differences in PPI APO sensitivity to differences in DA-stimulated signal transduction in the nucleus accumbens (NAC). Thus, SD versus LE differences in PPI APO sensitivity are accompanied by, and often correlate significantly with, differences in NAC [^{35}S]GTPγS binding (21) and in APO-induced inhibition of NAC phosphorylation of cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) (24) and FOS expression (22). F1 (SD × LE) rats exhibit intermediate phenotypes in PPI APO sensitivity (17,18,21) and in some measures of NAC DA-stimulated signal transduction (21,22).

Strain differences in NAC GTPγS binding, FOS expression, CREB phosphorylation, and PPI APO sensitivity might reflect differences in the expression of genes in SD versus LE rats that normally contribute to the regulation of DA-linked signal transduction in the NAC. Identification of these genes could provide potential molecular targets that contribute to the dopaminergic regulation of PPI in rodents and in several heritable psychiatric disorders, including schizophrenia and Tourette syndrome (25). Deoxyribonucleic acid microarrays provide a powerful means to investigate brain regional gene expression in rodents that exhibit differences in DA agonist sensitivity (26,27). To identify genes that might contribute to strain differences in sensitivity to the gating-disruptive effects of DA agonists, we compared NAC messenger RNA (mRNA) expression in SD and LE rats of approximately 700 transcripts associated with DA-regulated signal transduction and

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mRNA expression, and genes reproducibly shown to be relevant to schizophrenia. We focused exclusively on the NAC based on evidence that this region is critically involved in the dopaminergic regulation of PPI (8,12) and specifically in strain differences in this regulation (23) and in activity within DA-linked signal transduction pathways (21,22,24).

Methods and Materials

Twelve SD and twelve LE male rats (229–250 g) (Harlan Laboratories, San Diego, California) were housed and handled as per previous reports (14-24). All studies were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and approved by the University of California, San Diego (UCSD) Animal Subjects Committee (protocol #S01221).

PPI Testing

Startle chambers (SR-LAB; San Diego Instruments, San Diego, California) were located in a sound-attenuated room with a 60 dBA ambient noise. A brief startle session was used to form balanced drug groups according to average %PPI. Testing began a week later. Animals received either APO (.5 mg/kg, subcutaneous [SC]) or vehicle (.01% ascorbic acid) immediately before testing. Tests lasted approximately 19 min and included 5 min of 70 dBA background followed by six trial types: PULSE (120 dBA 40 msec noise burst), prepulse trials (5 msec noise burst, 15 dB above background followed 10, 20, 30, 60, or 120 msec later by PULSE), and a NOSTIM trial (no stimulus delivery). Seven days later, testing was repeated with APO and vehicle treatment reversed and treatment order balanced within and between rat strains.

Behavioral Data Analysis. One LE rat displayed negligible startle and was excluded from analyses. Prepulse inhibition was calculated as [100 − (startle amplitude on prepulse trials/startle amplitude on PULSE trials) × 100] and analyzed by analysis of variance (ANOVA) with strain as a between factor and drug and prepulse interval as within factors. Post hoc comparisons utilized the Fisher's protected least significant difference (PLSD) test. A measure of the magnitude of the APO effect (mean PPI after vehicle minus mean PPI after APO) was also used for strain comparisons; this value was previously shown effective in detecting differences in PPI drug sensitivity (16,21).

GeneChip Experiments. A 14-day interval was used to ensure both drug washout and a diminution of acute stress effects resulting from startle testing. As a result, this study compared basal gene expression in SD versus LE strains and not drug-induced gene activation patterns. Fourteen days after completion of PPI testing, animals were anesthetized (15–30 sec) with isoflurane, decapitated, and their brains were removed and placed in ice-cold saline for 30 sec. A 2 mm thick coronal tissue slab was cut with a wire tissue slicer and the NAC was removed bilaterally with a 2.5 mm diameter tissue punch and snap frozen in liquid nitrogen. Total RNA was isolated from tissue using RNeasy columns (Qiagen, Chatsworth, California). Ribonucleic acid quality was checked by Agilent Lab-on-a-chip (Agilent Technologies, Santa Clara, California) and spectrophotometry (260/280). Aliquots of total RNA (5 μg) were used to prepare complementary DNA (cDNA). Complementary DNA synthesis, complementary RNA (cRNA) amplification, hybridization to Affymetrix 230 2.0 Genechips (Affymetrix, Foster City, California), and subsequent washes and scanning (NIH Microarray Consortium) were performed according to the Affymetrix standard protocols (http://www.affymetrix.com/support/technical/manuals.affx).

DNA Microarray Data Analysis

Sprague Dawley versus LE expression patterns were compared across a focused set of approximately 700 of the >31,000 transcripts represented on the Affymetrix 230 2.0 rat chip. This focused set was selected prior to the onset of testing to include genes that are: 1) implicated in the control of either DA-related signal transduction pathways or DA metabolism; 2) regulated by DA agonists, antagonists, or DA depletion; or 3) most strongly associated with schizophrenia, based on published findings.

Raw array images were analyzed, with features extracted using GCOS 1.4 (Affymetrix). The resulting CEL files containing probe level information were normalized and converted to gene intensity values by the Robust Multi-Array Average (RMA) algorithm (28) with Gene Expression Console (Affymetrix). *T* tests (unequal variation) were performed on these normalized values (GeneSpring GX, Agilent Technologies, Santa Clara, California). Because corrections for multiple comparisons such as Bonferroni are very conservative, *p* values were chosen using both a very stringent ($p < .001$) and a more commonly used (26,29), less restrictive level ($p < .01$) to limit the number of false positives and negatives. Corresponding q-values were calculated, with significance analysis of microarrays (SAM) (30). The q-value measures significance by approximating false discovery rates (FDRs) rather than false-positive rates (31).

Hierarchical Cluster Analysis (GeneSpring GX) was performed, using genes that exhibited significant expression differences between SD and LE rats. Pearson correlations were used to 1) cluster genes that share common expression patterns into nearby places or branches in the gene tree, and 2) cluster samples with similar expression patterns in the condition tree. Canonical pathways were generated using Ingenuity Pathways Analysis (Ingenuity Systems, Redwood City, California, www.ingenuity.com). Right-tailed Fischer's exact test was used to calculate *p* values determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone. Netaffx (Affymetrix), Ingenuity Pathways Analysis, and GeneSpring GX were used to annotate genes.

Because PPI values were not normally distributed across both strains (based on high sensitivity in one strain and low sensitivity in another strain), Spearman correlations were performed for both baseline PPI and APO effects versus gene expression values for differentially expressed genes for individual animals in each strain. Prepulse intervals of 10, 20, and 120 msec were used in these correlational analyses, because these intervals are characterized by the most robust strain differences in APO sensitivity (LE most sensitive at 10–20 msec and SD most sensitive at 120 msec). Pearson correlations were performed on the DNA microarray and real-time reverse transcription polymerase chain reaction (RT-PCR) expression values for several genes.

Taqman RT-PCR

First strand cDNA was synthesized using Quantiscript Reverse Transcriptase (Qiagen, Chatsworth, California). For each sample, 1 μg of total RNA was reverse transcribed in a 20 μL reaction with a 50 ng mixture of poly A and random hexamer primers, Quantiscript RT buffer (Qiagen), ribonucleotides, and RNase H according to the manufacturer's protocol. Complementary DNAs representing the level of RNA expression were amplified by real-time RT-PCR using Applied Biosystems TaqMan Gene Expression Assays (Foster City, California) and performed on an Applied Biosystems 7300 in a 20 μL reaction with Universal PCR Master Mix (without AmpErase UNG) according to the manufacturer's protocol. Genes and assay identification (ID) numbers