Coregulation of genes in the mouse brain following treatment with clozapine, haloperidol, or olanzapine implicates altered potassium channel subunit expression in the mechanism of antipsychotic drug action

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Background Antipsychotic drugs are the most effective treatment for the psychotic symptoms of schizophrenia, yet their mechanism of action remains largely unknown.

Objectives Earlier studies have shown gene expression changes in rodent brains after treatment with antipsychotic drugs. We aimed to further characterize these changes using whole-genome transcript profiling to explore coregulation of genes after multiple antipsychotic drug treatment studies.

Methods This study involved transcript profile analysis after 7-day treatment of inbred C57BL/6 mice with conventional (haloperidol) or atypical (clozapine or olanzapine) antipsychotic drugs. Microarray analysis was undertaken using whole-brain mRNA on Affymetrix 430v2 arrays, with quantitative reverse transcriptase-PCR used to confirm gene expression changes. Western blotting was also used to explore translation of gene dysregulation to protein changes and to explore anatomical specificity of such changes.

Main results Thirteen genes showed verified regulation by multiple antipsychotic drugs – three genes significantly upregulated and 10 genes significantly downregulated by treatment. These genes encode proteins that function in various biological processes including neurogenesis, cell adhesion, and four genes are involved in voltage-gated ion channels: neural precursor cell developmentally downregulated gene 4 (*Nedd4*), K_v channel interacting protein 3 (KChip3), potassium voltage-gated channel, shaker-related subfamily, α1 (*Kcna1*) encoding $K_v1.1$ protein and β1 (*Kcnab1*) encoding $K_vβ1$ protein. The translation of these gene expression changes to protein dysregulation for $K_v1.1$, KCHIP3, and NEDD4 was confirmed by western blot, with regional protein analyses undertaken for $K_v1.1$ and KCHIP3.

Conclusion These results suggest that transcriptional regulation of ion channels, crucial for neurotransmission, may play a role in mediating antipsychotic drug effects. *Psychiatr Genet* 18:226–239 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Antipsychotic drugs (APDs) are the core treatment for schizophrenia because of their high efficacy in treating the psychotic symptoms of the illness (Kane, 1996) although the neurobiological changes underlying effective treatment are not well understood. Two main classes of APDs, conventional (typical) and modern (atypical), characterized by their neurotransmitter receptor-binding profiles are observed. Haloperidol is a conventional APD with high affinity for the dopamine D2 receptor. D2-receptor occupancy is associated with the main adverse effect of this class of APDs, extrapyramidal side effects that manifest as neurological movement disorders (Tauscher *et al.*, 2002). Clozapine and olanzapine are both atypical APDs. They exhibit broader neurotransmitter receptor-binding profiles than conventional APDs interacting, each with distinct affinities, at dopamine, serotonin, α-adrenergic, histaminergic, and muscarinic receptors (Miyamoto *et al.*, 2005). Clozapine and olanzapine are associated with reduced extrapyramidal side effects compared with conventional APDs (Leucht *et al.*, 2003) yet they have other adverse effects such as dramatic weight gain (Allison *et al.*, 1999). Clozapine, haloperidol, and olanzapine have distinct receptor profiles and yet all act to alleviate the psychotic symptoms of schizophrenia. As all APDs bind dopamine D2 receptors (Seeman *et al.*, 1975), this effect is undoubtedly mediated through dopamine and other neurotransmitters yet the underlying molecular mechanisms of therapeutic action after neurotransmitter modulations remain undefined.

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Microarray technology allows analysis of the expression profiles of many genes simultaneously. Earlier, this technology has been used to analyze gene expression changes in postmortem brain tissue from schizophrenic patients leading to the identification of genes involved in various metabolic pathways (Middleton *et al.*, 2002), myelination (Hakak *et al.*, 2001) apolipoprotein L family members (Mimmack *et al.*, 2002), and presynaptic function (Mirnics *et al.*, 2000; Vawter *et al.*, 2002), the latter of which was confirmed in a subsequent study that also found alterations in genes involved in glutamate function (Vawter *et al.*, 2002).

More recently, microarray technology has been used to transcript profile brain tissue derived from APD-treated animals. Genes involved in signal transduction, cell communication, metabolism, and transport were altered after chronic olanzapine treatment (Fatemi *et al.*, 2006) and chronic risperidone administration altered genes involved in neurotransmission and synaptic plasticity (Chen and Chen, 2005). Transcript profiling of mice treated acutely with clozapine-revealed changes in genes involved in neurotransmission, signaling, neuronal and glial cell development and function, transcription factors, and enzymatic regulators, in multiple schizophrenia-associated brain regions (Le-Niculescu *et al.*, 2007). Microarray analysis and downstream studies have also shown dysregulation of specific genes by APD treatment. An increase in synapsin II expression was seen after chronic haloperidol treatment (Chong *et al.*, 2002), and chronic clozapine treatment resulted in the upregulation of glucose-dependent insulinotropic peptide (Sondhi *et al.*, 2005).

Other neuropharmacological microarray studies have investigated the expression of genes regulated by multiple APDs. This type of analysis is presumed to reveal common genes involved in antipsychotic response, rather than the side effects of individual compounds. Gene expression analysis of rat frontal cortex after acute (1 day) treatment with clozapine or haloperidol showed coregulation of genes related to synaptic function (Kontkanen *et al.*, 2002). Two-week treatment of mice with the same APDs revealed altered expression of genes involved in metabolism, calcium homeostasis, and signal transduction (Thomas *et al.*, 2003). Chronic (26 days) treatment supported a role for clozapine and haloperidol in synaptic plasticity and protein phosphorylation in the rat frontal cortex (MacDonald *et al.*, 2005). Genes in these pathways were also altered by acute and chronic treatment with risperidone (Feher *et al.*, 2005).

The aim of this study was to further characterize the transcript profile resulting from treatment with three APDs: clozapine, haloperidol and olanzapine, at a time intermediate to acute and chronic treatment. Additionally, we aimed to identify patterns of common gene regulation between multiple APDs to uncover mechanisms of antispsychotic action. Earlier published studies of gene expression alterations by APDs in rodents have focused on either acute or chronic dysregulation, as described above. This study is unique in that a 7-day intermediate time point for APD treatment was used. Recent analysis suggests that this time point may be of considerable clinical relevance given that psychotic symptoms in patients with schizophrenia show the greatest reduction in the first week of treatment as compared with the subsequent 3 weeks of treatment, contrary to the earlier dogma of no amelioration of symptoms during the first 2 weeks (Agid *et al.*, 2006). Our microarray analysis and subsequent quantitative PCR validation revealed many genes in various biological pathways that were significantly regulated by two or more APDs. Western blot analysis revealed that APD regulation of genes involved in voltage-gated ion channels was translated into protein changes and that these changes were regionally specific. We hypothesize that genes altered in this study may be involved in the underlying molecular mechanisms of antipsychotic response, in particular via regulation of neuronal ion channels.

Methods

Animals

Male C57BL/6 mice (ARC, Perth, Australia) were housed in a controlled temperature (22°C) and humidity (52%) environment with a 12 h day–night cycle and maintained with standard chow and water *ad libitum* during a 1-week initial quarantine and subsequent treatment periods. For the collection of whole-brain tissue, animal studies were approved by the Garvan Institute of Medical Research/St Vincent's Hospital Animal Experimentation Ethics Committee (NSW, Australia, AEEC #03/09). For region-specific tissue collection, animal treatment studies were approved by the University of New South Wales Animal Care and Ethics Committee (ACEC# 07/22A). All 76 mice required for the experiments detailed here were weighed before each injection and were 8–10 weeks of age at the commencement of treatment.

Animal drug treatment and serum level evaluation

Clozapine and haloperidol were purchased from Sigma-Aldrich (Sydney, New South Wales, Australia). They were dissolved in glacial acetic acid, diluted with 0.9% saline, adjusted to pH 5.5 with sodium hydroxide (1 mol/l) and brought to a final concentration of 1 mg/ml for clozapine and 0.1 mg/ml for haloperidol. Olanzapine was purchased from Eli Lily (Sydney, Australia) and dissolved in 0.9% saline solution to a final concentration of 1 mg/ml.

Clozapine (10 mg/kg), olanzapine (10 mg/kg), or haloperidol (1 mg/kg) were administered via daily intraperitoneal injection for 7 days at 11:00 h each day. Control mice received intraperitoneal injections once daily of 10 ml/kg sterile 0.9% saline solution for 7 days. Five hours after the last injection on the seventh day of treatment, mice were euthanased under 4% halothane anesthesia to allow

cardiac puncture and collection of approximately 1 ml of blood from each animal. The whole brain was immediately removed, snap frozen in liquid nitrogen and stored at −80°C until required. Blood samples taken from the treated animals were assayed for serum concentration for clozapine (Central Sydney Area Health Service, Royal Prince Alfred Hospital, Sydney, Australia) and olanzapine (SouthPath, Adelaide, Australia) to mimic human therapeutic levels (102–771 mg/l for clozapine; 5–75 mg/l for olanzapine).

Preparation of total RNA and protein from mouse whole brain

Total RNA and protein were extracted from mouse whole-brain tissue in TRIzol Reagent (Invitrogen, Melbourne, Australia). Thawed whole brains were homogenized in TRIzol Reagent solution (1 ml/100 mg tissue) for 5 min using a polypropylene hand homogeniser (Sigma-Aldrich). Total RNA was precipitated from the supernatant layer using standard phenol/chloroform extraction, resuspended in RNase-free water (Sigma-Aldrich) and purified using RNeasy Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The concentration of purified total RNA samples was determined by measuring the absorbance at 260 nm using a GeneQuant UV spectrophotometer (Pharmacia Biotech, Sydney, Australia) and quality was checked by visualization of RNA samples on a 0.8% formaldehyde-denaturing agarose gel. Protein was precipitated from the phenol layer of the TRIzol product and dissolved in 10% sodium dodecyl sulfate (SDS). Protein concentration was assayed using the RC DC Protein Assay kit (Bio-Rad Laboratories, California, USA) following the manufacturer's instructions. The absorbance of samples and bovine serum albumin standards was measured at 650 nm using SpectraMax250 plate reader with SoftmaxPro v1.1 software (Molecular Devices Corp., Sunnyvale, California, USA).

Target preparation and microarray hybridization

High-quality individual total RNA samples were prepared from 64 mice, 16 from each treatment group (saline, clozapine, haloperidol, or olanzapine). Within each treatment group, eight individual mouse total RNA samples were randomly selected and pooled together to form two separate pools. Total RNA was used as template to generate high-fidelity cDNA using Superscript Double-Stranded cDNA Synthesis Kit (Invitrogen), which was modified at the 3′ end to contain an initiation site for T7 RNA polymerase as per the Affymetrix Expression Analysis Technical manual (Affymetrix, Santa Clara, California, USA). Biotinylated cRNA probes were transcribed *in vitro* from cDNA using the BioArray High Yield RNA Transcription Labeling Kit (Affymetrix), following the manufacturer's instructions, purified as above and fragmented into 35–200 bp fragments. Microarray cRNA probes were prepared for each separate pooled sample in all four groups (control, clozapine, haloperidol, olanzapine). These eight cRNA probes were initially hybridized to Affymetrix GeneChip Test3 microarrays. The quality of each sample was assessed using the 3′/5′ ratios for mouse housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin. After quality control assessment, cRNA probes were hybridized to Affymetrix GeneChip Mouse Genome 430 2.0 Array microarrays (Affymetrix) after the protocol outlined in the Affymetrix GeneChip Expression Analysis Technical manual (Affymetrix). Microarrays were washed using a semiautomated GeneChip Fluidics Station 400 (Affymetrix) and hybridization quantified using an Affymetrix GeneChip Scanner 3000 (Affymetrix).

Microarray data analysis

Using GeneChip Operating Software (GCOS) v1.2 software (Affymetrix), the fluorescent signal intensities of 45 101 transcripts contained on the Affymetrix GeneChip Mouse Genome 430 2.0 Array were determined. The raw intensity values were normalized to the mean intensity of all probe sets. Genes were initially filtered for a 'present' call as assigned by the GCOS software for each of the hybridized microarray chips and selected for further analysis if they were called as 'present' on all eight microarrays. Differentially expressed genes were determined by calculating the mean signal intensity from the two array chips for each gene in each group (control, clozapine-treated, haloperidol-treated, or olanzapine-treated). The fold-change signal ratio was then calculated for each transcript as an average normalized expression ratio of intensity for treatment versus control arrays. Transcripts were further selected that had $\geq$ 1.5-fold difference in expression between treatment and control groups (or $\geq$ 2-fold change for haloperidol downregulated transcripts because of excess regulation).

Real-time quantitative reverse transcriptase-PCR

The differential gene expression detected by microarray analysis was validated using real-time quantitative reverse transcriptase (RT)-PCR (QPCR) analysis of cDNA from total RNA that was extracted earlier. Genomic DNA contamination was removed from total RNA using RQ1 RNase-free DNase (Promega, Sydney, Australia) following manufacturer's instructions. RT was performed using 2 μg of total RNA with Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen) and 2 mmol/l oligo$(dT)_{20}$ primer (Invitrogen) following manufacturer's instructions. After cDNA synthesis RNA was removed from the reaction by incubation with two units of RNase H (Invitrogen) at 37°C for 20 min.

Prepared cDNA was diluted in two parts to five and added to a PCR reaction mixture containing 1X Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) and forward and reverse primers (200 nmol/l each). PCR primers were designed using Primer3 (Rozen and

Table 1 Oligonucleotide primers used to amplify mouse cDNAs by real-time quantitative reverse transcriptase-PCR

Gene	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	Size (base pair)	T_{an} (°C)
GAPDH	AACTTTGGCATTGTGGAAGGG	TCATCATACTTGGCAGGTTTCTCC	272	58
Kcna1	AGTATCCCCGATGCTTTC	GGTCACTGTCAGAGGCTAAG	250	58
Numbl	GGGAGGGATGTTGACTCTGT	TTCCTTTATTGGCTGTCCTTG	165	65
S100a9	TTACTTCCCACAGCCTTTGC	TCAGACAAATGGTGGAAGCA	230	62
AK036626	TTGAACCCCTACCCTCCTCT	GAGCCAAAATGTCCTGCTTC	208	62
Plp	GTGTTCTCCCATGGAATGCT	GTTTAAGGACGGCGAAGTTG	186	64
Rln4	TTACGTTGGTGCCTTGTTCA	ATTCTGCTTTGCGCTTCAAT	191	60
Dclk	ACAAGGGGACTCCTCTCCAT	CCCGTGACCGTTTCTTTTTA	212	64
Serpini1	GCTGTTCCTCTCCAAAGCTG	CCTCAAAGTCATGGCCACTT	236	65
Sdfr1	AAAAACTTGCGCCAGAGAAA	GGCATGCTTTAGACGGTCAT	160	62
Sypl	TTGTGGTTGGTGAGTTCCTC	AAGCATTTCCTCCCCAAAGT	206	62
Mal	TCCCTGACTTGCTCTTCGTT	TGTGGCTGCCCTATTTTACC	156	64
Kns2	CAGCTGGAGGAGGAGAAGAA	ACTACTGTGCTGCTGCTGGA	174	64
Cspg3	GTAGGGTGTGAACCCTGGT	TTGTGCGTGTGTTGGAGAAT	179	60
Nedd4	GGATGATGGATTCGGAAAAA	TCGTCAAAGGATTCGTAGGG	205	60
Rapgef4	GGATCCTTCCAGAAACCACA	TATTAGCTTGAGCGGCATCC	233	63
Bat2	AGCCCTGGACCTCCTAATTC	CAGAAACTGGCCCTTGAGAG	195	64
Gabra1	AAAAGCGTGGTTCCAGAAAA	CGATTTTGCTGACGCTGTTA	220	63
Kchip3	CTGGAGCATGTGGAGAGGTT	CAGGATTGAGGCTTCTCTGG	198	64
Catnb	CCAGTTCCCTCTTCAGGACA	ATGCTCCATCATAGGGTCCA	176	62
Kcnab1	GACCTCTCTCCAATCGCTGA	CCTTTTTGCTGTAGGGCTTG	223	64

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; T_{an}, annealing temperature.

Skaletsky, 2000) and synthesized by Sigma Genosys (Sydney, Australia) (Table 1). Amplification conditions were as follows: uracil-DNA glycosylase treatment at 50°C for 2 min, denaturation at 95°C for 2 min, and 45 cycles of amplification (denaturation at 95°C for 5 s, annealing for 15 s, and extension at 72°C for 15 s). SYBR Green I fluorescence intensity was measured at the end of the annealing step. After amplification, samples were dissociated by incremental heating between 72 and 99°C, at a rate of 0.2°C/s. During this dissociation, SYBR Green I fluorescence was constantly measured and a melting curve plotted to confirm single amplicon amplification. Amplification was performed in a Rotor-Gene 3000 PCR machine (Corbett Research, Sydney, Australia).

Quantitative PCR critical cycle threshold (C_t) values for each sample were determined using Rotor-Gene v5.0.37 software (Corbett Research). Serial dilutions of DNA standards of known concentration were used for each gene to quantitate the number of mRNA copies in the unknown samples. Amplification was done in triplicate for each gene, using 10 randomly selected cDNA samples (from a total of 16) for each treatment group (clozapine, haloperidol, and olanzapine) and controls. The relative expression of the gene of interest (GOI) for each sample was expressed as a ratio of the number of GOI mRNA copies to the number of copies of GAPDH mRNA, giving a normalized expression value.

Western blot analysis of whole-brain lysates

Western blotting was used to measure protein expression of three genes: shaker-related subfamily, member 1 (*Kcna1*) encoding $K_v1.1$, K_v channel-interacting protein 3 (*KChip3*), and neural precursor cell expressed, developmentally downregulated gene 4 (*Nedd4*). Approximately 20 μg of protein samples prepared from whole mouse brain tissue were heated at 95°C for 10 min. For $K_v1.1$ and NEDD4, sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed using a NuPAGE 4–12% BisTris Gel (Invitrogen) after transfer to a nitrocellulose membrane using the Xcell SureLock Mini-Cell Western Transfer apparatus (Invitrogen) at 30 V for 60 min in accordance with the manufacturer's instructions. For KCHIP3, sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed using a Criterion Cell 10% Tris–HCl gel (Bio-Rad Laboratories) after by transfer to a nitrocellulose membrane using the Criterion Blotter system at 100 V for 22 min (Bio-Rad Laboratories) in accordance with the manufacturer's instructions. Immunoblots were blocked for 1 h at room temperature with agitation in 5% skim milk powder and appropriate buffer, as indicated below. Blots were then incubated with primary antibody overnight at 4°C with agitation. A 1 : 300 dilution of rabbit polyclonal antihuman $K_v1.1$ IgG (sc-25680, Santa Cruz Biotechnology, Santa Cruz, California, USA) was used to detect $K_v1.1$ protein levels. A 1 : 250 dilution of rabbit polyclonal antihuman CSEN IgG (ab41717, Abcam, Cambridge, UK) was used to detect KCHIP3 protein levels. A 1 : 5000 dilution of rabbit polyclonal antirat NEDD4 IgG (ab14592, Abcam) was used to detect NEDD4 protein levels. To bind the secondary antibody, blots were incubated for 1 h at room temperature with agitation in a 1 : 10000 dilution of biotinylated goat antirabbit IgG (Vector Laboratories, California, USA). After each step in this process $K_v1.1$ and KCHIP3 immunoblots were washed in 0.1% Tween-20/Tris buffered saline (TBS) and NEDD4 immunoblots were washed in 0.05% Triton-X100/TBS buffer. To normalize the amount of protein loaded onto each lane membranes were additionally blotted with an antibody to β-actin (ab6276, Abcam).

After this staining procedure, protein bands were detected using the ECL Plus detection kit (Amersham Biosciences, Piscataway, New Jersey, USA). Visualization of chemiluminescent bands, with background deducted, was undertaken using the Molecular Imager ChemiDoc XRS System (Bio-Rad Laboratories) with QuantityOne v4.5.1 analysis software used for quantification (Bio-Rad Laboratories). This procedure, from electrophoresis to transfer to antibody staining and detection, was completed in duplicate for each protein of interest.

Microdissection and protein extraction of mouse brains for regional analysis

Anatomically defined brain dissections were performed on six saline-treated mice and six mice treated with haloperidol (1 mg/kg/day) for 1 week. The regional dissections were accomplished after the procedure described by Lazar and Blum (1992) with minor modifications. Mice were killed by cervical dislocation and decapitated. The brain was removed from the skull and chilled in saline on ice. A region corresponding to the infralimbic cortex and rostral cingulate cortex (bregma 1.98 mm Paxinos and Franklin, 2001) was removed first from a coronal slab. From the adjacent coronal slab, the caudate putamen was removed between the lateral ventricles and corpus callosum (bregma 0.38 to 1.70 mm Paxinos and Franklin, 2001). The midbrain was separated and the substantia nigra and ventral tegmental area were dissected out with angular cuts in the ventral tegmentum. The hippocampus was removed whole by lifting from the external capsule and transecting the fimbria fornix.

Protein extraction from brain regions

Protein extraction from regional lysates was undertaken using two solutions: solution 1 was 0.05% triton X-100 in TBS with Complete EDTA-free protease inhibitor cocktail (Roche Molecular Biochemicals, Basel, Switzerland) facilitating inhibition of chymotrypsin, thermolysin, papain, pronase, and trypsin. Solution 2 contained 0.05 mol/l Tris pH 8.0, 2% SDS in TBS with Complete EDTA-free protease inhibitor cocktail (Roche Molecular Biochemicals). For protein extraction, tissues were homogenized in microcentrifuge tubes with solution 1 (100 µl/10 mg) using a hand-held homogenizer for 5 min. After centrifugation at 16 000*g* at 4°C for 15 min, the lysate was removed. The remaining tissue was resuspended in solution 2 (100 µl/10 mg) and homogenized for a further 5 min, centrifuged at RT at 160 000*g* for 15 min and lysate was collected.

Western blots of regional protein lysates

For region-specific analyses, 20 µg of protein from each mouse brain in each region was loaded onto the gels for $K_v1.1$ detection and 25 µg of protein from each mouse brain in each region for KCHIP3 detection. Western blot analysis was carried out as described above except that KCHIP3 was used at a dilution of 1 : 200. Furthermore, the BioRad western blotting and transfer system was used for both $K_v1.1$ and KCHIP3, with electrophoretic separation of proteins undertaken for 60 min at 200 V and western transfer for 22 min at 100 V.

Statistical analysis

For QPCR analysis the GAPDH normalized expression levels for the GOI was statistically compared between APD-treated and control animals using a two-tailed Student's *t*-test with unequal variance. For whole-brain regional analysis, the β-actin normalized expression level for the protein of interest was statistically compared between APD-treated and control animals using a two-tailed Student's *t*-test with unequal variance. For the regional western blot analysis, given we knew the direction of expected change, a one-tailed test was used on protein expression levels normalized to β-actin and expressed as an averaged ratio of total protein expression levels in saline-treated animals. Statistical significance was set at a P value of less than 0.05.

Results

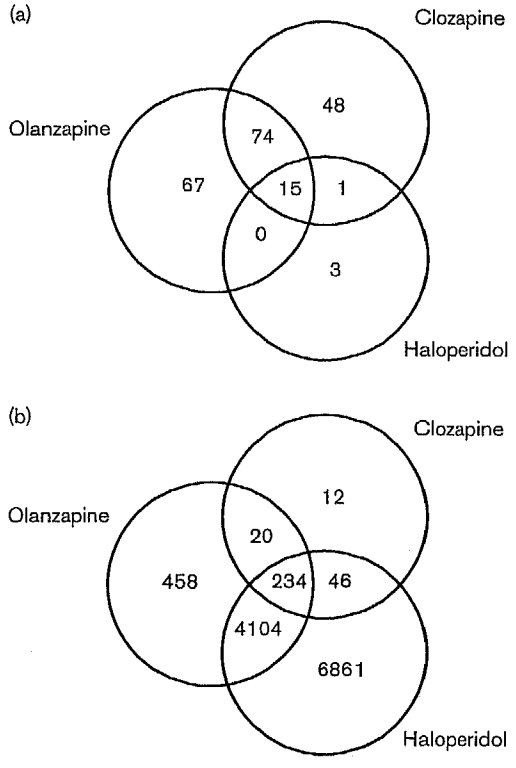
Animal model of intermediate antipsychotic drug treatment

An animal model of APD action was developed by 7-day treatment with saline or one of three APDs: clozapine, haloperidol, or olanzapine. Olanzapine dose trials were based on earlier studies (Schreiber *et al.*, 1999; Wang *et al.*, 2004) and dose was selected at 10 mg/kg based on endpoint serum drug concentrations of 5–75 ng/ml (the human therapeutic range) after a 1-week trial period (data not shown). Clozapine dose (10 mg/kg) was based on endpoint serum drug concentrations of low human therapeutic dosage (100 ng/ml) after a 7-day trial period (data not shown) and concentrations used in earlier studies (Kontkanen *et al.*, 2002; Simosky *et al.*, 2003; MacDonald *et al.*, 2005). Higher doses showed adverse effects on treated animals. The dose used for haloperidol treatment (1 mg/kg/day) was based on earlier studies (Kontkanen *et al.*, 2002; Simosky *et al.*, 2003; Emamian *et al.*, 2004).

Microarray analysis

Analysis of gene expression profiles in brain RNA from 16 animals per treatment group was undertaken using whole-genome Affymetrix GeneChip microarrays (Affymetrix). Two microarrays were used per treatment group, with equal amounts of RNA from eight mice pooled onto each microarray. Data analysis using GCOS revealed a total of 11 941 out of over 39 000 transcripts showed greater than 1.5-fold change by at least one APD compared with saline-treated controls. Further analysis was undertaken to identify genes that were regulated by multiple APDs, which was approximately 37% of the dysregulated transcripts (Fig. 1). Specifically, 90 of the 206 transcripts upregulated by drug treatment were

Fig. 1



Venn diagrams indicate transcripts (a) upregulated and (b) downregulated by individual antipsychotic drugs and coregulated by multiple drug treatments compared with controls as seen by whole genome microarray analysis.

altered by at least two APDs and 4404 of the 16093 transcripts downregulated by drug treatment were altered by at least two APDs. In total, 249 transcripts were coregulated by all three APDs in this analysis.

On account of the large number of genes downregulated by haloperidol, further analysis used a two-fold change cut-off for this regulation. Gene ontology searches performed for all significantly altered transcripts revealed that these are involved in diverse biological processes including signal transduction, protein metabolism, neurogenesis, and synaptic transmission (full list available upon request). After undergoing bioinformatic analysis of the significantly dysregulated transcripts, 20 candidate genes were chosen for further expression analysis based on their regulation by multiple APDs, the chromosomal location of their human homologs, pattern of expression, or relevant neurobiological function (Table 2). All candidate genes were altered by more than 1.5-fold change in expression by at least two APDs compared with saline-treated controls in the microarray analysis.

Validation by real-time quantitative reverse transcriptase-PCR

A total of 13 out of 20 dysregulated genes had a statistically significant change in gene expression by one or more APDs as determined by real-time QPCR. Statistically significant upregulation by drug treatment was found for two known genes: potassium voltage-gated channel, *Kcna1* and doublecortin-like kinase I (*Dclk1*) and an expressed sequence tag: (*AK036626*) (Fig. 2a). Ten genes were statistically significantly downregulated by drug treatment (Fig. 2b). These are calgranulin B (*S100a9*), rap guanine nucleotide exchange factor 4 (*Rapgef4*), myelin and lymphocyte protein (*Mal*), *Nedd4*, human leukocyte antigen-B-associated transcript 2 (*Bat2*), *β-catenin*, *KChip3*, potassium voltage-gated channel shaker-related subfamily β member 1 (*Kcnab1*), chondroitin sulfate proteoglycan 3 (*Cspg3*), and numb-like (*Numbl*).

Whole-brain protein expression analysis

To determine whether changes in gene expression after APD treatment in our animal model were translated into protein changes, three verified dysregulated genes were selected for further analysis. Western blotting of protein samples from APD-treated animals compared with controls showed significant upregulation of $K_v1.1$ by haloperidol (Fig. 3a). This was true for both protein species, at 57 and 59 kDa, comprising the recognized doublet for $K_v1.1$ cell surface protein expression (Deal *et al.*, 1994). No change in whole-brain $K_v1.1$ protein expression was detected following clozapine or olanzapine treatment. Significant downregulation of KCHIP3 protein was shown in whole-brain lysates after both haloperidol and olanzapine treatment (Fig. 3b), with no change detected in the clozapine-treated group. Western blot analysis also showed significant downregulation of NEDD4 protein after olanzapine treatment (Fig. 3c), with haloperidol treatment effecting no change in whole-brain NEDD4 levels. Proteins from clozapine-treated animals were not assayed as *Nedd4* gene expression change was not verified by QPCR analysis.

Protein expression analysis in brain regions implicated in schizophrenia

We carried out further western blot analysis to define the regional specificity in altered protein expression for $K_v1.1$ and KCHIP3 in saline and haloperidol-treated animals.

Regional western blots of $K_v1.1$ confirmed the presence of a 57–59 kDa dimer (data not shown). Triton X-100 protein extraction revealed the presence of the lower molecular weight protein, approximately 57 kDa. This species was significantly increased in the ventral tegmental area and hippocampus in haloperidol-treated mice compared with controls (Fig. 4a). The 59 kDa $K_v1.1$ band was also present in the Triton X-100 fraction and this species was significantly increased in the striatum and

Table 2 Microarray analysis results for selected genes in various biological pathways

Gene	Genbank ID	Microarray fold-change Clo	Hal	Ola	Gene ontology	Human genetic linkage[a]	Genetic association studies[b]
Genes upregulated by antipsychotic drug treatment							
Potassium voltage-gated channel, shaker-related subfamily, member 1 (*Kcna1*)	NM_010595	**1.62**	1.13	**1.65**	Ion transport; synaptic transmission	No	No
(Expressed sequence tag)	AK036626	**2.18**	1.30	**1.97**	Unknown	No	N/A
Doublecortin-like kinase I (Dclk1)	BC050903	**2.34**	1.30	**2.93**	Neurogenesis; neuronal migration	No	No
Stromal cell derived factor receptor I (*Sdfr1*)	NM_009145	**1.93**	0.90	**2.26**	Receptor activity	Weak	Yes (trend)
Genes downregulated by antipsychotic drug treatment							
Numb-like (*Numbl*)	NM_010950	**-1.65**	**-3.61**	**-3.14**	Neurogenesis	No	Yes (2 positive)
S100 calcium-binding protein A9 (*S100a9*)	NM_009114	**-2.55**	**-1.71**	**-2.55**	Calcium binding; Signal transduction	Strong	No
Myelin proteolipid protein (*Plp*)	NM_011123	**-1.57**	**-4.59**	**-2.11**	Integral membrane; myelination	No	Yes (positive)
Reticulon 4 (*Rtn4*) aka Nogo	AY102286	-1.46	**-4.36**	**-1.52**	Neurogenesis; apoptosis	Strong	Yes (two positive, five negative)
Serine proteinase inhibitor, clade I, member 1 (*Serpini1*)	NM_009250	-1.46	**-4.14**	**-1.52**	CNS development	No	No
Synaptophysin-like protein (Sypl)	AF081501	-1.37	**-3.61**	**-2.03**	Synaptic transmission	No	No
Myelin and lymphocyte protein (Mal)	NM_010762	**-1.83**	1.37	**-3.25**	Myelination	No	No
Kinesin II (Kns2)	BC055744	-1.46	**-5.96**	**-2.98**	Microtubule associated	No	No
Chondroitin sulfate proteoglycan 3 (*Cspg3*)	NM_007789	**-1.80**	**-5.56**	-1.41	Cell adhesion; calcium binding	No	No
Neural precursor cell expressed, developmentally downregulated gene 4 (*Nedd4*)	NM_010890	**-1.54**	**-4.29**	**-2.00**	Ubiquitin protein ligase activity	Weak	No
Rap guanine nucleotide exchange factor 4 (*Rapgef4*)	NM_019688	**-1.65**	**-4.44**	**-1.87**	cAMP-mediated signal transduction	No	No
GABA-receptor alpha-1 subunit (*Gabra1*)	NM_010250	-1.06	**-4.09**	-1.29	Neuronal signaling	Strong	Yes (3 positive, 2 negative)
HLA-B associated transcript 2 (*Bat2*)	NM_020027	**-1.62**	**-3.36**	**-3.61**	Unknown	Strong	Yes (negative)
Kv channel interacting protein 3 (*KChip3*) aka Csen, Dream	NM_019789	-1.19	**-3.42**	**-2.34**	Ion transport; transcriptional regulation	Strong	No
Catenin (cadherin associated protein)-β(β-*catenin*)	NM_007614	-1.49	**-4.07**	**-1.90**	Cell adhesion; signal transduction	No	No
Potassium voltage-gated channel, shaker-related subfamily, β member 1 (*Kcnab1*)	NM_010597	-1.32	**-5.76**	**-1.62**	Ion transport; synaptic transmission	No	No

Bold type indicates values more than 1.5-fold change in gene expression between antipsychotic-treated and saline-treated array groups.
[a]Corresponding to chromosomal regions of schizophrenia susceptibility from meta-analysis (Lewis *et al.*, 2003).
[b]According to the SZ gene database (*www.schizophreniaforum.org/res/sczgene/*).
Clo, clozapine; Hal, haloperidol; Ola, olanzapine.

hippocampus of haloperidol-treated mice compared with controls (Fig. 4a). The higher molecular weight protein was additionally seen in a second SDS-extracted fraction although there were no changes in the expression of $K_v1.1$ in this fraction (data not shown).

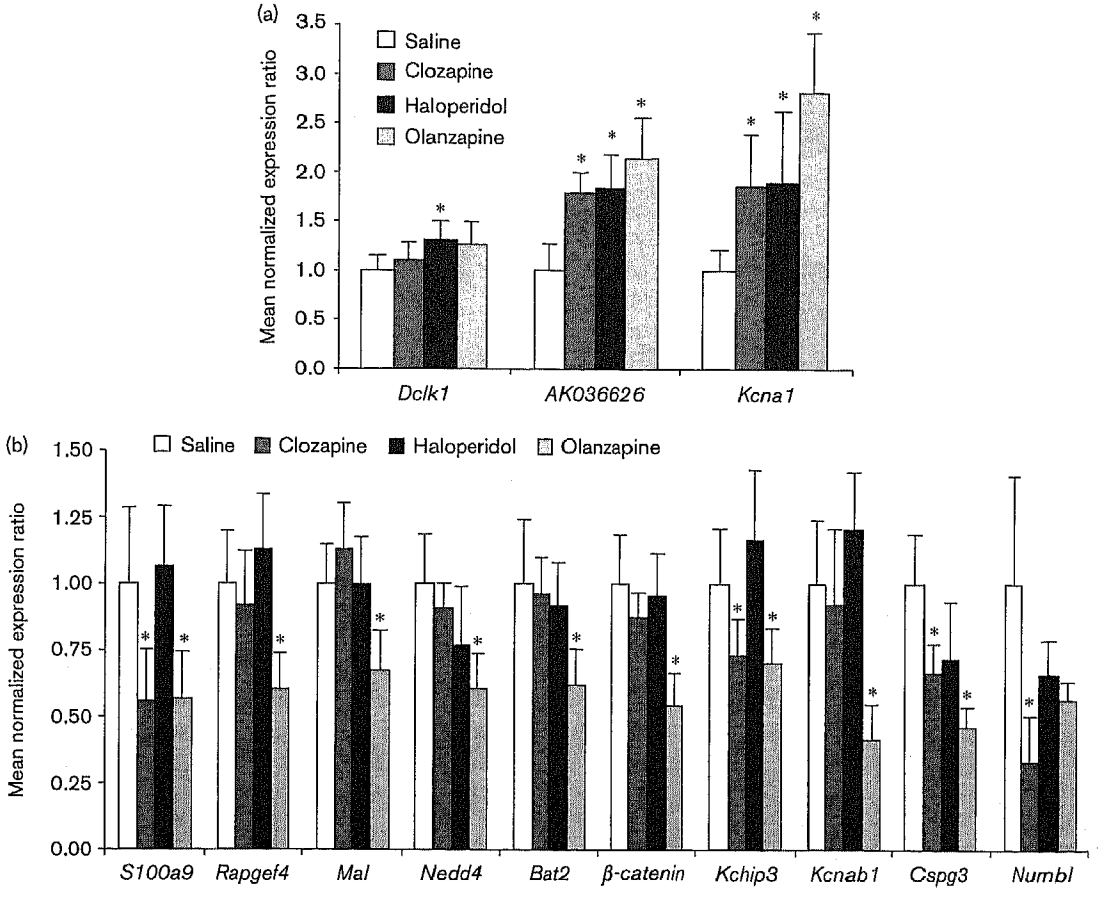
In our regional analysis of KCHIP3 protein, we found no significant alteration in levels of the monomer 27 kDa protein. A significant decrease in an approximately 55 kDa species detected by blotting with anti-KCHIP3 in the prefrontal cortex, ventral tegmental area, and hippocampus was, however, observed (Fig. 4b). This band is likely to be a dimer of KCHIP3, which occurs when the protein is bound to calcium (Osawa *et al.*, 2001), as EDTA chelation decreased the presence of this protein band in a dose-dependent manner (data not shown).

Differential regional abundance of the multiple molecular weight species of both KCHIP3 and $K_v1.1$ in the saline-treated control animals in our regions of interest was observed (Table 3).

Discussion

This study analyzed genes that are coregulated by up to three APDs after 7-day treatment in an animal model using whole genome microarray analysis. It has recently been found that although the full benefits of APD treatment on schizophrenic patients may take several weeks to realize, a greater reduction in psychotic symptoms is seen in the first 2 weeks of treatment when compared with any subsequent 2 weeks during chronic treatment (Agid *et al.*, 2006). This indicates that clinically relevant changes in brain biochemistry resulting from APD administration may occur earlier than originally thought. This study aimed to identify gene expression changes that occur after acute effects have stabilized, yet before deleterious effects of chronic medication appear in rodents such as tardive dyskinesia and metabolic

Fig. 2



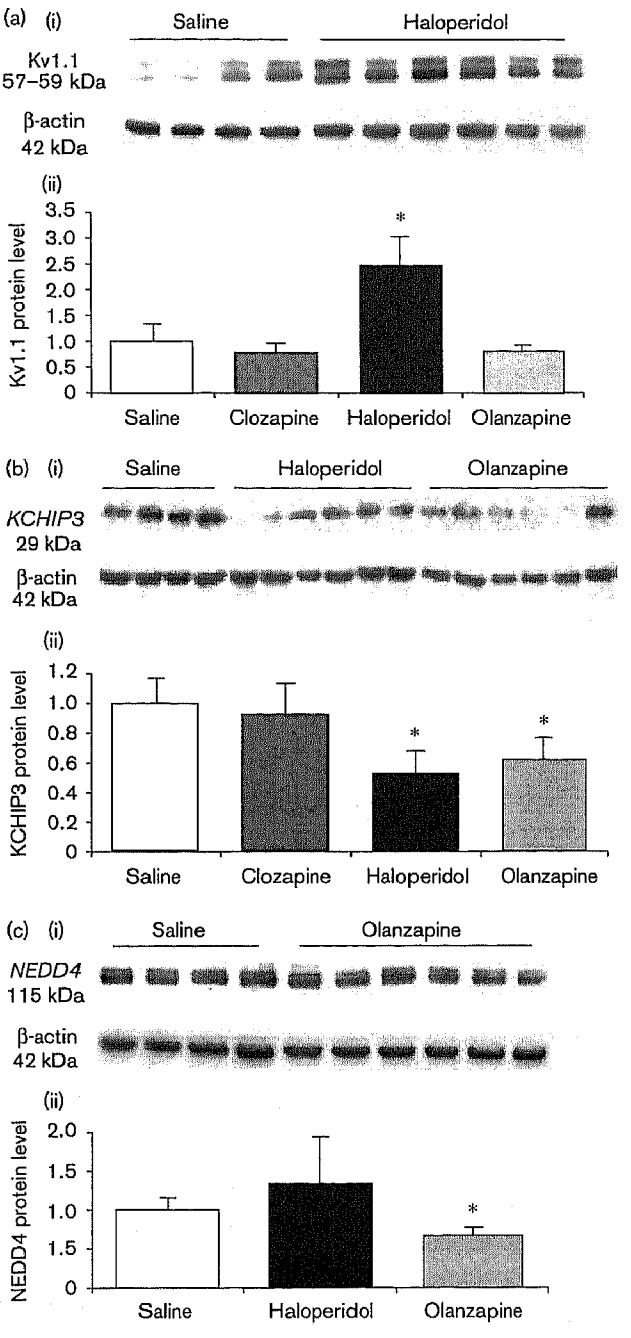
Real-time quantitative reverse transcriptase-polymerase chain reaction validates significantly (a) upregulated and (b) downregulated by 7-day antipsychotic drug treatment. Columns represent the mean expression (95% confidence interval) of eight mice/treatment group normalized to glyceraldehyde-3-phosphate dehydrogenase expression. *Significant change between antipsychotic-treated and saline-treated whole-brain mRNA expression at P value less than 0.05.

syndrome (Waddington, 1990; Coccurello *et al.*, 2006). Our intermediate time point will not reflect long-term changes in gene expression that may occur with the chronic administration of APDs to schizophrenia patients yet it may provide novel insights into the molecular mechanism of therapeutic action at 1 week.

In this study, we chose to pool the RNA from genetically identical mice that had undergone the same treatment onto a single microarray chip. Pooling, particularly with equal contribution from each RNA sample as in this study, is a statistically valid method for maximizing power whereas minimizing the cost of microarray analysis (Peng *et al.*, 2003). We used whole-brain tissue in this microarray study as this provides an unbiased approach to screen for major changes in gene expression pertaining to a disorder without a definitive neuropathology and also allows for the widespread nature of neuronal interaction. Selection of whole-brain tissue may lead to more false negatives; with real changes in expression in one region diluted by minimal changes in other regions or by opposing changes in different regions leading to no observable change in the whole-brain tissue. We also chose to use average fold-change method for identifying differentially expressed genes, which is a useful way of determining biologically relevant microarray changes, yet does not provide a significant estimate for observed changes (Breitling *et al.*, 2004).

This transcript profiling study revealed many genes altered by treatment with individual drugs in our animal model as well as transcripts that were regulated by multiple APDs. An obvious disparity in the number of downregulated transcripts for each treatment group with clozapine having very few altered transcripts compared with haloperidol and clozapine is observed. This may be

Fig. 3



Western blots of (a) $K_v1.1$ protein (about 57–59 kDa) (b) KCHIP3 protein (about 29 kDa) and (c) NEDD4 protein (about 115 kDa) in whole-brain protein isolates. *i/*, Immunoblots showing bands for proteins of interest in control and antipsychotic drug-treated animals. β-actin (about 42 kDa) was used to control equal protein loading. *ii/*, Graphs showing mean protein expression in control ($n=4$) and antipsychotic drug-treated animals ($n=6$/group) normalized to β-actin protein expression. *Significant change between antipsychotic-treated and saline-treated whole-brain protein level at P value less than 0.05.

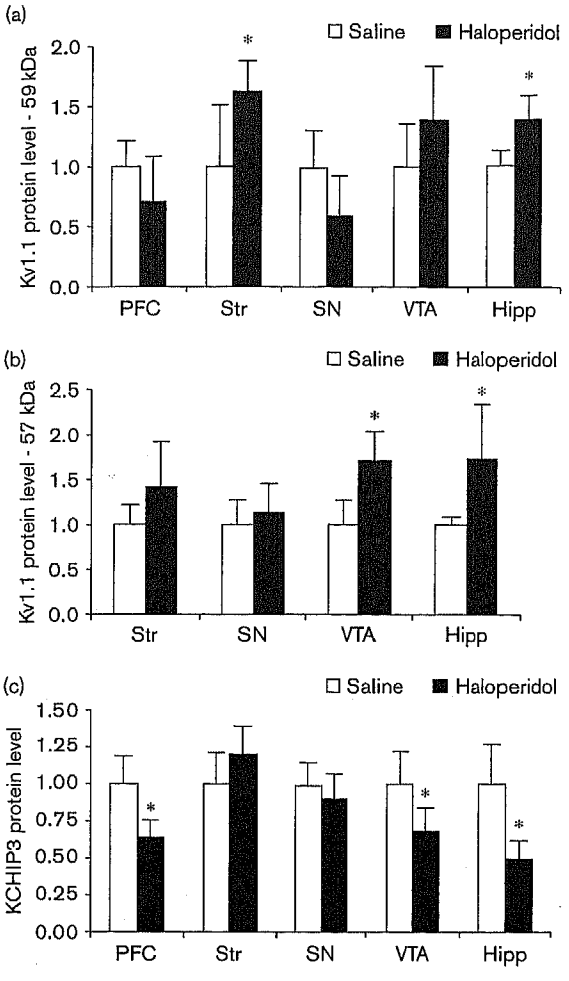
a result of the dose used, which was mid-range for clozapine (10 mg/kg), high-range for haloperidol (1 mg/kg) and maximal range for olanzapine (10 mg/kg) compared with other mouse antipsychotic treatment studies (Schreiber *et al.*, 1999; Chong *et al.*, 2002; Kontkanen *et al.*, 2002; Wang *et al.*, 2004; MacDonald *et al.*, 2005; Sondhi *et al.*, 2005). Despite this, we have identified many transcripts downregulated by multiple APDs. Interestingly, more transcripts were upregulated by both atypical APDs (clozapine and olanzapine) than between either/both atypical APDs and the typical APD haloperidol. These results may provide evidence that atypical APDs share common mechanisms in addition to their receptor-binding profiles.

From the list of coregulated genes, some were chosen for further validation based on their mRNA and protein expression patterns, biological function, and whether their human homolog is located within a chromosomal region earlier linked to schizophrenia susceptibility (Lewis *et al.*, 2003). One or more APDs produced a statistically significant change in gene expression in 65% of transcripts examined using QPCR analysis, although the alterations were not always consistent with array results. This is to be expected given that microarray analysis is associated with a high rate of false positives as well as false negatives (Mirnics *et al.*, 2006). The gene validation rate is consistent with false discovery rate estimation of 30% and is typical for similarly performed studies (Kontkanen *et al.*, 2002; Chen and Chen, 2005; MacDonald *et al.*, 2005; Fatemi *et al.*, 2006).

Some of the genes that were regulated by APD treatment in this study have been earlier implicated in similar studies or in studies of postmortem tissue from schizophrenia patients (Table 4) (Higgs *et al.*, 2006; Elashoff *et al.*, 2007). In particular, the expression of *Dclk1*, *S100a9* (Calgranulin B), *Bat2*, *KChip3*, and *Kcnab1* in post mortem brain tissue has been shown to be dependent on medication status in patients and this study provides additional evidence for their dysregulation by APDs. Furthermore, both *Kcna1* and *S100a9* have earlier shown similar regulation by chronic APD treatment in animals (Sondhi *et al.*, 2005; Fatemi *et al.*, 2006) as we see in this intermediate treatment study. Acute (24-h) APD treatment also revealed alterations in *Mal*, *KChip3*, and *Kcnab1* (Le-Niculescu *et al.*, 2007).

It is well recognized that gene expression changes from microarray studies do not necessarily translate into protein changes, with concordance being particularly low in brain tissue (Mirnics *et al.*, 2006). From the list of validated dysregulated genes, we chose *Kcna1*, *KChip3*, and *Nedd4* for further expression analysis, as they had verified regulation by multiple APDs and had commercial antibodies for their protein products available. By western blot analysis of whole-brain lysates, we were able to confirm translation of gene expression changes into significant protein expression changes after haloperidol

Fig. 4



Graphs showing quantification from western blots of specific regions in the brains of mice treated with saline or haloperidol for 7 days. Values represent an average expression of six mice per treatment group, normalized to β-actin, expressed as a ratio to total normalized saline-treated protein amount (a) $K_v1.1$ regional protein expression of a 59 kDa protein from Triton X-100-extracted lysates (b) $K_v1.1$ regional protein expression of a 57 kDa protein from Triton X-100-extracted lysates (c) KCHIP3 regional protein expression of a dimer band (about 56 kDa) in sodium dodecyl sulfate-extracted lysates. Hipp, hippocampus; PFC, prefrontal cortex; SN, substantia nigra; Str, striatum; VTA, ventral tegmental area. *Significant change between haloperidol-treated and saline-treated regional protein levels at P value less than 0.05.

Table 3 Abundance of $K_v1.1$ and KCHIP3 in mouse brain regions analyzed by western blot with protein levels normalized to β-actin expression

Protein	PFC	Str	SN	VTA	Hipp
$K_v1.1$					
59 kDa band	1.8	1.1	2.7	0.5	2.0
57 kDa band		0.4	1.8	0.6	0.3
KChip3					
27 kDa monomer		0.7	1.4	1.0	0.6
55 kDa dimer	0.5	1.7	1.9	0.2	0.2

Hipp, hippocampus; MWt, molecular weight; PFC, prefrontal cortex; SN, substantia nigra; Str, striatum; VTA, ventral tegmental area.

($K_v1.1$ and KCHIP3) and olanzapine (KCHIP3 and NEDD4) treatment.

Additionally, we examined the region-specific changes for two of these proteins, $K_v1.1$ and KCHIP3, after haloperidol treatment as this was the treatment group with the most consistent changes in analysis of whole-brain tissue. We chose to focus on brain regions important in dopamine transmission as all APDs target dopamine D2 receptors (Seeman *et al.*, 1975). Specifically, we looked at the origin of dopamine neurons in the midbrain, separated into substantia nigra and ventral tegmental area, and the regions of projection of dopamine neurons – the striatum and prefrontal cortex. We also examined the hippocampus as it is a major contributor to whole-brain measurements and has been implicated in schizophrenia pathogenesis (for review, see Harrison, 2004). We observed regional differences in expression of $K_v1.1$ and KCHIP3 in control animals in our brain regions of interest. Both proteins were robustly expressed in the substantia nigra (when protein amount was normalized), suggesting that they have a direct role in dopaminergic neurotransmission. Regional differences in the regulation of $K_v1.1$ and KCHIP3, and their multiple molecular weight species, by haloperidol were also observed. After confirming increased expression of a dimer of $K_v1.1$ in whole-brain lysates, we attempted to localize these changes in a more region-specific manner. We found the two molecular weight $K_v1.1$ species to be differentially extracted, as the higher molecular weight band was less readily soluble. This is consistent with the higher molecular weight species being the palmitoylated form of the $K_v1.1$ protein, which is more tightly associated with the lipid membrane, as suggested earlier (Deal *et al.*, 1994; Gubitosi-Klug *et al.*, 2005). Differential increases in protein expression after haloperidol treatment were seen between the two molecular species which may indicate that both pretranslationally and posttranslationally modified $K_v1.1$ may be important in the action of APDs.

This regional protein analysis also revealed alterations in a band corresponding to the KCHIP3 dimerized protein, with no change in the monomer form. KCHIP3 is also known as Dynorphin response element antagonist modulator and calsenilin and diverse functions have been ascribed to each of these pseudonyms in the mammalian brain. Yet it is the dimerized form of the KCHIP3 protein that is believed to associate with potassium channel subunits (Osawa *et al.*, 2001), so it is this potassium channel regulatory function that may be targeted by APDs.

Role for voltage-gated ion channels in antipsychotic drug effects

The specific effects of multiple APDs on four genes regulating voltage-gated ion channels – *Kcna1*, *Kcnab1*, *KChip3* and *Nedd4* – may indicate a role for these neuronal regulators in APD action.

Table 4 Previous evidence for dysregulation of verified candidate genes in this study by APD treatment and/or in postmortem brain tissue

Gene	Previous animal APD treatment studies	Human postmortem brain analysis[a]		
		mRNA expression change	Psychiatric drug effect	Other studies
Genes upregulated in this study				
Kcna1	↑ chronic clozapine treatment (Sondhi *et al.*, 2005); atypical APD pimozide blocks Kv1.1 (Zhang *et al.*, 2003)	↓ DLPFC in BP and SZ patients (Bahn)	Lithium ↓ in BP	
Dclk1	–	↓ DLPFC in BP (Stanley consortium)	Lifetime APD ↓ in BP; valproate ↓ in SZ; lithium ↑ in SZ	
S100a9	↑ chronic olanzapine treatment (Fatemi *et al.*, 2006)	↑ DLPFC in BP (Stanley consortium), ↑ in SZ (Stanley array)	Lifetime APD ↑ in SZ and BP	↓ CSF in SZ (Poltorak *et al.*, 1995); ↑ blood in SZ (Tsuang *et al.*, 2005); no change blood in SZ (Yao *et al.*, 2008)
Genes downregulated in this study				
Rapgef4	–	↓ DLPFC in BP and MD (Altar), ↑ DLPFC in BP and MD (Altar)	MS ↑ in SZ, lithium ↓ in BP	
Mal	↓ acute clozapine and PCP treatment (Le-Niculescu *et al.*, 2007)	↓ DLPFC in SZ (Hakak *et al.*, 2001), ↓ DLPFC in BP and MD (Altar), ↑ DLPFC in SZ (Stanley array)	No effect found	↓ blood in BP (Middleton *et al.*, 2005)
Nedd4	–	↓ DLPFC in BP (Sklar)	MS ↑ in SZ	
Bat2	–	↓ DLPFC in BP (Altar), ↑ DLPFC in BP (Altar)	Lifetime APD ↑ and AD ↓ in SZ	
β-catenin	APDs ↑ protein in rat midbrain, hippocampus, prefrontal cortex and striatum (Alimohamad *et al.*, 2005a,b); Hal ↑*in vitro* (Sutton *et al.*, 2007)	↑ DLPFC in BP and MD (Altar)	No effect found	↓ cytosolic protein in hippocampus in SZ (Cotter *et al.*, 1998); No change PFC in SZ (Beasley *et al.*, 2001)
Kchip3	↑ acute clozapine and PCP treatment (Le-Niculescu *et al.*, 2007)	↓ DLPFC in BP and MD (Altar)	Lifetime APD ↑ in BP	
Kcnab1	↑ by acute clozapine, ↓ by acute PCP treatment (Le-Niculescu *et al.*, 2007)	↓ DLPFC in BP (Stanley array and consortium), ↓ DLPFC in SZ (Stanley array)	APD, MS ↓ in BP; APD, MS, lithium ↑ in SZ; valproate ↓ in SZ	
Cspg3	–	↓ DLPFC in MD (Altar)	No effect found	
Numbl	–	↓ DLPFC in BP (Dobrin), ↑ DLPFC in MD (Altar)	No effect found	

↓, decreased expression; ↑, increased expression; AD, antidepressants; APD, antipsychotic drugs; BP, bipolar affective disorder patients; DLPFC, dorsal lateral prefrontal cortex; MD, major depression patients; MS, mood stabilizers; PCP, phencyclidine; SZ, schizophrenia patients.
[a]Stanley Medical Research Institute online genomics database of a meta-analysis undertaken on 12-array studies using the consortium collection containing 15/15/15 SZ/BP/MD brains and array collection containing 35/35/35 SZ/BP/MD brains.
Name in brackets indicates specific study where a change was found if not in the array or consortium meta-analyses

Voltage-gated potassium (K_v) channels modulate the electrical activity of neurons and are comprised of pore-forming α-subunits and auxiliary β-subunits that regulate K_v channel activity (Rettig *et al.*, 1994). In this study we found APD regulation of two genes encoding subunits of the K_v1 channel: potassium voltage-gated channel, shaker-related subfamily, member 1 (*Kcna1*), and beta member 1 (*Kcnab1*). *Kcna1* encodes $K_v1.1$, one of seven α subunits that is highly expressed in caudal regions of the adult rat brain, particularly the midbrain, cerebellum, cerebral cortex, and hippocampus (Beckh and Pongs, 1990). *Kcnab1* encodes three isoforms of which $K_vβ1$ is the major species in the rodent nervous system. *Kcnab1* is highly expressed in the hippocampus and caudate putamen as well as in the thalamic nuclei, neocortex, and cerebellum (Rettig *et al.*, 1994). Immunohistochemical experiments reveal that $K_vβ1$ and $K_v1.1$ colocalize in neurons of the rat brain, particularly in the molecular layer of the dentate gyrus and interneurons in CA1–CA3 hippocampal fields (Rhodes *et al.*, 1997). $K_vβ1$ and $K_v1.1$ interact *in vitro* and coinjection of these subunit mRNAs in *Xenopus* oocytes showed that this interaction results in modulation of the K_v channel gating properties (Rettig *et al.*, 1994).

Both molecular weight species of $K_v1.1$ protein were increased in the hippocampus, where $K_v1.1$ has earlier been implicated as a factor involved in neurogenesis (Almgren *et al.*, 2007). This is particularly interesting with accumulating evidence for altered adult neurogenesis in schizophrenia (Barbeau *et al.*, 1995; Reif *et al.*, 2006), although the possible effect of APD treatment on neurogenesis remains controversial (Halim *et al.*, 2004; Toro and Deakin, 2007).

This study also found alterations in *KChip3*, with decreased mRNA and protein localized to the ventral tegmental area, hippocampus, and prefrontal cortex, after 7 days of APD treatment. KCHIP3 is the third annotated member of a family of K_v channel-interacting proteins that modulate K_v4 α-subunits and increasing A-type K_v currents that are important in neuronal excitability (An *et al.*, 2000). Furthermore, there is evidence to suggest a direct correlation between *KChip3* mRNA expression

levels in dopaminergic neurons in the substantia nigra and A-type potassium channel densities that control spontaneous electrical activity of these midbrain dopaminergic neurons (Liss *et al.*, 2001). Given the various neuronal functions of KCHIP3, its dysregulation by multiple APDs could have many effects in the brains of APD-treated mice. These regional data suggest that KCHIP3 dysregulation by haloperidol may play a role in altering mesocortical dopamine neuronal firing, which is believed to be dysfunctional in schizophrenia and may underlie cognitive deficits (Abi-Dargham, 2004). Additionally, although KCHIP3 already has low levels of expression in wild-type mice it is further downregulated in the hippocampus by haloperidol treatment.

Nedd4 mRNA and protein were decreased in whole mouse brain by 7-day APD treatment in this study. *Nedd4* is highly expressed in granular cells of the olfactory bulb, cerebellum (Kumar *et al.*, 1997), hippocampus, and cortical layers II, IV in adult mouse brain (*www.brain-map.org*). NEDD4 interacts with some of the pore-forming α-subunits of neuronal voltage-gated sodium channels (Na_v) in *Xenopus* oocytes, resulting in Na_v channel inhibition, which is dependent upon the enzymatic activity of NEDD4 (Fotia *et al.*, 2004). Na_v channels are crucial for neuronal cell development and plasticity as they control action potential initiation in excitable membranes (Catterall, 2000).

Part of the mechanism of action of currently prescribed APDs may be the modulation of neuronal voltage-gated ion channels, in particular potassium and sodium channels, which are integral for neuronal electrical activity and neurotransmission (Trimmer and Rhodes, 2004). This modulation has been explored earlier using electrophysiological techniques (Ogata *et al.*, 1989; Zhou *et al.*, 2006). Of particular relevance is the inhibition of the $K_v1.1$ channel current upon treatment of Chinese Hamster Ovary cells with pimozide, a typical APD (Zhang *et al.*, 2003). This is contradictory to our findings of an upregulation of $K_v1.1$ mRNA and protein after APD treatment. When coexpressed with $K_v\beta1$ the K_v1 current inhibition was reversed (Zhang *et al.*, 2003). These data suggest this may be because of opposing regulation of these subunit gene expressions by APDs. Electrophysiological studies with haloperidol have found a suppression of Na currents (Ito *et al.*, 1997) and currents from $K_v1.1$ and $K_v1.4$ channels in rat ganglion cells (Akamine *et al.*, 2002), the latter of which would be consistent with decreased *KChip3* mRNA expression. A separate study in *Xenopus* oocytes, however, found minimal inhibition of these channel subtypes by haloperidol (Suessbrich *et al.*, 1997).

In summary, 7-day treatment of mice with one of three APDs and subsequent transcript profiling of their brain tissue has revealed regulation of genes involved in various biological functions. In particular, the regulation of genes by multiple APDs may indicate antipsychotic-specific effects on neuronal voltage-gated ion channels. Although APDs have earlier been associated with the modulation of voltage-gated ion currents, this study is the first to investigate regulation of channel gene and protein expression by these compounds. Further molecular studies are required to explain the role of voltage-gated ion channels, crucial for neurotransmission, in APD activity. In particular, further investigation into the regional and cellular localization of these changes, as well as their electrophysiological implications, may reveal aspects of the mechanism of action of APDs in modulating psychosis.

Supplementary data

Supplementary data are available at *The Psychiatric Genetics Journal Online* (*http://www.psychgenetics.com*).

Acknowledgements

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