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SCIENCE DIRECT

Molecular Brain Research 140 (2005) 142 – 149

MOLECULAR BRAIN RESEARCH

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Short Communication

Gene expression profiling in whole cerebral cortices of phencyclidine- or methamphetamine-treated rats

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Accepted 18 July 2005
Available online 24 August 2005

Abstract

Both phencyclidine (PCP) and methamphetamine (MAP) can cause schizophrenia-like symptoms. To identify the molecules relating to the drug-induced psychotic state, we used serial analysis of gene expression in rodent cerebral cortices isolated 1 h after intraperitoneal injection of saline, PCP (10 mg/kg), or MAP (4 mg/kg). We analyzed a total of 150,000 tags and found significantly up- or down-regulated genes. The number of MAP-, PCP-, and MAP and PCP-reactive tags were 229, 215, and 41, respectively.

Theme: Disorders of the nervous system
Topic: Neuropsychiatric diseases

Keywords: Phencyclidine; Methamphetamine; Schizophrenia: serial analysis of gene expression (SAGE); Cerebral cortex

In humans, an acute administration of phencyclidine (PCP), an NMDA receptor antagonist, causes both positive-like symptoms (hallucinations, delusions, bizarre behavior, and thought disorder) and negative-like symptoms (affective flattening, alogia, apathy, and asociality) of schizophrenia [27]. Methamphetamine (MAP), a dopamine transporter inhibitor, mainly induces positive-like symptoms in the acute phase [10]. Sometimes, these symptoms after single administration persist for 1 month, which is longer than serum half-life (MAP = about 13 h, PCP = about 1 h) and urinary positive period (MAP = about 5 days, PCP = about 2 weeks) [1,14]. Thus, these symptoms are thought to be caused not only by direct effects of these drugs but also by continuing organic changes containing gene expression alterations. In order to widely figure out acute pathological conditions and to identify the molecules which might be related to PCP- and/or MAP-induced psychotic state, we investigated gene expression profiles in cerebral cortices of rats injected with PCP or MAP by serial analysis of gene expression (SAGE) [23,26].

Nine male Wistar rats (8 weeks old, 180–210 g) (Japan SLC Inc., Hamamatsu, Japan) were used in this study. The study was conducted in accordance with guidelines for the care and use of laboratory animals of Tohoku University School of Medicine and the NIH guidelines on animal care (NIH Publications No. 80-23) revised 1996. For drug treatment, rats were injected intraperitoneally with saline [0.9% (w/v) NaCl, 1 ml/kg, $n = 3$], MAP (4 mg/kg, $n = 3$), or PCP (10 mg/kg, $n = 3$) during the light period of a 12:12-h light/dark cycle. MAP (Dainippon, Osaka, Japan) and PCP were dissolved in saline. PCP was generously provided by Prof. T. Nabeshima (Nagoya University Graduate School of Medicine, Nagoya, Japan). Rats were decapitated 1 h after the injection. The intact cerebral cortices were rapidly isolated on ice [6] and pooled. The total RNAs were isolated by using Isogen (Nippon Gene, Tokyo, Japan). Poly(A)$^+$ RNAs (7.5 μg) were further isolated from 150 μg of total RNA with the Dynabeads mRNA Isolation Kit (Dynal Biotech, Oslo, Norway). Poly(A)$^+$ RNAs were converted to double-stranded complementary DNA (cDNA) with a BRL

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doi:10.1016/j.molbrainres.2005.07.011

cDNA synthesis kit (Gibco BRL, Rockville, MD) using 5′ biotinylated oligo(dT)$_{20}$ (Roche Molecular Biochemicals, Mannheim, Germany) as a primer. The remaining procedures for SAGE were performed according to the Detailed Protocol version 1.0 d, and data were analyzed with SAGE software 4.0.0.0, both of which were generously provided by K.W. Kinzler (Johns Hopkins University, Baltimore, MD). For identification of corresponding genes, a SAGE tag database file ("SAGEmap_tag_ug-rel", UniGene Build #116) was downloaded from the NCBI FTP site (ftp://ftp.ncbi.nih.gov/pub/sage/map/Rn/NlaIII/) and linked to our SAGE data. All procedures were conducted according to the manufacturer's protocols. We used the SAGE analysis software to count the frequency of each tag. The software was also used to calculate the *P*-chance values with a Monte Carlo simulation method [26].

SAGE libraries were constructed from cerebral cortices of saline-, PCP-, or MAP-injected rats. The numbers of total tags were 49,918, 50,567, and 50,517, respectively. After excluding linker-derived sequences, 49,188, 49,809, and 50,174 tags remained. We identified 18,636, 18,640, and 18,926 unique tags, respectively. We then compared the drug-treatment libraries to the saline library and calculated *P*-chance values and ratios with SAGE software. Classified by major functions of corresponding genes, we found 229 MAP-reactive, 215 PCP-reactive, and 41 both MAP- and PCP-reactive tags with significant changes ($P < 0.05$). Tables 1–5 show a part of them, excepting tags that correspond to ESTs, match with multiple genes or do not match with any gene. (Complete data are shown in Supplemental tables.)

To confirm the validity of our SAGE results, we examined expression of two housekeeping genes (α-tubulin and ornithine decarboxylase; ODC) and 12 genes randomly chosen from Tables 1–4 (indicated in boldface). RNAs from the same pooled samples were used. All procedures for synthesis of the first-strand cDNAs were same as those in

Table 1
Genes up-regulated by PCP injection

Tag sequence	Saline	PCP	Ratio	Transcript (UniGene ID)
(A) Cell damage				
GAATAATAAA	42	78	1.8	Heat shock cognate protein 70 (Rn.3672)
(B) Cellular metabolism and energy production				
TGTCCCGGCT	0	5	9.9	β-4*N*-acetylgalactosaminyltransferase (Rn.10119)
AGCACTGCAG	1	9	8.9	*N*-myristoyltransferase 1 (Rn.830)
CCTGTGGATA	1	7	6.9	2-Oxoglutarate carrier (Rn.853)
AGTCTGCTAT	1	7	6.9	Guanine deaminase (Rn.24783)
AAGCTACTAT	3	11	3.6	Sterol carrier protein 2, liver (Rn.31887)
AACGAGGAGA	17	31	1.8	ATPase Na^+/K^+ transporting β1 polypeptide (Rn.8925)
TTCCAGCTGC	24	42	1.7	Phosphoglycerate mutase 1 (Rn.1383)
(C) Intercellular signaling				
TGTAATACAA	0	6	12	Granulin (Rn.5820)
ATTCAAAAA	0	5	9.9	Neuropeptide Y (Rn.9714)
GCCAACAATA	2	9	4.4	Nuclear receptor subfamily 2, group F, member 6 (Rn.25840)
TGCTCACACG	3	12	4	Neurofascin (Rn.3048)
ACTCCTGTCC	17	34	2	Thyrotropin-releasing hormone receptor (Rn.9962)
AATACGCAGA	21	37	1.7	Transthyretin (prealbumin, amyloidosis type I) (Rn.1404)
(D) Intracellular signaling				
ATCAATGAAG	0	8	16	Calmodulin 2 (phosphorylase kinase, δ) (Rn.5968)
GTGGGGAAAG	1	11	11	Ras homolog enriched in brain (Rn.859)
TGGGCATTGC	**0**	**5**	**9.9**	**Synaptojanin 2 (SYNJ2) (Rn.10868)**
AAGGGGGGCA	0	5	9.9	*Rattus norvegicus* mRNA for hnRNP protein, partial (Rn.54869)
AAACCGTGCT	**1**	**8**	**7.9**	**Microtubule-associated protein 6 (MTAP6) (Rn.37490)**
AGCCAGGGCT	**2**	**13**	**6.4**	**Brain-specific angiogenesis inhibitor 1-associated protein 2 (BAIAP2) (Rn.13017)**
ATTTAGTGTG	2	9	4.4	Calmodulin 1 (phosphorylase kinase, δ) (Rn.4166)
TACTTGTGTT	5	18	3.6	Stromal cell-derived factor receptor 1 (Rn.37476)
TTTGTGACTG	5	15	3.0	C-terminal-binding protein 1 (Rn.3946)
TACGGAGTAT	7	17	2.4	Glial fibrillary acidic protein (Rn.91512)
TTGCTGTTGA	39	60	1.5	Calmodulin 2 (phosphorylase kinase, δ) (Rn.5968)
(E) Others				
GCTTCAGAGA	3	10	3.3	Siah-binding protein 1; FBP interacting repressor; pyrimidine tract binding splicing factor; Ro ribonucleoprotein-binding protein 1 (Rn.21553)
TAACTTTAAG	23	40	1.7	Olfactomedin related ER localized protein (Rn.11005)
CTCTGACTTT	23	38	1.6	Basigin (Rn.2269)