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Research Report

Nicotinic modulation of gene expression in SH-SY5Y neuroblastoma cells

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dTC, D-tubocurarine
nAChR, nicotinic acetylcholine receptor(s)
RT-PCR, reverse transcription-polymerase chain reaction
qRT-PCR, quantitative real-time RT-PCR

ABSTRACT

Exposure to nicotine has a broad range of physiological and psychological effects that can be long lasting and contribute to nicotine dependence. On a time course longer than that needed to activate nicotinic acetylcholine receptor (nAChR) function, nicotine exposure induces functional inactivation of nAChR, upregulation of nAChR radioligand binding sites, and other alterations of cellular functions. To identify possible mechanisms underlying nicotine-induced changes in nAChR numbers and function, we defined changes in gene expression in neuron-like, SH-SY5Y human neuroblastoma cells following 24 h of continuous exposure to 1 mM nicotine. This treatment condition produces both functional inactivation and upregulation of nAChR. Repeat and cross-controlled microarray (~5000 genes queried) analyses revealed 163 genes whose expression was consistently altered at the $p<0.01$ level following nicotine treatment. Quantitative, real-time, reverse transcription-polymerase chain reaction analyses confirmed altered expression of thirteen out of fourteen of these genes chosen for further study, including contactin 1, myozenin 2, and ubiquitin-conjugating enzymes E2C and E2S. Inhibition or reversal of these effects by the general nAChR antagonist, D-tubocurarine, indicated that gene expression changes are dependent on nAChR activation. Studies using other nAChR subtype-selective antagonists identified gene expression changes that required activation of both α7- and α3*-nAChR, α7-nAChR alone, or either α7- or α3β4*-nAChR, suggesting some convergent and some divergent pathways of gene activation coupled to these nAChR subtypes. These results suggest that longer-term physiological and psychological effects of nicotine exposure and changes in nAChR expression may be due in part to effects on gene expression initiated by interactions with nAChR.

1. Introduction

Nicotinic acetylcholine receptors (nAChR) exist as a family of diverse subtypes and are prototypes of the ligand-gated ion channel superfamily of neurotransmitter receptors (Jensen et al., 2005; Lukas and Bencherif, 2006). nAChR have been implicated in a variety of processes in the nervous system including attention and cognition (Levin and Simon, 1998), mood and emotion (Shytle et al., 2002), synaptic plasticity (Ji et al., 2001), and cell survival (Donnely-Roberts and Brioni, 1999). Some of these effects simply reflect nAChR mediation of transmembrane ion flux altering cellular electrical excitability (Lukas and Bencherif, 2006). However, evidence is emerging that at least some nAChR subtypes are engaged in

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intracellular signaling cascades with a broad range of consequences, such as changes in gene expression (Dajas-Bailador et al., 2002; Sharma and Vijayaraghavan, 2002; Brunzell et al., 2003; Dunckley and Lukas, 2003; Li et al., 2003, 2004; Sun et al., 2003).

Whereas nicotine exposure initially induces opening of nAChR ion channels, longer-term exposure leads to other changes. Seconds to hours of nicotine exposure induces nAChR functional desensitization and longer-lasting functional inactivation, whereas hours to days of nicotine exposure produce increases in numbers of nAChR-like radioliogand binding sites ("upregulation;" Gentry and Lukas, 2002). However, cell specificity of some of these effects suggests that non-nAChR proteins could be involved (Gentry and Lukas, 2002), and the fact that some of these effects occur on time-scales much longer than those involved in acute activation of receptor suggests engagement of other mechanisms in nicotine-induced changes in nAChR numbers and function.

We previously reported that 1 h of treatment with 1 mM nicotine acts through nAChR to alter expression of a select set of genes in the neuron-like, SH-SY5Y neuroblastoma cell line (Dunckley and Lukas, 2003). We postulated that some of these effects could contribute to the loss of nAChR function seen for exposure to nicotine under these conditions. In the current studies, we used cDNA microarray analyses to identify candidate genes in SH-SY5Y cells sensitive to 1 day of nicotine exposure and possibly involved in nicotine-induced upregulation of nAChR radioligand binding sites. We then used quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) analyses to validate microarray findings and to extend the study to an examination of the pharmacology and nAChR subtype specificity/selectivity of gene expression effects. The results show reproducible and significant effects on gene expression that may underlie some of the effects of nicotine exposure on nAChR numbers and function and perhaps on other nervous system processes.

2. Results

2.1. *Twenty four hours of nicotine exposure affects gene expression*

To determine the extent to which nicotine exposure, under conditions that produce both functional inactivation of nAChR and upregulation of nAChR-like radioligand binding sites, affects gene expression in neuron-like SH-SY5Y cells naturally expressing human $\alpha3^*$- and $\alpha7$-nAChR, we treated cells under control conditions or in the continuous presence of 1 mM nicotine for 24 h and determined gene expression levels using a cDNA microarray representing ~5000 unique genes. Based on our previous results investigating the effects of 1-hour nicotine exposure on gene expression (Dunckley and Lukas, 2003; Ke et al., 1998), we anticipated that chronic nicotine treatment would have subtle effects on gene expression in the SH-SY5Y cell line. For this reason, we performed reciprocal fluorescent labeling of the control and nicotine-treated mRNA populations in duplicate, yielding a data set consisting of four independently hybridized, cross-controlled microarrays (see Experimental procedures).

Using a significance cut-off value of $p<0.01$, we observed expression changes for 82 genes and found 163 genes with

Table 1 – Microarray based analysis of nicotine-mediated gene expression changes

Gene name	Genbank #	Array 1 Ratio	Array 2 Ratio	Array 3 Ratio	Array 4 Ratio	Average Ratio	SD	*p* value
cadherin-like 23, *CDH23*	R91296	1.61	1.36	2.10	1.55	1.66	0.32	0.001
Complement component 7, *C7*	AA598478	1.31	1.17	1.29	1.34	1.30	0.09	0.001
Contactin 1, *CNTN1*	R25234	1.15	1.19	1.35	1.24	1.23	0.09	0.01
Eukaryotic translation initiation factor 4A isoform 2	W21081	1.34	1.45	1.34	1.42	1.39	0.06	0.0001
Golgi autoantigen, golgin subfamily a, 4, *golgA4*	AA460981	1.56	1.42	1.82	1.49	1.57	0.17	0.0001
Hypothetical zinc finger protein *MGC2396*	R08755	1.47	1.40	1.61	1.57	1.51	0.10	0.0001
Myeloid/lymphoid or mixed-lineage leukemia 3, *MLL3*	N91302	1.69	1.10	1.02	1.29	1.27	0.29	>0.01
myozenin 2 (calcineurin-binding protein calsarcin-1)	AA064973	1.75	1.76	1.33	1.90	1.69	0.25	0.001
Ninein (GSK3B interacting protein), *NIN*	R16456	1.24	1.20	1.65	1.59	1.42	0.23	0.01
RAD50 homolog, *RAD50*	H99196	1.29	1.30	1.29	1.34	1.30	0.03	0.001
Son of sevenless homolog 1, *SOS1*	H643241	1.31	1.46	1.37	1.40	1.39	0.06	0.0001
Stress-induced-phosphoprotein 1, *STIP1*	AA487635	0.78	0.82	0.68	0.81	0.76	0.06	0.01
Ubiquitin-conjugating enzyme E2C, *UBE2C*	AA430504	0.85	0.88	0.75	0.85	0.83	0.05	0.01
Ubiquitin-conjugating enzyme E2S, *UBE2S*	AA464729	0.85	0.87	0.82	0.81	0.83	0.03	0.01
Glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*	H16958	1.06	1.01	0.75	0.87	0.92	0.14	>0.01

Microarray analyses reveal numerous, consistent alterations of gene expression following 24 h of chronic nicotine treatment. Microarray analyses were conducted as described in Experimental procedures to identify genes whose expression was changed relative to untreated control samples by 24 h of exposure to 1 mM nicotine. Indicated (columns left-to-right) are the names of nicotine-sensitive genes identified and subjected to further investigation, their Genbank accession numbers, expression ratios determined in each array study, the average ratio for gene changes across all four microarray hybridizations, the standard deviation of those averages (SD), and the significance value of the observed gene expression change. The expression ratio corresponds to the normalized value of fluorescence intensities of the nicotine-treated sample divided by that of the control sample. Therefore, a ratio greater than 1 indicates nicotine-induced upregulation of gene expression, whereas a ratio less than one indicates repression. The Genbank accession numbers correspond to those for the cDNAs printed on the microarray, not to the full-length mRNAs for each gene.