Nicotine Modulates The Expression Of A Diverse Set Of Genes In The Neuronal Sh-Sy5Y Cell Line

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Nicotine exposure can have long lasting effects on nervous system function, some of which must contribute to nicotine dependence. Up-regulation, an increase in numbers of radioligand-binding nicotinic acetylcholine receptors (nAChR), occurs on exposure to nicotine at high concentrations. To determine whether altered gene expression might account for long term changes and up-regulation following nicotine exposure, we assessed effects of 1 h of 1 mM nicotine exposure on alteration of gene expression in the neuron-like SH-SY5Y neuroblastoma clonal line. Repeat and cross-controlled microarray analyses yielded a list of 17 genes from the initially screened ~5,000 whose expression was consistently altered following nicotine treatment. Subsequent quantitative, real time reverse transcriptase PCR analyses confirmed altered expression in 14 of 16 genes tested. Further, the general nAChR antagonist, d-tubocurarine, blocked all but two of the observed changes in gene expression, indicating that these changes are dependent on nAChR activation. Use of other antagonists revealed that nAChR subtypes can differentially affect gene expression. The genes affected code for proteins that may be broadly categorized into four groups: transcription factors, protein processing factors, RNA-binding proteins, and plasma membrane-associated proteins. Our results suggest that nicotinic activation of nAChR may have a broad role in affecting cellular physiology through modulating gene expression.

Nicotine is known to affect the expression of several genes. Among these is the gene coding for tyrosine hydroxylase, which is involved in a rate-limiting step in catecholamine synthesis (5, 6), as well as genes involved in the regulation of food intake and energy expenditure, such as neuropeptide Y, orexins, and their receptors (7, 8). In addition, nicotine, like other substances of abuse, such as cocaine and alcohol, induces the expression of immediate early genes such as c-fos and junB in various brain regions (9–11). Nicotine also up-regulates the mRNA levels of c-fos and c-jun in the neuronal SH-SY5Y cell line. Because these immediate early genes function as transcription factors, their nicotine-mediated up-regulation suggests that nicotine may regulate the expression of additional genes in SH-SY5Y cells.

Nicotine activates the mitogen-activated protein kinase (MAPK) signaling pathway in a variety of tissues and cell types (12–15). Recent work indicates that nicotine also activates this signaling pathway in SH-SY5Y cells (16). Further, nicotine and MAPK signaling pathways affect many of the same cellular processes, such as cell survival and memory processing (1, 3, 17, 18).

Beyond the role of nicotine in activating the MAPK cascade and early immediate gene expression, little is known about the specific genes that nicotine may regulate. We therefore investigated the effects of nicotine exposure on gene expression in the SH-SY5Y cell line using a microarray-based approach to identify candidate nicotine-regulated genes. We show that nicotine, at a concentration that induces up-regulation of nAChR, a process that has been implicated in nicotine dependence and tolerance, affects the expression of a wide range of genes that code for proteins with seemingly diverse functions. Collectively, these results demonstrate that nicotine can modulate the gene expression profile of a neuron-like cell line and suggest that some of the cellular and physiological effects of nicotine may result from these nAChR-mediated effects.
Nicotine Consistently Alters the Expression of a Diverse Set of Genes—To assess the extent to which nicotine modulates gene expression in neuronal cells, we have performed analyses using a microarray containing cDNAs corresponding to ~5,000 different genes. These results provide the foundation for more exhaustive whole-genome screening. However, here we have focused on a smaller number to identify novel nicotine-regulated genes and firmly establish the extent to which nicotine alters the expression of these genes. For these analyses, the neuroblastoma-derived SH-SY5Y cell line was treated with 1 mM nicotine for 1 h. Treatment with 1 mM nicotine maximally alters the expression of these genes. For these analyses, the neuroblastoma-derived SH-SY5Y cell line was treated with 1 mM nicotine for 1 h. Treatment with 1 mM nicotine maximally alters the expression of these genes.
were added to the analysis. The genes identified and the significance of their sensitivity to nicotine exposure will be discussed in greater detail below. However, it is notable that there were increases in expression of only 3 of the 17 genes, whereas expression of the other 14 genes was repressed by nicotine treatment. Also notable was the low magnitude of these changes resulting from nicotine treatment, including 26–38% increases in gene expression for the three up-regulated genes and a greater than 2-fold decrease in expression for only one of the 14 down-regulated genes.

**Nicotine Alters Gene Expression through nAChR Activation**—We used a pharmacological approach both as another way to test the significance and specificity of changes in gene expression and, because nicotine readily crosses the cell membrane, to assess whether nicotine altered gene expression through nAChR-dependent or nAChR-independent pathways. In the course of these studies, we also obtained sample replicates and analyzed them by real time RT-PCR to confirm the results of the microarray surveys. The SH-SY5Y cells were treated with 1 mM nicotine alone, 100 μM d-TC (a general nAChR antagonist) alone, or the combination of 100 μM d-TC plus 1 mM nicotine. We then utilized quantitative, real-time RT-PCR using total RNA as the template to verify and replicate the gene expression changes observed in the microarray experiments (see “Materials and Methods”). Vimentin mRNA was chosen as the control for normalization because its expression level was unchanged in the microarray analyses (1.03 ± 0.31). In addition, subsequent RT-PCR experiments wherein the levels of vimentin mRNA were compared with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA showed that both mRNAs were present at comparable levels in all drug-treated samples relative to the control sample (Fig. 1 and Table II (GAPDH row)). Further, when normalized to the amount of total RNA in each sample, none of the drug treatments altered the expression of either GAPDH or vimentin. This result is consistent with previous studies using human coronary artery endothelial cells where GAPDH expression was unchanged in response to nicotine (23). These combined results indicated that vimentin mRNA expression was unaltered in response to nicotine.

The RT-PCR analyses confirmed statistically significant changes in expression of 14 of the 16 consistently altered genes that were tested (Table II; RAB6A and regulatory factor X-associated protein, RFXAP). Each primer pair was checked for specificity by melting curve analysis and agarose gel electrophoresis to ensure that only specific product was quantitated. Each primer pair was checked for specificity by melting curve analysis and agarose gel electrophoresis to ensure that only specific product was quantitated. Each primer pair was checked for specificity by melting curve analysis and agarose gel electrophoresis to ensure that only specific product was quantitated. Each primer pair was checked for specificity by melting curve analysis and agarose gel electrophoresis to ensure that only specific product was quantitated. Each primer pair was checked for specificity by melting curve analysis and agarose gel electrophoresis to ensure that only specific product was quantitated. 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Real time RT-PCR experiments were performed as described under “Materials and Methods.” Matrin3 (mRNA) and Contactin 1 (mRNA) samples represent reactions performed using the same samples that were used in the microarray hybridizations. All of the other results are from a replicate experiment wherein total RNA was isolated and used as the template for the RT reactions. Column labels are as follows: Nicotine, samples treated with 1 mM nicotine for 1 h; d-TC, samples treated with 100 μM d-tubocurarine for 1 h; d-TC + nicotine, samples treated with 1 mM nicotine and 100 μM d-tubocurarine for 1 h. The numbers in bold represent the average fold changes across multiple independent PCRs (from three to eight reactions). The standard deviation of the ΔK values (see “Materials and Methods”) was used to calculate a range of fold induction or repression for each sample to determine the reliability of the results. Each range column represents the range of values for the drug-treated sample to the left of that column.

| Gene name       | Nicotine | Range ± S.D. | d-TC | Range ± S.D. | d-TC + nicotine | Range ± S.D. |
|-----------------|----------|--------------|------|--------------|-----------------|--------------|
| Matrin3 (mRNA)  | -1.82<sup>a</sup> | -2.17/-1.52  | 1.52<sup>b</sup> | 1.36/1.72     | 1.88             | 1.40/2.51    |
| Contactin 1 (mRNA) | -2.76<sup>a</sup> | -3.32/-2.28  | 1.41  | 1.15/1.74    | 1.59             | 1.29/1.96    |
| Matrin3         | -1.58<sup>a</sup> | -1.89/-1.31  | 1.34  | -1.58/-1.13  | -1.63            | -2.35/-1.14  |
| FEZ1 variant 1  | -1.34<sup>a</sup> | -1.48/-1.32  | -1.75 | -2.60/-1.18  | -2.72<sup>b</sup> | -2.99/-2.46  |
| FEZ1 variant 2  | -1.69<sup>a</sup> | -2.07/-1.39  | -1.18 | -1.36/-1.03  | -1.30            | -1.66/-1.02  |
| TFPI2           | -1.57<sup>b</sup> | -1.85/-1.33  | -1.12 | -1.51/1.21   | -1.07            | -1.51/1.32   |
| Czor2           | -1.94<sup>a</sup> | -2.58/-1.44  | -1.33 | -1.51/-1.17  | -1.55<sup>b</sup> | -1.75/-1.37  |
| ZFR             | -2.61<sup>b</sup> | -3.20/-2.11  | -1.08 | -1.26/1.08   | -1.03            | -1.31/1.22   |
| PTPx            | -1.67<sup>b</sup> | -1.91/-1.46  | -1.01 | -1.18/1.16   | -1.16            | -1.55/1.14   |
| RAB6A           | 1.15      | 1.12/1.48    | 1.04  | -1.23/1.15   | -1.18            | -1.60/1.15   |
| PIG7/LITAF1     | -1.56<sup>a</sup> | -1.85/-1.31  | 1.04  | 1.13/1.19    | -1.01            | -1.44/1.40   |
| DLL3            | -1.48<sup>a</sup> | -1.75/-1.24  | 1.16<sup>b</sup> | 1.13/1.19    | -1.01            | -1.44/1.40   |
| DHFR            | -1.70<sup>b</sup> | -2.00/-1.45  | -1.48<sup>b</sup> | -1.79/-1.23  | -1.18            | -1.67/1.20   |
| RFXAP           | -1.15      | -1.40/1.05   | -1.01 | 1.18/1.16    | 1.09             | 1.02/1.17    |
| UBE3A           | -1.43<sup>a</sup> | -1.71/-1.21  | 1.28<sup>b</sup> | 1.20/1.38   | 1.44             | 1.09/2.27    |
| RBPB6           | -3.75<sup>b</sup> | -4.79/-2.95  | -1.03 | -1.18/1.12   | 1.09             | 1.02/1.17    |
| cDNA DKFZp564F112 | -1.86<sup>b</sup> | -2.06/-1.69  | -1.05 | -1.14/1.04   | 1.21<sup>b</sup> | 1.15/1.27    |
| EGR1            | 1.91<sup>a</sup> | 1.68/2.50    | -1.05 | -1.74/1.45   | 1.10             | 1.27/1.53    |
| GAPDH           | 1.00      | 1.05/1.06    | -1.01 | -1.07/1.04   | -1.02            | -1.06/1.03   |

<sup>a</sup> Significance at the p < 0.05 level as measured by paired t test.
<sup>b</sup> Significance at the p < 0.01 level.

**Fig. 2. The RT-PCR products are specific for each gene.** Each PCR generates only one product of the predicted size, indicating the specificity of the primers used. The specific genes targeted in the PCRs are indicated above the lanes. –nic, untreated control; +nic, samples treated with 1 mM nicotine for 1 h; d-TC, samples treated with 100 μM d-tubocurarine for 1 h; d-TC + nic, samples treated with 100 μM d-tubocurarine for 1 h. Quantitation of the amount of product was performed in real time as described under “Materials and Methods.” Calculated expression ratios for each drug-treated sample relative to the control sample are indicated beneath each lane. These values represent the average values over at least three PCRs.

In some cases, the modulation of gene expression observed using quantitative, real time RT-PCR was in the opposite direction to that observed in the microarray analysis (compare matrin3, FEZ1, TFPI2, and EGR1 in Tables I and II). Importantly, this apparent discrepancy does not reflect variation between the mRNA samples used for the microarrays and the replicate total RNA samples used for RT-PCR studies, because matrin3, which showed slight induction on nicotine exposure in the microarray experiments, was found in RT-PCR analyses of both mRNA and total RNA samples to be comparably repressed by nicotine treatment (Table II). Expression ratios were low for these four genes but still in the range for the 10 other genes for which RT-PCR and microarray studies showed concordance. Absolute expression levels were low (data not shown) for FEZ1, TFPI2, and EGR1 but not for matrin3. However, the 1.9-fold increase in EGR1 expression that is seen here in the quantitative, real time RT-PCR experiments with SH-SY5Y cells following 1 h of 1 mM nicotine treatment (Table II) is in concordance with results of another study showing 2.8-fold induction of EGR1 in PC12 cells following 1 h of 200 μM nicotine treatment (25). Although we cannot fully explain the differences between the microarray and RT-PCR results, we consider that findings from quantitative, real time RT-PCR analyses, especially when corroborated by antagonist sensitivity of the effects of nicotine, are more reliable than more raw microarray results. Therefore, we used RT-PCR findings for our interpretations if there was ambiguity in results from the two types of analyses. Nevertheless, our observations underline the importance of secondary verification of microarray results and indicate potential complications in deriving gene expression profiling conclusions based solely on microarray analyses.

**Nicotine Modulates the Levels of a Variety of mRNAs Coding for Plasma Membrane-associated Proteins**—The RT-PCR analyses confirmed that nicotine treatment subtly but significantly altered the levels of a variety of classes of mRNAs. Nicotine repressed the expression of mRNAs that code for plasma membrane associated proteins, including contactin 1 and protein-tyrosine phosphatase receptor α (PTPα). Interestingly, the contactin 1 protein physically interacts with PTPα (26) as well as with voltage-gated Na<sup>+</sup> channels (27, 28). These observations suggest interesting possibilities for roles of contactin and PTPα in nAChR up-regulation (see “Discussion”). Another interesting observation is that whereas nicotine exposure repressed expression, d-TC significantly induced UBE3A expression and showed a distinct trend to induction of contactin 1 expression (Table II). This induction was not reversed when nicotine and d-TC were used in conjunction, suggesting at least two possible
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Nicotine also alters the expression of two RNA-binding proteins, matrin3 and ZFR, potentially implicating nAChR in RNA processing events. The ZFR protein is essential for murine embryonic development (42). However, the human ZFR mRNA is highly expressed in adult brain (43), suggesting that this gene has important functions beyond development. Matrin3 is a member of a large family of RNA-binding proteins and is a nuclear matrix protein known to be involved in the nuclear retention of A-to-I edited mRNA (44). The potential role of these genes in nAChR function and in the physiological effects of nicotine is currently unclear. Regardless, repression of these mRNAs in response to nicotine suggests that nicotine may affect RNA metabolism.

Different nAChR Subtypes Have Varying Effects on Gene Expression—The SH-SY5Y cell line expresses a3, a5, a7, b2, and b4 nAChR subunits that assemble to form various a3*-nAChR subtypes or homomeric a7-nAChR (19, 45). To determine which nAChR subtypes mediate the observed gene expression changes, we treated SH-SY5Y cells with nicotine in the presence of either 1 M a-cobratoxin or 3 M mecamylamine. a-Cobratoxin is thought to be a specific ligand for nAChR containing a1 or a7 subunits. A 1 M dose of a-cobratoxin will specifically inhibit responses to nicotine of a7-nAChR expressed by SH-SY5Y cells. Although interactions of mecamylamine (or of d-tubocurarine) at non-nAChR targets cannot be entirely discounted, mecamylamine at low micromolar doses selectively inhibits nAChR containing a3 or a4 subunits. The mecamylamine inhibition profile for SH-SY5Y cells suggests that a3b4*-nAChR represent the primary a3*-nAChR subtype present (19). Mecamylamine at a concentration of 3 M would be expected to inhibit about 80% of the nicotinic responses of a3b4*-nAChR and only 20% of a7-nAChR responses to nicotine (46).

Results showed that a-cobratoxin blocked nicotine-mediated repression of C2orf2 and RBBP6 (Table III). This result suggests that specific activation of a7-nAChR is both necessary and sufficient to down-regulate both of these mRNAs. In contrast, none of the observed changes in C2orf2 or RBBP6 gene expression were specifically blocked by mecamylamine at a 3 M concentration. However, either a-cobratoxin or mecamylamine affected the nicotine-mediated repression of multiple genes. For contactin1, MLL3, UBE3A, ZFR, and DHFR, both a-cobratoxin and mecamylamine prevented nicotine-dependent repression of gene expression. These results implied that simultaneous signaling through both a7-nAChR and a3*-nAChR subtypes was required for nicotine-dependent repression of these mRNAs. In addition, MLL3 showed a trend toward induction in response to the combination of mecamylamine plus nicotine.

Antagonist effects on nicotine repression or induction of other genes were more complex. Repression of PTPα, PIG7, and cDNA DFR2p564F112 (represented by GenBank access number N28268) were not blocked by either a-cobratoxin or mecamylamine. The combined set of observations that PTPα, PIG7, and N28268 repression are blocked when all nAChR subtypes are inhibited by d-TC (Table II) but not when a7- or a3b4*-nAChR are inhibited alone suggests that nicotine-mediated signaling through either receptor subtype is sufficient to modulate expression of these genes. However, it should be noted that we cannot rule out the possibility that the remaining 20% of functional a3b4*-nAChR in the presence of 3 M mecamylamine may be sufficient to repress these genes in response to nicotine. Alternatively, repression of these genes could occur through additional a3*- subtypes that are not inhibited by mecamylamine at a 3 M concentration.
There were instances where α-cobratoxin exposure alone affected mRNA expression comparably to the effects of nicotine (Table III). This effect of α-cobratoxin alone also complicates the interpretation of findings for contactin 1, MLL3, DHFR, and UBE3A, where a block of the effects of nicotine by α-cobratoxin was seen. Similarly, there were instances where mecamylamine exposure alone affected mRNA expression comparably to nicotine, thereby possibly explaining why mecamylamine did not block the effects of nicotine on C2orf2. Additionally, the effect of mecamylamine alone complicates the interpretation of findings for DHFR and UBE3A, where a block of the effects of nicotine by mecamylamine was seen. These results imply that the combined effects of agonist and antagonist acting at nAChR can have differing effects on gene expression than either agonist or antagonist acting alone. Further, assuming that there are no effects of these antagonists on other targets, these results suggest that changes in the conformation of the nAChR that result from antagonist or agonist binding may play a role in activating signaling pathways that ultimately result in alterations of gene expression.

**DISCUSSION**

**Microarray Analyses Can Elucidate Nicotine-dependent Changes in Gene Expression**—The current findings indicate that exposure of neuronal SH-SY5Y cells to nicotine at a concentration that produces maximal nAChR up-regulation has relatively subtle effects on the expression of a range of genes coding for proteins with diverse functions. These effects can be identified using a microarray-based approach provided that sufficient replicate, reciprocal labeling experiments are performed to separate the true responses from the background noise. The microarray approach is useful for generating hypothesis about what genes are affected by a given treatment. However, subsequent RT-PCR experiments are required to independently validate the microarray results, especially when there is a low test/control expression ratio or when mRNA levels are only slightly above background. Further validation of results using pharmacological studies also is suggested when possible. In this study, quantitative, real time RT-PCR experiments confirmed altered expression following nicotine exposure for 14 of the 16 genes tested base on microarray findings (Table II). Ten of these genes are altered in a manner consistent with the observed expression ratios from the microarray analyses. In contrast, RT-PCR experiments to detect the mRNAs for matrin3, FEZ1, TFFP2, and EGR1 showed that the expression levels of these genes were altered in a direction opposite to that observed in the microarray analyses. This finding could reflect low expression ratios (Table I) and, except for matrin3, low absolute levels of mRNA expression for these genes in SH-SY5Y cells (data not shown). Nevertheless, pharmacological studies indicated nicotinic receptor antagonist sensitivity of effects of nicotine on expression of 13 of the 14 genes confirmed by RT-PCR analyses. Collectively, the results of this study illustrate the utility of microarrays as screening devices. Moreover, the results also underscore the need for more comprehensive RT-PCR studies to validate and extend the results of microarray analyses. In addition, pharmacological approaches can be valuable to provide further verification and illumination of observations.

**Effects of Nicotine on Gene Expression Involve nAChR Activation**—Nicotine rapidly crosses the plasma membrane and therefore could affect gene expression either through a nAChR-dependent signaling pathway or through a nAChR-independent pathway. To distinguish between these possibilities, we determined whether nicotine could modulate gene expression when nAChR activity was blocked by the general nAChR antagonist d-TC. The critical observation suggesting that nAChR
Individual nAChR Subtypes Differentially Affect Gene Expression—The SH-SY5Y cells express homomeric α7-nAChR and heteromeric α3*-nAChR subtypes. Experiments wherein α7-nAChR and α3*-nAChR subtypes were differentially inhibited using either 1 μM α-cobratoxin or 3 μM mecamylamine yielded four important observations (see Table IV for a summary of antagonist studies in RT-PCR analyses). First, α-cobratoxin prevented the nicotine-dependent down-regulation of both C2orf2 and RBBP6 mRNAs. This result indicates that down-regulation of these mRNAs results exclusively from activation of the α7-nAChR. Second, both α-cobratoxin and mecamylamine blocked, reduced, or reversed the repression of matrin3, contactin1, TFP12, ML33, UBE3A, DHFR, and ZFR mRNAs (Table IV). This finding suggests that simultaneous nicotinic activation of both α7-nAChR and α3*-nAChR is required to repress these mRNAs. Third, neither antagonist prevented nicotine-mediated repression of the N28268 (cDNA DKK2 in Table IV) and PIG7 genes. However, d-TC, a general nAChR antagonist, prevented nicotinic effects on expression of these genes, suggesting that signaling through either α7- or α3*-nAChR is sufficient to affect expression of these messages. Nevertheless, we cannot currently rule out either of the possibilities that the 20% of active α3β4-nAChR that remain functional in the presence of 3 μM mecamylamine are sufficient to regulate these genes or that additional α3β subtypes may regulate these genes. Fourth, the three nAChR antagonists alone were able to alter the expression of some of the genes identified (see Tables II–IV for a summary of results). Although there is a formal possibility that antagonists could be affecting ongoing non-nAChR signaling that modulates gene expression, this result implies that there exists a nAChR-dependent pathway to repress gene expression that is independent of nAChR channel opening.

The assessment in this study of effects of nAChR antagonists alone should be, but is not, routine practice. Many studies showing the effects of antagonist plus agonist treatments, such as blockade of or failure to block nicotine agonist effects and synergy with nicotinic agonist effects, need to be replicated with concomitant assessments of antagonist effects alone to help elucidate the bases for ligand actions. Had our studies not examined the effects of antagonists alone, interpretation of the results would have been misleadingly simplified.

**Table IV**

| Gene Name | Nicotine | d-TC | cobt | mec | dTC | cobt | mec |
|-----------|----------|------|------|-----|-----|------|-----|
| Matrin3   | ↓        | ↑    |      |     |     |      |     |
| Contactin1| ↓        |      |      |     |     |      |     |
| TFP12     | ↓        |      |      |     |     |      |     |
| ML33      | ↓        |      |      |     |     |      |     |
| DHFR      | ↓        |      |      |     |     |      |     |
| UBE3A     | ↓        |      |      |     |     |      |     |
| C2orf2    | ↓        |      |      |     |     |      |     |
| RBBP6     | ↓        |      |      |     |     |      |     |
| PTP6      | ↓        |      |      |     |     |      |     |
| PIG7/TIATAF1| ↓    |      |      |     |     |      |     |
| cDNA DKK2 | ↓        |      |      |     |     |      |     |
| EGR1      | ↑        |      |      |     |     |      |     |

| Group 1   | Group 2   | Group 3   |
|-----------|-----------|-----------|
|            |           |           |

**What Are the Signaling Pathways Leading from nAChR Activation to Altered Gene Expression?**—Our data suggest that at least two initial nAChR-mediated signals can modulate gene expression. Although d-TC blocked the majority of the effects of nicotine on gene expression, the levels of several mRNAs were affected by d-TC alone, and this effect was not reversed when nicotine and d-TC were used in conjunction. For DHFR, d-TC repressed expression comparably to nicotine. For contactin1, matrin3, and UBE3A, d-TC elicited an increase in mRNA levels, whereas nicotine reduced mRNA levels. Additionally, α-cobratoxin and mecamylamine alone affected the expression of multiple genes. These observations suggest several possibilities. First, there may be at least two nAChR-dependent signaling pathways. One pathway may be dependent on channel opening and subsequent ion flow, and a second pathway may be activated by changes in nAChR conformation that result from either agonist or antagonist binding. In support of the first possibility, α7-nAChR are highly permeable to calcium, and in the SH-SY5Y cell, activation of α7-nAChR has been shown to activate the extracellular signal-regulated kinase 1/2 through a calcium-dependent mechanism (16). Nicotine has been shown to activate this pathway, which ultimately affects gene expression. In contrast, the observation that antagonists alone can significantly repress expression of some genes supports the view that alternative conformational states of the nAChR can affect the expression of some genes. Previous studies have not determined the effects of nAChR antagonists on activation of the MAPK pathway. It is therefore unclear whether mecamylamine, d-TC, or α-cobratoxin could activate the MAPK signaling pathway, thereby possibly explaining their effects on gene expression through a common signaling pathway.

**Could Changes in Contactin 1 mRNA Levels Be Involved in Nicotine-induced nAChR Up-regulation?**—One of the interesting aspects of nAChR function is the phenomenon of up-regulation. When SH-SY5Y cells are continuously exposed to nicotine, there is an early transient decrease in the total number of assembled, cell surface nAChR that display radioligand binding. However, numbers of total radioligand-binding sites increase immediately, reflecting an increase in intracellular pools (22). Over time, the decline in surface receptor numbers reverses, perhaps reflecting renewal and then later up-regulation of cell surface pools replenished from the increased intracellular pool of precursors. The mechanisms underlying this response are poorly understood.

Nicotine treatment significantly reduced the expression of the contactin 1 mRNA in as little as 1 h (Tables I and II and Fig. 2). Contactin physically interacts with many different proteins on the cell surface, including voltage-gated Na + channels (26, 27), protein-tyrosine phosphatase receptors (25, 47–49),...
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down-regulated following 1 h nicotine exposure, a time in which surface expression of NaChR is regulated. Consistent with this model, contactin mRNA would result in reduced surface expression of NaChR. Gated Na
expressions of nAChR in a manner similar to that of the voltage-gated Na channels, then one would predict that repression of contactin 1 mRNA would result in reduced surface expression of nAChR. Consistent with this model, contactin mRNA is down-regulated following 1 h of nicotine exposure, a time in which surface expression of nAChR is also significantly reduced. Interestingly, after 24 h of nicotine exposure surface nAChR have returned nearly to pretreatment levels (22). This correlates with a 1.4-fold up-regulation of contactin 1 mRNA following 24 h of continuous exposure to 1 mM nicotine (data not shown). Future work will be aimed at elucidating the potential role of contactin 1 in the regulation of nAChR function.

Summary—Nicotine exposure has reproducible, but sometimes relatively subtle, effects on gene expression in a neuron-like cell line. These gene expression changes can be classified into three general groups based on the effects of nAChR antagonists. Further, many of these effects are pharmacologically specific and appear to be mediated by traditional nAChR channel function. Other effects of nicotine on gene expression may result from alternative, yet nAChR-dependent, mechanisms. Our results demonstrate the utility of microarrays in this type of analysis to identify candidate genes where subtle changes in gene expression, as would be predicted to result from drug exposure, occur. Our results also highlight some of the caveats in interpreting the results from such an approach, emphasizing the importance of secondarily verifying consistent changes in gene expression. From these studies come tangible suggestions and targets for future investigation as to how nicotine affects gene expression in the nervous system, potentially adding to ways in which this drug exerts its physiologically relevant effects.

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