The Epigenetic Effect of Nicotine on Dopamine D1 Receptor Expression in Rat Prefrontal Cortex

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KEY WORDS epigenetic; nicotine; dopamine; histone acetylation; prefrontal cortex; DRD1

ABSTRACT Nicotine is a highly addictive drug and exerts its effect partially through causing dopamine release, thereby increasing intrasynaptic dopamine levels in the brain reward systems. Dopaine D1 receptor (DRD1) mRNAs and receptors are localized in reward-related brain regions, which receive cholinergic input. The aim of this study is to evaluate whether nicotine administration affects the expression of DRD1s, and if so, whether epigenetic mechanisms, such as histone acetylation, are involved. Twenty Male Sprague Dawley rats received nicotine (0.4 mg/kg/day, s.c.) or saline injections for 15 days. After nicotine/saline treatment, rats were perfused with saline; prefrontal cortex (PFC), corpus striatum (STR), and ventral tegmental area (VTA) were dissected. Homogenates were divided into two parts for total RNA isolation and histone H4 acetylation studies. DRD1 mRNA expression was significantly higher in the PFC of the nicotine-treated group compared with controls; similar trends were observed in the VTA and STR. To study epigenetic regulation, the 2kb upstream region of the *DRD1* gene promoter was investigated for histone H4 acetylation in PFC samples. After chromatin immunoprecipitation with anti-acetyl histone H4 antibody, we found an increase in histone acetylation by two different primer pairs which amplified the −1365 to −1202 ($P < 0.005$) and −170 to +12 ($P < 0.05$) upstream regions of the DRD1 promoter. Our results suggest that intermittent subcutaneous nicotine administration increases the expression of DRD1 mRNA in the PFC of rats, and this increase may be due to changes in histone H4 acetylation of the 2kb promoter of the *DRD1* gene. **Synapse 00:000–000, 2013.** © 2013 Wiley Periodicals, Inc.

INTRODUCTION

Dopamine plays a major role in reward pathways and mediates in addiction to drugs, including nicotine (reviewed by Balfour (2004)). Nicotine, a highly addictive drug, regulates the brain's reward system, as well as other psychomotor and cognitive processes, via nicotinic acetylcholine receptors (nAChRs) (reviewed by Wonnacott et al. (2005)). The nAChRs are expressed in the ventral tegmental area (VTA), striatum (STR), and frontal cortex (reviewed by Gotti et al. (2006)). Acute systemic nicotine injections increase dopamine outflow and dopaminergic activity throughout the reward pathways (Wise and Bozarth, 1987). This treatment increases the firing rates and bursting patterns of dopamine neurons in the VTA and substantia nigra pars compacta (Grenhoff et al., 1986); enhances dopamine outflow in the Nucleus Accumbens (NAc) (Di Chiara and Imperato, 1988; Imperato et al., 1986; Nisell et al., 1996; Pontieri et al., 1996; Schilstrom et al., 1998), dorsal STR (Benwell and Balfour, 1997; Janhunen and Ahtee, 2004), and frontal cortex (Liang et al., 2008; Tsukada et al., 2005); and augments locomotor activity (Kanyt et al., 1999; Leikola-Pelho and Jackson, 1992; Reavill and Stolerman, 1990). Systemic nicotine, administered acutely to rats, increases glucose metabolism, an index of local function, in some brain regions including the VTA (London et al., 1988). On the other

Contract grant sponsor: Ege University Research Fund Grant; Contract grant number: 2010-BAUM-001.

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Received 14 December 2012; Accepted 22 February 2013

DOI: 10.1002/syn.21659

Published online 28 February 2013 in Wiley Online Library (wileyonlinelibrary.com).

hand, chronic treatment with nicotine results in the sensitization of locomotor activity and dopamine release in the NAc (Benwell and Balfour, 1992; Benwell et al., 1995; Cadoni and Di Chiara, 2000; Le Foll et al., 2003; Lecca et al., 2006), dorsal striatum (Marshall et al., 1997), and prefrontal cortex (PFC; Nisell et al., 1996). Dopamine D1 receptor antagonists (Corrigall and Coen, 1991; Damaj and Martin, 1993) or mesolimbic dopaminergic system lesions (Clarke et al., 1988) block the locomotor stimulant effects of nicotine. Increased dopaminergic neurotransmission in the mesocorticolimbic system during chronic nicotine exposure may underlie the development of nicotine addiction (Balfour, 2004; Di Chiara, 2000).

The D1 receptor belongs to the D1-like dopamine receptor subfamily and is the most abundant type of dopamine receptor in the brain. DRD1 mRNAs and receptors are localized in reward-related brain regions that receive cholinergic input. VTA, NAc, dorsal STR, and PFC contain variable levels of D1-like dopamine receptors (Bergson et al., 1995; Gaspar et al., 1995; Lidow et al., 2003; Wong et al., 1999). D1 receptors expressed in these regions (Caine et al., 1995; McGregor and Roberts, 1993; Ranaldi and Wise, 2001) are implicated in the reinforcing and rewarding properties of psychostimulants, including nicotine (Spina et al., 2006). Also, D1 receptors in the NAc and dorsal STR control motor behavior (David et al., 2005). Dopaminergic transmissions in the NAc and dorsal STR are involved in the formation of conditioned reinforcement and habit learning, respectively, both of which lead to the compulsive drug-seeking and drug-taking behavior during addiction (Everitt and Robbins, 2005). Additionally, D1 receptors expressed in the PFC have important roles in higher cognitive functions such as working memory (Seamans and Yang, 2004). Selective inhibition of D1 receptors by administering a D1 antagonist significantly decreases nicotine self-administration in rats (Corrigall and Coen, 1991), and blocking D1 receptors in the NAc shell hinder the acquisition of nicotine-induced conditioned place preference (CPP) (Spina et al., 2006). These findings suggest that D1 receptors are involved in the reinforcing properties of nicotine (Le Foll et al., 2009). Similarly, in humans, dopamine D1 receptor polymorphisms are associated with nicotine dependence (Huang et al., 2008).

Recent studies suggest that epigenetic mechanisms may have a role in drug addiction (Renthal and Nestler, 2008), for example, through covalent modification of histones in nucleosomes. Modification through histone acetylation allows the transcription initiation machinery and gene regulatory proteins to bind to the DNA (Alberts et al., 2002). To date, few studies have investigated the possible effects of nicotine on histone acetylation (Landais et al., 2005; Levine et al., 2011; Pastor et al., 2011). To the best of our knowledge, the effects of nicotine treatment on histone acetylation at the *DRD1* gene have not been evaluated.

In this study, we aimed to determine the regulation of the dopamine D1 receptors by nicotine, considering that this may be one of the neurobiological substrates that mediate nicotine addiction. We applied repeated intermittent nicotine or saline injections daily, for 15 days. We planned our study in two stages: first, we investigated the effects of nicotine exposure on DRD1 mRNA expression in rat STR, PFC, and VTA, using quantitative real-time PCR (qPCR). Based on our findings, in the second stage, we studied the possible effects of intermittent nicotine treatment on histone H4 acetylation in the *DRD1* gene promoter in the most significantly regulated brain area, employing chromatin immunoprecipitation (ChIP).

MATERIALS AND METHODS

Animals

Adult male (250–300 g) Sprague Dawley rats ($n = 20$, 10 nicotine treated) were housed (3–4 rats/cage) with food and water provided ad libitum. Animals were maintained on 12:12 h light:dark cycle (lights on from 07:00 to 19:00). Manipulations of the rats were performed under the rules of the Institutional Animal Ethics Committee of Ege University, Izmir, Turkey, complying with the European Communities Council Directive (2003/003) and guided by the "International Guiding Principles for Biomedical Research Involving Animals" developed by the Council for International Organizations of Medical Sciences (NIH).

Animals were divided into two groups receiving either saline or nicotine (−nicotine hydrogen tartrate, 0.4 mg/kg, calculated as base; Sigma) administered subcutaneously daily for 15 days. Repeated intermittent daily 0.4 ± 0.1 mg/kg nicotine injections have been used in behavioral studies, shown to increase dopamine outflow in the NAc, and regulate nACh receptors in the brain (reviewed by Matta et al. (2007) and Vezina et al. (2007)). Following subcutaneous injection, peak brain levels of nicotine are seen within approximately 15 min and the $t_{1/2}$ is 45 min in rats; nicotine is totally cleared before the next injection in a daily injection schedule (Matta et al., 2007). Therefore, to exclude the probable acute effects, we sacrificed the rats on day after the last injection. On the 16th day, rats were anesthetized with thiopental sodium (40 mg/kg/i.p.) and perfused with saline (pH 7.4). The brains were removed and dissected according to visual anatomical landmarks and the atlas of Paxinos and Watson (1998). PFC, STR, and VTA were dissected immediately on ice. Brain regions were first minced using a surgical blade and then divided into

two parts to be used either for histone acetylation or qPCR analysis.

RNA extraction and cDNA synthesis

Total RNA was isolated using the Trizol reagent (Invitrogen) according to the manufacturer protocol. Purity of extracted RNA was measured at optical densities of 230, 260, and 280 nm with a NanoDrop2000C (Thermo Scientific). The RNA integrity was verified using ethidium bromide staining of 28S and 18S ribosomal RNA bands on 2% agarose gel. RNA was treated with amplification-grade DNaseI (Invitrogen) to degrade any genomic DNA present in the sample. cDNAs were generated from total RNA using anchored-oligo(dT) primers and Transcriptor First-Strand cDNA Synthesis Kit (Roche Applied Science) according to the manufacturer protocol. The first-strand cDNA was synthesized at 55°C for 30 min. cDNA chains were denatured and reverse transcriptase activity was arrested by heating to 95°C for 5 min.

PCR standards

The PCR standards for DRD1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) consisted of known numbers of molecules of purified PCR products (Dhanasekaran et al., 2010). PCR product was purified by gel electrophoresis, followed by excision of the band of the correct molecular weight and purification of the DNA using Qiagen Minelute PCR purification Kit (Qiagen, California, USA) according to manufacturer instructions (Dhanasekaran et al., 2010). The concentration of the purified PCR product DNA was estimated by OD_{260} and the number of copies per microliter of standards were calculated for each amplifier using the following formula: copies/$\mu l = [(6.02 \times 10^{23}$ copies) $\times$ (C $\times$ OD_{260} g/μl)/[base pairs $\times$ (660 Daltons/base)] where C = 5 $\times$ 10^5 g/ml for DNA samples (Godornes et al., 2007; Yin et al., 2001). Standards were made to a concentration of 10^8 copies/μl for DRD1 and 10^9 copies/μl for GAPDH, aliquoted and stored at −20°C.

Quantitative real time-PCR

qPCR was done in a LightCycler 1.5 System using LightCycler Taqman Master mix (Roche Applied Science) and the thermocycler conditions recommended by the manufacturer. A two-step qRT-PCR method was used. A nine-point, five-fold dilution standard curve for DRD1 (10^8–10^4) and GAPDH (10^8–10^4) was performed in duplicate in two independent experiments. Standard curves were generated by plotting the threshold cycles versus the $\log_{10}$ copies of the PCR products, and results were saved as external curves. The slope and linear correlation coefficient (R_2) were determined by linear regression analysis using LightCycler 2.0 Software (Roche Applied Science). In every PCR run, three points of each standard curve were included for curve fit. R_2, an indicator of fit for the standard curve, was plotted to the standard data points of both genes, yielding values of 0.987 and 0.998 for DRD1 and GAPDH, respectively. The calculated slope values were 3.281 and 3.353 for DRD1 and GAPDH, respectively. Primers and Universal ProbeLibrary probes for amplification of DRD1 and GAPDH are listed in Table I. Cycling conditions were 94°C for 10 min, followed by 40 cycles of 95°C for 10 s, 60°C for 40 s, and 72°C for 1 s, during which data acquisition was performed. The quantity of DRD1 in each sample was normalized to the reference gene *GAPDH*.

TABLE I. Nucleotide sequences of the primers and TaqMan probes designed for specific amplification of DRD1 and GAPDH

Primer/Probe	Sequence (5′-3′)	Position	Amplicon size
DRD1-F	cgaactgtatggtgcccttc (sense)	931–950	62bp
DRD1-R	gatggaatcgatgcagaatg	973–992	
UPL Probe #21 (Roche Diagnostics)	tggctctg (sense)	953–960	
GAPDH-F	ctgcaccaccaactgcttag (sense)	520–539	92bp
GAPDH-R	tgatggcatggactgtgg	594–611	
UPL Probe #58 (Roche Diagnostics)	tttggcatcgtg (sense)	569–580	

Chromatin immunoprecipitation

ChIP assays were performed using a ChIP Assay Kit (EMD Millipore) according to manufacturer instructions. Antibodies against acetylated histone H4 (H4Ac; catalog no. 06–866) were obtained from EMD Millipore. Brain tissue samples were treated with 1% formaldehyde to cross-link DNA to protein and sonicated to an average size of 500 bp. Sonicated chromatin was then diluted 10-fold in ChIP dilution buffer (200 µl), precleared with 80 µl salmon sperm DNA/Protein A Agarose for 1 h at 4°C, with rotation. A portion of the Protein A-purified chromatin (20 µl) was used to prepare DNA as the "input" sample. Antibodies (2–5 µl) were added to the clarified chromatin (160 µl) and immunoprecipitated overnight, with rotation. To the anti-histone mix, 60 µl Protein A Agarose was added, and then incubated at 4°C for 1 h, with rotation. The Protein A Agarose/histone complex was collected by gentle centrifugation and washed three times with ChIP buffers and once with TE buffer. The bound chromatin was then eluted in 500 µl aliquots with elution buffers. After shearing cross-linked DNA and checking by gel electrophoresis that the size of the sonicated DNA was between 200 and 1000 base pairs in length, 20 µl of 5 M NaCl was added. The protein–DNA cross-linking was reversed by heating at 65°C for 4 h. Samples were treated

TABLE II. Primers designed for amplification of three different promoter regions of DRD1 *gene*

Primer	Sequence (5′-3′)	Position upstream of TSS	Amplicon size
−1792APP-F	ggttatatgtctgatcacgagtc	−1792 to −1770	161bp
−1792APP-R	gctctcctaattagccagc	−1650 to −1632	
−1365APP-F	aggtgttgtgtctatgctc	−1365 to −1347	163bp
−1365APP-R	acgctaacacagtagctaatg	−1223 to −1203	
−170APP-F	tgtcaagctcagcccac	−170 to 154	182bp
−170APP-R	tcgttcttagggcaggac	−6 to +12	

with proteinase K, purified by Mini-Elute PCR purification kit (QIAGEN), and then eluted in 50 μl of TE buffer.

qPCR was used to assess the degree of histone H4 acetylation in the DRD1 promoter. For each of the precipitated samples, the ratio of immunoprecipitation was determined by comparison with the input chromatin, which represented the total DNA of each sample before the addition of antibodies against the acetylated H4Ac antibody.

Quantitative PCR analysis of immunoprecipitated chromatin

Levels of specific histone modifications in the *DRD1* gene promoter were determined by measuring the amount of the amplified promoter sequence of *DRD1* gene in chromatin immunoprecipitates by use of qPCR (LightCycler 2.0; Roche Applied Science) (see Table II for primers). Input DNA (non-immunoprecipitated) and immunoprecipitated DNA were PCR amplified in triplicate using LightCycler DNA Master SYBR Green I according to manufacturer instructions. Ct values from each sample were obtained and relative quantification of amplified template was performed as described earlier by Chakrabarti et al. (2002). Each real-time PCR reaction was repeated at least twice, independently.

Statistical analyses

SPSS (v17.0) software was used for all statistical analyses. Two-way ANOVAs were performed, with brain regions (STR, PFC, and VTA) and treatment (control, nicotine) as factors and normalized expression levels (relative to GAPDH) for dopamine DRD1 mRNA as the dependent variable. Nonparametric independent samples Mann–Whitney U tests were performed to determine the difference between control and nicotine-treated rats in each brain region. DRD1 mRNA levels and quantitative PCR results of the ChIP assays were calculated as percentage of control of the same brain region.

RESULTS

DRD1 mRNA expression in the three brain regions

Quantitative PCR was performed to measure DRD1 mRNA levels in the PFC, STR, and VTA in saline- and nicotine-treated rats. DRD1 mRNA levels were normalized to the levels of GAPDH mRNA as the endogenous control. All assays were run in triplicate. Normalized basal expression levels (relative to GAPDH) for dopamine DRD1 mRNA in the three brain regions are given in Table III.

TABLE III. Dopamine DRD1 mRNA expression

	Control	Nicotine
STR	101.93 ± 8.89	130.64 ± 14.15
PFC	22.72 ± 2.34	29.23 ± 1.55[a]
VTA	5.03 ± 0.63	5.89 ±0.54

Normalized basal expression levels (relative to GAPDH) of dopamine DRD1 mRNA (mean ± SEM) in saline- (control) and nicotine-treated rats in three brain regions of rat brain: Striatum (STR), prefrontal cortex (PFC), and ventral tegmental area (VTA).
[a]Different from control ($P < 0.05$).

We performed a two-way ANOVA with brain regions (STR, PFC, and VTA) and treatment (control, nicotine) as the factors and normalized DRD1 mRNA levels (relative to GAPDH) as the dependent variable. Regions [$F(2,53) = 133.75$, $P < 0.0001$] and treatment [$F(1,54) = 4.12$, $P < 0.05$] emerged as significant main effects; expression was highest in the STR and lowest in the VTA, and expression in nicotine-treated rats were higher than saline-treated controls. Independent samples Mann–Whitney U tests were performed to see the differences in expression between control and nicotine-treated rats for each brain region separately. The only significant difference was observed in the PFC ($P = 0.034$); nicotine-treated rats had higher expression than saline-treated controls.

Nicotine treatment increased histone H4 acetylation in the DRD1 gene promoter in the PFC

An increase in the acetylation of histone H4 has been correlated with both gene transcription and active DNA control elements such as response elements and enhancers (Shahbazian et al., 2005). To determine whether nicotine treatment induces distinct histone acetylation profiles on distal and proximal promoter elements, we designed primers to amplify three different segments of the DRD1 promoter. We found that nicotine treatment increases histone H4 acetylation more than three-fold in the sequence amplified by −170APP compared with controls [$t\ (9) = 2.38$, $P = 0.044$]. Similarly, histone H4 acetylation increased more than two-fold compared with controls in the promoter sequence amplified by

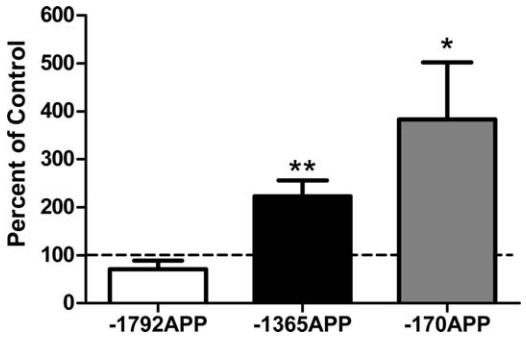

Fig. 1. Effect of chronic nicotine treatment on histone H4 acetylation in DRD1 promoter. The bars show average ± SEM. Different from saline-treated control: $^{**}P < 0.005$. $^{*}P < 0.05$.

−1365APP [t (9) = 3.63, $P = 0.005$]. There was no significant change in histone H4 acetylation of the promoter sequence amplified by −1792APP [t (9) = 1.64, $P = 0.162$] (Figure 1).

Transcription factor binding site analysis of sequences amplified by −170APP and −1365APP primer pairs

Transcription factor binding sites (TFBSs) in 2kb upstream regulatory region of the *DRD1* gene was analyzed by using MatInspector, a software tool for promoter analysis (Cartharius et al., 2005). The analysis revealed that the sequence amplified by −170APP includes a transcription binding site for EGR1 (−136 to −120, ggacgcggAGGCggggt), whereas the sequence amplified by −1365APP contained a TFBS for SP1 (−1316 to −1300, agaacGGG Cggaggagc).

DISCUSSION

In the first stage of this study, qPCR results showed that repeated intermittent nicotine treatment resulted in approximately 30% increase in DRD1 mRNA in the PFC. Therefore, in the following stage of this study, only the PFC samples were used for further analysis. ChIP assay in combination with qPCR demonstrated that nicotine treatment increased histone H4 acetylation at two different segments of the DRD1 promoter in the PFC, from nucleotide −1365 to −1202 and −170 to +12. These results suggest that these two segments became available for the binding of gene regulatory proteins after nicotine treatment. To the best of our knowledge, this is the first study, which demonstrates that repeated intermittent nicotine treatment increased histone H4 acetylation at the *DRD1* gene promoter.

Studies report that acute systemic and intra-PFC nicotine administrations increase dopamine release in the PFC (Carboni et al., 2010; Shearman et al., 2008, 2005; Tsukada et al., 2005). Nicotine, when injected systemically, may regulate dopamine release in the PFC through the nicotinic receptors expressed on dopaminergic cell bodies in the VTA, as well as on dopaminergic terminals in the PFC (Livingstone and Wonnacott, 2009). The nicotine exposure protocol used in our study has been shown to upregulate nicotinic binding sites and increase nicotine-induced dopamine release in the prefrontal cortex (PFC; Fredrickson et al., 2003) leading to sensitized dopamine response and locomotor activity (Nisell et al., 1996). Fredrickson et al. (2003) reported decreased release after chronic nicotine pretreatment, although the values are still two-fold greater in nicotine-treated animals compared with controls. On the other hand, Jacobs et al. (2002) did not observe an effect of chronic nicotine pretreatment on dopamine release in the PFC.

In the PFC, dopamine D1 receptors are expressed on pyramidal neurons and GABAergic interneurons (Seamans and Yang, 2004). Dopaminergic axon terminals in the PFC originate mainly from the VTA (Heidbreder and Groenewegen, 2003; Steketee, 2003). Dopamine released from these axon terminals may regulate the excitability of the pyramidal neurons directly through D1 receptors expressed on the pyramidal neurons themselves and indirectly through D1 receptors expressed on GABAergic interneurons. One of the major findings of this study is that repeated intermittent nicotine treatment upregulated DRD1 mRNA expression in the PFC. There are few studies that examined the effects of chronic nicotine treatment on DRD1 levels in the PFC. Similar to our findings, Boules et al. (2011) reported that nicotine self-administration increased DRD1 mRNA in the PFC, while Levin et al. (1997) reported that chronic nicotine treatment did not regulate D1 receptor binding in the frontal cortex. However, Levin et al. (1997) performed continuous nicotine infusions instead of intermittent administration, which was used in our study. Upregulation of D1 receptors in the PFC by chronic nicotine exposure may enhance dopamine signaling in the PFC and lead to nicotine addiction.

Dopamine D1 receptors expressed in the PFC are implicated in the reinforcing and rewarding properties of some commonly abused drugs (McGregor and Roberts, 1993; Pierce and Kumaresan, 2006). Studies show that D1 receptors are expressed on pyramidal neurons, which project to the NAc (Brenhouse et al., 2008). Addictive drugs may transiently regulate genes through activating D1 receptors. Cocaine or amphetamine increases dopamine levels and stimulates D1 receptors in the NAc and STR (reviewed by Hyman (2005)). D1 receptors expressed on glutamatergic output neurons in the PFC are suggested to play an important role in drug-related associative learning (Brenhouse et al., 2008; Kalivas et al., 2005). Additionally, chronic nicotine treatment is reported to enhance working memory (Levin and

Simon, 1998) and D1 receptors in the PFC have an important role in the regulation of working memory performance (Seamans and Yang, 2004). Therefore, dopamine D1 receptor upregulation in the PFC after nicotine exposure may also contribute to the regulation of working memory performance.

In the PFC, ChIP in combination with qPCR revealed that intermittent nicotine exposure for 15 days increased histone H4 acetylation in two segments of the DRD1 promoter sequence, the −1365 to −1202 region, amplified by the −1365APP primer pair, and the −170 to +12 region, amplified by the −170APP primer pair. This finding shows that these two segments of the DRD1 promoter sequence are available for the binding of response elements and enhancers such as transcription factors. This is the first study that demonstrated the effects of nicotine treatment on histone acetylation in the DRD1 promoter. Analysis of the sequences amplified by these primer pairs revealed binding sites for EGR-1 (Early growth response protein-1, zif268) and Sp1 (Specificity Protein 1). These transcription factors were previously shown to be regulated by nicotine treatment in the nervous system. Belluardo et al. (2005) reported that acute intermittent nicotine treatment upregulated EGR-1 and EGR-2 gene expression and mRNA in the rat cerebral cortex and hippocampal formation. Li et al. (2004) showed that chronic nicotine treatment upregulated Sp1 gene expression in the PFC. Furthermore, EGR-1 (Takeuchi et al., 2002) and Sp1 (Dunah et al., 2002; Goold et al., 2007; Yang et al., 2000) are reported to activate the dopamine receptor gene promoter and increase dopamine receptor gene transcription.

The epigenetic regulation of the dopaminergic system has been studied in major psychiatric disorders (Abdolmaleky et al., 2008). Smoking is considered to be a gateway to using other abused drugs. Levine et al. (2011) studied the effects of prior nicotine exposure on responses to cocaine. In addition to enhancing behavioral responses to cocaine, nicotine-inhibited histone deacetylase, resulting in increased activation of FosB gene by cocaine; inhibiting histone deacetylase had the same effect as nicotine. Based on these results, nicotine appears to enhance histone acetylation by inhibiting deacetylation which would silence genes (discussed by Volkow (2011)). Pastor, also used a histone deacetylase inhibitor and showed that the preference for nicotine was significantly reduced in a CPP protocol. Along the same lines, the recent report by Chase and Sharma (2012) suggested that nicotine treatment can increase histone acetylation in primary cortical neuronal cultures through decreasing histone methlytransferase. The authors describe this epigenetic effect of nicotine as "leading to a more permissive epigenomic environment."

This study investigated the epigenetic regulation of the *DRD1* gene promoter by repeated intermittent nicotine treatment. Although we did not use a mechanistic approach to elucidate the underlying mechanism, we demonstrated that 15-day nicotine exposure increased the transcription of D1 receptors in the PFC. Our findings suggest that this upregulation of D1 receptor transcription may be due to increased histone H4 acetylation in the DRD1 promoter region. Upregulated D1 receptor levels in the PFC, in turn, may contribute to the nicotine-induced sensitization of the dopamine-regulated mesocorticolimbic system, leading to addiction.

ACKNOWLEDGMENT

The authors thank Jacqueline Renee Gutenkunst (Izmir University of Economics, School of Foreign Languages), for her effort in refining the language of this article.

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