Neuroscience Letters 637 (2017) 75–79

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Gene expression of pro-opiomelanocortin and melanocortin receptors is regulated in the hypothalamus and mesocorticolimbic system following nicotine administration

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HIGHLIGHTS

- 0.6 mg/kg nicotine increased POMC mRNA in the hypothalamus.
- 0.6 mg/kg nicotine increased MC4R mRNA in the medial prefrontal cortex.
- 0.2, 0.4 and 0.6 mg/kg nicotine increased MC3R mRNA in the ventral tegmental area.

ARTICLE INFO

Article history:
Received 10 October 2016
Received in revised form
22 November 2016
Accepted 23 November 2016
Available online 24 November 2016

Keywords:
Nicotine
POMC
Melanocortin receptor
Mesocorticolimbic system

ABSTRACT

Pro-opiomelanocortin (POMC)-derived peptides and their receptors have been shown to play important roles in natural and drug-induced reward and reinforcement. Reward process may involve the regulation of POMC gene expression and the gene expression of POMC-derived peptide receptors. The present study investigated the alterations observed in the transcript levels of POMC, melanocortin 3 (MC3R), melanocortin 4 (MC4R) and mu-opioid receptors (MOR) in the hypothalamus and mesocorticolimbic system during nicotine exposure. Rats were injected subcutaneously for 5 days with one of the three doses (0.2, 0.4 or 0.6 mg/kg/day, free base) of nicotine and were decapitated one hour after a challenge dose on the sixth day. mRNA levels of POMC in the hypothalamus, MC3R in the ventral tegmental area (VTA), MC4R and MOR in the medial prefrontal cortex (mPFC), nucleus accumbens, dorsal striatum, amygdala, lateral hypothalamic area and VTA were measured by quantitative real-time PCR. Our results showed that treatment with 0.6 mg/kg/day nicotine upregulated POMC mRNA in the hypothalamus and MC4R mRNA in the mPFC. Additionally, all three nicotine doses increased MC3R mRNA expression in the VTA. On the other hand, none of the nicotine doses altered MOR mRNA levels in the mesocorticolimbic system and associated limbic structures. These results suggest that nicotine may enhance melanocortin signaling in the mesocorticolimbic system and this alteration may be an important mechanism mediating nicotine reward.

1. Introduction

Nicotine exerts its rewarding effects through activation of nicotinic receptors (nAChR) localized in the mesocorticolimbic system of the brain [1]. This system involves the neural pathway which connects the ventral tegmental area (VTA) with two major targets, nucleus accumbens (NAc) and the prefrontal cortex (PFC). Increased dopamine signaling in the mesocorticolimbic system has long been associated with reward processing and drug addiction

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http://dx.doi.org/10.1016/j.neulet.2016.11.049
0304-3940/

[1,2]. Repeated daily nicotine injections lead to dopamine overflow in the NAc and induce reward-related behavior [3,4].

In the brain, pro-opiomelanocortin (POMC) is synthesized primarily in the arcuate nucleus (ARC) of medial basal hypothalamus [5]. POMC is post-translationally processed to yield biologically active melanocortin peptides [α-melanocyte stimulating hormone (α-MSH), γ-MSH, adrenocorticotropic hormone (ACTH)] and the opioid peptide, β-endorphin [6]. The actions of melanocortins are mediated by melanocortin receptors. The main melanocortin receptors expressed in the brain are melanocortin 3 (MC3R) and melanocortin 4 (MC4R) receptors [7]. A growing body of evidence supports the notion that melanocortin peptides have important roles in food and drug reward [8]. Studies report that α-MSH and signaling through melanocortin receptors increase dopamine release and dopamine turnover in the NAc and dorsal striatum (DST) [9–11]. Administration of melanocortin receptor agonists into the VTA decrease, while melanocortin receptor antagonists increase food intake [8]. Melanocortinergic neurotransmission also regulates ethanol, amphetamine and cocaine-induced reward [8,12,13]. However, the role of melanocortins in nicotine reward is not known.

β-endorphin binds to the opioid receptors, showing the highest affinity to mu-opioid receptors (MOR) [14]. MOR mRNA is found abundantly in the mesocorticolimbic system [15]. β-endorphin and MOR activation enhance dopamine release in the NAc and DST [16]. MOR signaling is implicated in the rewarding effects of nicotine (reviewed in [17]). Nicotine-induced conditioned place preference is abolished in MOR knock-out mice [14], or by administration of MOR antagonists [18]. MOR antagonists also reduce nicotine infusions in a rat model of drug self-administration [19].

The present study aimed to investigate the possible regulatory effects of nicotine on the gene expression of POMC and POMC-derived peptide receptors. After repeated daily exposure to nicotine for 6 days, POMC mRNA expression in the hypothalamus, MC3R, MC4R and MOR mRNA expressions in the mesocorticolimbic system [medial PFC (mPFC), NAc, DST, VTA] and associated limbic regions [amygdala (AMG), lateral hypothalamus (LHA)] were measured by quantitative real-time PCR (qPCR) analysis.

2. Methods

2.1. Experimental animals

Adult male (4 months old, 300–450 g) Sprague Dawley rats were housed (3–4 rats/cage) in standard plastic cages with food and water provided ad libitum during the habituation period for four weeks. Animals were maintained on 12:12 h light: dark cycle (lights on 07:00–19:00).

The animals were handled under the prescriptions for animal care and experimentation of the pertinent European Communities Council Directive (86/609/EEC), and all the procedures were approved by the Institutional Animal Ethics Committee of Ege University, Izmir, Turkey.

In this study, the weight of the animals were measured both on the first day of nicotine injections and on the last day, before the decapitation. However, there were no significant differences between the two measurements showing that nicotine treatment did not affect the body weight of the rats.

2.2. Nicotine treatment

Nicotine (hydrogen tartrate salt, Sigma) was injected subcutaneously between 08:00 and 10:00 a.m. for 6 days. Rats received either saline (1 ml/kg) or one of the three doses of nicotine (0.2, 0.4 or 0.6 mg/kg, free base) and assigned to saline, 0.2, 0.4 and 0.6 mg/kg Nic groups.

Numerous studies have shown that the nicotine treatment regimen described above enhanced dopamine release in the mesocorticolimbic system, increased locomotor activity and induced behavioral sensitization [3,20,21]. Our previous studies have also demonstrated that the selected nicotine doses induced reward-related behaviors such as increased locomotor activity and conditioned place preference [20,22].

2.3. Tissue dissection

Rats were sacrificed by decapitation between 09:00 and 11:00 am on day 6, one hour after the last injection. Following decapitation, brains were removed. Four coronal brain slices containing mPFC, NAc, DST, AMG, LHA, medial hypothalamic area (MHA) and VTA were cut according to the stereotaxic coordinates depicted in the rat brain atlas by Paxinos and Watson (Fig. 1) [23]. The coronal brain slices were cut fresh on ice using a steel matrix (Ted Pella Inc., #15007) and steel blades. The dissection area for mPFC involved only the anterior cingulate, prelimbic, medial orbital, and infralimbic cortices. NAc, DST, AMG and VTA were dissected by micropunch technique. All of the selected brain regions were dissected bilaterally on ice.

2.4. RNA Extraction and cDNA synthesis

qPCR analysis of POMC mRNA was carried out in the MHA, which involves the ARC nucleus. In the hypothalamus, the presence of POMC mRNA is restricted to the ARC nucleus [5]. qPCR analysis of MOR mRNA was performed in mPFC, NAc, DST, AMG, LHA and VTA, where high density of MOR mRNA expression is present [15]. For the qPCR analysis of MC4R mRNA expression, mPFC, NAc, DST, AMG and LHA samples were used. Intense/moderate MC4R mRNA expression is observed in these brain regions [24]. Compared with MC4R, the expression pattern of MC3R mRNA is more restricted in the mesocorticolimbic system with an intense expression in the VTA [8]. Therefore, analysis of MC3R gene expression was done in the VTA.

The total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA). RNA concentration and purity was determined spectrophotometrically. The RNA integrity was verified using ethidium bromide staining of 28S and 18S ribosomal RNA bands on 1% agarose gel. One microgram RNA was conveyed to cDNA with the using anchored-oligo (dT) primers.

2.5. Quantitative real-time PCR (qPCR)

Primers and probes for amplification of Pomc, Oprm1, Mc3r, Mc4r and Actb were designed using the Primer3 software. For each gene of interest, forward, reverse and probe sequences were: Pomc (NM_139326), ctccatagacgtgtggag (F), cggggattttcagtcaag (R), ctgttcatctccgttgcctgg (P); Oprm1 (NM_001038597), agaggagaatatcagacg (F), ctaggtaggctgtaactg (R), tgccgctcttctctggttcc (P); Mc3r (NM_001025270), gccgataaccatgaactc (F), tctggcttgatgaaaacc (R), cctcttatccgacgctgcctaa (P); Mc4r, (NM_013099), gagtgaatactacggctaa (F), tgctcatcatttctttagaag (R), ctctcggctgaccagtctgc (P); Actb (NM_031144), cccgcgagtacaaccttct (F), cgtcatccatggcgaact (R), agctcctccgtcgccggtcca (P). qPCR was performed in a LightCycler 480 System using LightCycler 480 Probes Master (Roche Applied Science) according to conditions recommended by the manufacturer. Two-step qPCR was performed on samples in duplicate, to control for PCR variations. Specific PCR amplification efficiencies for each gene were generated using Ct slope method. The linear correlation coefficients (R^2) were all between 94% and 99%. Cycling conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 1 s during which data acquisition was performed. The quantity of each measured cDNA sample was