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Repeated nicotine exposure modulates prodynorphin and pronociceptin levels in the reward pathway

Lucia Carboni[a,*], Benedetto Romoli[b,1], Patrizia Romualdi[a,2], Michele Zoli[b,2]

[a] Department of Pharmacy and Biotechnology, Alma Mater Studiorum University of Bologna, Bologna, Italy
[b] Department of Biomedical, Metabolic and Neural Sciences, Center for Neuroscience and Neurotechnology, University of Modena and Reggio Emilia, Modena, Italy

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ABSTRACT

Background: Nicotine dependence is maintained by neurobiological adaptations in the dopaminergic brain reward pathway with the contribution of opioidergic circuits. This study assessed the role of opioid peptides and receptors on the molecular changes associated with nicotine dependence. To this aim we analysed nicotine effects on opioid gene and receptor expression in the reward pathway in a nicotine sensitization model.
Methods: Sprague-Dawley rats received nicotine administrations for five days and locomotor activity assessment showed the development of sensitization. The mRNA expression of prodynorphin (pdyn), pronociceptin (pnoc) and the respective receptors was measured by quantitative PCR in the ventral midbrain (VM), the nucleus accumbens (NAc), the caudate-putamen (CPu), the pre-frontal cortex (PFCx), and the hippocampus.
Results: A significant positive effect of sensitization on pdyn mRNA levels was detected in the CPu. This effect was supported by a significant and selective correlation between the two parameters in this region. Moreover, chronic but not acute nicotine treatment significantly decreased pdyn mRNA levels in the NAc and increased expression in the PFCx. Pnoc mRNA was significantly increased in the VM and the PFCx after sub-chronic administration of nicotine, whereas no alterations were observed after acute treatment. No treatment associated changes were detected in κ-opioid receptor or nociceptin receptor mRNAs.
Conclusions: This experiment revealed an effect of nicotine administration that was distinguishable from the effect of nicotine sensitization. While several pnoc and pdyn changes were associated to nicotine administration, the only significant effect of sensitization was a significant increase in pdyn in the CPu.

1. Introduction

Tobacco smoking, one of the primary causes of preventable disease and premature death (Hatsukami et al., 2008), is partially motivated by an addiction to nicotine. Nicotine, like other addictive substances, produces its behavioural effects by inducing long-term changes in the dopaminergic brain reward pathway. In particular, the mesocorticolimbic pathway, originating in the ventral tegmental area in the midbrain and projecting to the ventral striatum and pre-frontal cortex (PFCx), is enriched with α4/α6/β2-containing nicotinic receptors that are responsible for nicotine addictive properties (Picciotto and Mineur, 2014; Pistillo et al., 2015). The addiction process develops through a series of neuroadaptations that take place in the reward pathway by modulating intracellular pathways and ultimately regulating gene expression (Picciotto and Mineur, 2014; Pistillo et al., 2015).

Compelling evidence demonstrates that opioidergic circuits contribute to the neurobiological adaptations and hence support nicotine dependence (Charbogne et al., 2014; Hadjiconstantinou and Neff, 2011). Opioid receptors and peptides are expressed in the mesocorticolimbic circuits that are relevant to addiction and nicotine administration can influence the expression and release of opioid peptides. In addition, the administration of opioid agonists and antagonists affects nicotine-induced dopamine release in the nucleus accumbens (NAc), and regulates nicotine rewarding effects, as measured in animal models of nicotine dependence. In line with these findings, the important role exerted by the opioid circuit

* Corresponding author at: Department of Pharmacy and Biotechnology, via Irnerio 48, 40126 Bologna, Italy.
E-mail address: lucia.carboni4@unibo.it (L. Carboni).
[1] Present address: Department of Psychiatry, University of California, San Diego, USA
[2] These authors share last authorship.

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was demonstrated using genetic models where opioid receptor or peptides had been eliminated by gene knockout (Charbogne et al., 2014; Hadjiconstantinou and Neff, 2011).

Within the opioidergic signalling system, the dynorphin/κ-opioid receptor (KOP) system is composed of the neuropeptides derived from the precursor prodynorphin and the respective seven-transmembrane receptor KOP. This system was initially discovered due to its role in analgesia and was subsequently characterised by assessing its importance in stress-induced behaviours, including the negative emotional state occurring during withdrawal and the stress-induced reinstatement of drug taking (Bruchas et al., 2010; Chavkin, 2013; Koob et al., 2014). The significance of the dynorphin/KOP system for nicotine addiction is supported by studies showing that nicotine withdrawal signs were attenuated by pre-treatment with KOR antagonists (Jackson et al., 2015). Moreover, both endogenous dynorphin release due to acute stress and KOP agonist treatment are able to potentiate nicotine-induced place preference (Smith et al., 2012). However, place preference was not altered in pdyn knock-out mice; instead, these animals displayed higher sensitivity to the reinforcing properties of nicotine in a self-administration paradigm (Galeote et al., 2009). Nevertheless, KOP antagonist administration showed no effect on nicotine self-administration (Liu and Jernigan, 2011), while agonist treatment increased or decreased this operant behaviour depending on the agonist dose (Ismayilova and Shoaib, 2010). After extinction, KOP receptors appear to mediate stress-induced reinstatement of nicotine-induced place preference (Grella et al., 2014). Overall, these findings suggest that the dynorphin/KOP system is involved in nicotine addiction, possibly by mediating aversive and dysphoric states which are alleviated by nicotine, thus contributing to its rewarding effects.

The nociceptin system, composed of the neuropeptide nociceptin and the nociceptin receptor (NOP), exhibits a characteristic and specific profile of pharmacological activity (Mogil and Pasternak, 2001). In analogy with the putative role attributed to the dynorphin/KOP system, several lines of evidence support the argument that nociceptin and NOP exert important functions in addiction, in particular in negative affective states that drive reinforcement (Schank et al., 2012). Although available data support the activity on alcohol and opioid dependence, there is sparse evidence to suggest that it has a role in nicotine addiction. Indeed, in NOP knockout mice nicotine treatments induce hypersensitive responses, including tolerance and withdrawal, suggesting that endogenous nociceptin may modulate nicotine addiction (Sakoori and Murphy, 2009).

The aim of this study was to investigate the role of opioid peptides and receptors related to the stress response by assessing the effect of nicotine on their expression in brain regions belonging to the reward pathway. A nicotine sensitization model was adopted since the sensitization behaviour is assumed to be supported by molecular changes in brain circuits that reflect the process of compulsive drug craving in addiction (Robinson and Berridge, 2008).

2. Material and methods

2.1. Animals

Experiments were carried out in male Sprague-Dawley rats (Charles-River, Calco, Italy) weighing 250–280 g. Rats were group housed with free access to water and food in controlled light conditions (from 7.00 a.m. to 7.00 p.m.), temperature (22 ± 2 °C) and humidity (65%). Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and National Ministry of Health laws and policies (project A137, Prot.n.100/2013/B) at the University of Modena and Reggio Emilia. Procedures received approval by the local Ethical Committee and animals were monitored by the University Veterinary Service. All efforts were made to reduce the number of animals and minimize their discomfort. This study is compliant with the ARRIVE guidelines (Kilkenny et al., 2010).

2.2. Nicotine treatments

Rats (28, n = 7/group) received intraperitoneal administrations of vehicle or nicotine ((−) nicotine hydrogen tartrate dissolved in saline solution: NaCl 0.9%, pH 7.4) at the dose of 0.4 mg/kg of nicotine base in 2 ml/kg saline solution during the late morning. The four experimental groups included acute treatments (sacrifice 4 h after nicotine or vehicle administration) and sub-chronic treatments (nicotine or vehicle administered once daily for 5 days, sacrifice 4 h after last treatment).

2.3. Test of locomotor activity

Locomotor activity was assessed in motility cages Macrolon III, 38 × 20 × 16 cm equipped with four infrared beams for detection of horizontal movements and four infrared beams for vertical movements (Med-Associates Inc., St Albans). Inter-beam distance was 8 cm horizontally and 6 cm vertically. Activity was recorded for 120 min in 5 min intervals (MED-PC Software) and nicotine or vehicle was injected after 60 min habituation to the motility cage. For all animals locomotor activity was assessed after a single nicotine or vehicle injection (acute groups) or for 5 consecutive days after each daily nicotine or vehicle injection (sub-chronic groups). This latter procedure has been shown to lead to the development of locomotor sensitization to nicotine treatment (Ferrari et al., 2002).

2.4. Sample collection

Rats were guillotined and their brains removed and placed on ice so that entire brain regions could be dissected out. First, the two hippocampi were manually isolated and dissected out and then brain slices of different sizes were taken. A slice from the rostral end of striatum (corresponding to bregma level 2.5 mm) to the optic chiasm (corresponding to bregma level 0 mm) was used to dissect caudate-putamen (CPu) and nucleus accumbens (NAc). These were manually isolated using a tweezer. The prefrontal cortex (PFCx) is defined as being the portion of cortex rostral to the striatal slice (including, prelimbic, infralimbic, orbital, rostral cingulate, rostral motor and sensory cortices). A slice of midbrain, from caudal mammillary bodies (corresponding to bregma level −4.5 mm) to the caudal end of the externally visible interpeduncular nucleus (corresponding to bregma level −6.5 mm) was cut and, after excision of the interpeduncular nucleus, the ventral portion was dissected out (ventral midbrain, VM, corresponding to the dopamine neuron-enriched regions substantia nigra pars compacta and reticulata and ventral tegmental area). The dissected regions were quickly frozen on dry ice and stored at −80 °C.

2.5. qPCR

RNA extraction, reverse transcription and real-time PCR by Sybr green technology was performed as previously described (Lattanzio et al., 2014), using the following primers: KOP Forward 5′- TTGGCTACTGGCATCATCTG −3′; Reverse 5′- ACACTCTTCAAGCGCAGGAT-3′; PDyn Forward 5′- CCTGTCCTTGTGTTCCCTGT-3′; Reverse 5′-AGAGGCAGTCAGGGTGAGAA −3′; NOP Forward 5′- GGCCTCTGTTGTCGGTGTTC −3′; Reverse 5′- GTAGCAGACAGAGATGACGAGCAC −3′; PNoc Forward 5′- TGCAGCACCTGAAGAGAATG −3′; Reverse 5′- CAACTTCCGGGCTGACTTC −3′; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Forward 5′- GGTCGGAGT-