Rat Nicotine Sensitization Modulates mRNA Levels of Stress Peptides and Receptors in the Reward Pathway

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Nicotine addiction is at the basis of tobacco smoking, one of the main preventable causes of disease and premature death. Addiction is considered as a three-stage cycle based on the phases of binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation. The cycle is sustained by two main drivers: positive and negative reinforcement. Nicotine addiction develops through long-term neuroadaptations in the brain reward circuit by modulating intracellular pathways and ultimately regulating gene expression. In particular, the negative reinforcement driver is supposed to derive from neurobiological adaptations in stress-related circuits, thus contributing to the establishment and maintenance of dependence.

This study was aimed at assessing the role of stress peptides and receptors in the molecular changes associated with nicotine dependence. To achieve this objective we analysed the gene expression of stress peptides and receptors in regions of the reward circuit in a nicotine sensitization model, since this behaviour is supported by molecular changes reflecting the process of compulsive drug craving.

Sprague-Dawley rats received i.p. nicotine administrations at 0.4 mg/kg for 1 day or 5 days. Locomotor activity was assessed to evaluate the development of sensitization. The mRNA expression of prodynorphin (pdyn) and its receptor KOP, pronociceptin (pnoc) and its receptor NOP, corticotropin-releasing factor (CRF) and its receptors CRFR1 and CRFR2 was measured by qPCR in pre-frontal cortex (PFCx), Nucleus accumbens (NAc), and Caudate-Putamen (CPu).

Acute nicotine administration increased locomotor activity in rats previously habituated to the test cage. In the 5-day treated group, locomotor activity progressively increased upon daily nicotine administration, reaching a clear sensitization by the 5th day. A significant positive effect of sensitization on pdyn mRNA levels was detected in CPu (time x treatment $F(1,19)=4.9$ $p=0.039$), with chronic nicotine treatment raising mRNA levels by 60% with no changes after acute treatment. In NAc nicotine treatment significantly decreased pdyn mRNA levels overall (treatment $F(1,19)=6.8$ $p=0.017$), whereas in PFCx a trend for a treatment effect was detected. KOP mRNA expression was not modified in any condition. Significantly increased pnoc mRNA was detected in PFCx (treatment $F(1,19)=7.8$ $p=0.012$), whereas no effect was observed in NAc and a trend for increased pnoc induced by repeated manipulations was detected in CPu. Nicotine did not affect NOP expression, while a significant decrease was induced in NAc by repeated manipulations (time $F(1,18)=7.6$ $p=0.013$). Significant effects of sensitization on CRF mRNA levels were detected in CPu and NAc in opposite directions: increased in CPu (time x treatment $F(1,17)=6.2$ $p=0.023$) and decreased in NAc (time x treatment $F(1,18)=6.6$ $p=0.019$). A significant decrease was induced in PFCx by manipulations (time $F(1,17)=13.41$ $p=0.002$). A similar time-associated reduction was detected in CRFR1 mRNA in PFCx and NAc (time $F(1,18)=5.7$ $p=0.03$; $F(1,18)=5.2$ $p=0.035$,

respectively). No changes were detected in CPu. Significantly increased CRFR2 mRNA levels after sensitization were detected in PFCx (time x treatment $F(1,17)=6.3$ $p=0.022$), with chronic nicotine treatment raising mRNA levels by 90%. Treatment and time-induced increases were detected in CPu (treatment $F(1,17)=7.19$ $p=0.016$; time $F(1,17)=5.5$ $p=0.032$), with no effect in NAc.

The experimental design allowed three patterns of significant change to be resolved from each other: the effect of repeated manipulations, the effect of nicotine administration and the effect of nicotine sensitization. The study distinguished region- and gene-specific effects of nicotine administration from those of nicotine sensitization. While pdyn, pnoc and CRFR2 changes were observed after nicotine administration, significant alterations in pdyn, CRF and CRFR2 were specifically associated to the development of sensitization.