Nociceptin and dynorphin systems alterations in rats chronically exposed to cocaine during adolescence

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Cocaine promotes molecular and cellular alterations underlying addiction-related neural plasticity phenomena. Previous studies demonstrated the involvement of different neural systems in the cocaine-reward effects including the endogenous opioid following chronic administration as well as during abstinence (1, 2). In particular, prodynorphin-kappa opioid receptor (pDYN-KOP) and pronociceptin/orphaninFQ-nociceptin receptor (pN/OFQ-NOP) systems play important roles in rewarding stimuli and stress mechanisms related to cocaine-addiction, since specific genetic and epigenetic changes have been reported (3). In this context, several studies focused on 'adolescence' as a crucial vulnerability period for cocaine addiction (4). In view of the crucial role played by the opioid systems in cocaine addiction, the study aimed to evaluate whether gene expression alterations of pDYN-KOP and pN/OFQ-NOP systems occur differently depending on the time of analysis. Rats were exposed to 20mg/kg of cocaine from post-natal day (PND) 28 to PND42, which roughly approximates adolescence in humans, and sacrificed at two different time points: 3days (PND45) and 48 days (PND90) after last cocaine injection. The gene expression was assessed by Real-Time PCR using the Delta-Delta Ct (DDCt) method in the following brain areas known to be involved in the reward mechanism: striatum (CPu), nucleus accumbens (Nac) and hippocampus (HIPPO).

The qRT-PCR analysis of pDYN-KOP and pN/OFQ-NOP gene expression in the CPu area showed no significant alterations at PND45 interval. In contrast, long-term abstinence from developmental exposure to cocaine (PND90) caused a significant up-regulation of pN/OFQ (1.63±0.11 *vs.* 1.00±0.12; $p<0.01$) and NOP (1.15±0.04 *vs.* 1.00±0.05; $p<0.05$) mRNA levels; no changes of pDYN-KOP at this interval were observed.In the Nac, gene expression data showed a selective up-regulation of pDYN at PND45 (1.35±0.12 *vs.*1.00±0.08; $p<0.05$). At PND90 cocaine exposure does not induce any alteration in this region.Gene expression analysis of HIPPO showed a significant down-regulation of pDYN mRNA level at PND45 (0.76±0.045 *vs.* control 1.00±0.09; $p<0.05$), whereas no changes occurred for KOP and pN/OFQ-NOP system. At PND90 cocaine exposure does not induce changes anymore.

These data suggest the involvement of pDYN/KOP and pN/OFQ-NOP systems in two different withdrawal phases, since PND45 could correspond to the phase-1 ('crash' day 1 to 4) and the PND90 to the phase-2 (week 1 to 10) in humans abstinence (5). Indeed, the pDYN system was altered at PND45, whereas the pN/OFQ-NOP was up-regulated at PND90.

Notably, the pattern of expression of pDYN parallels that of the neurotrophin BDNF. In fact an increase of BDNF in NAc and a decrease in HIPPO have been associated to depression in animal models (6,7). Since BDNF dysregulation has been associated with depression and given that DYN has been proposed as a downstream effector of BDNF (8), we can speculate that the changes found in pDYN expression may play a role in the depression-like symptoms observed early in cocaine withdrawal. Conversely, the abstinence-induced up-regulation of pN/OFQ-NOP system in the CPu, indicates this system as an important player in the long-lasting alterations induced by cocaine early in development suggesting a possible role in the relapse phenomena.

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