Opioid system gene expression alterations following poly addictive substance exposure in the amygdala of msP rats: ethanol and 3,4-Methylenedioxymethamphetamine (MDMA) effects.

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The excessive use of one addictive drug may increase the probability to use other psychoactive substances. Poly-drug abuse often entails higher risks than a single drug due to an increase of side effects and heightened propensity to develop addiction. Despite the increase of subjects who abuse multiple drugs, little research has been carried out to explore the comorbidity among specific substance-use disorders and the effects of multiple addictive drugs exposure.
Alcohol is the most common substance abused with other addictive drugs, although any combination of different drugs can be experienced (1, 2). Several studies have revealed that the psychoactive substances induce molecular changes and neurobehavioral adaptations that are critical in shaping addiction trajectory (3).
Among multiple brain circuitries involved, the endogenous opioid system represents an important neurobiological substrate regulating emotional aspects linked to drugs of abuse. In particular, the dynorphin (DYN) and the nociceptin/orphaninFQ (N/OFQ) systems have been implicated in mediating negative emotional states (4), and decrease in drug reward (5), respectively. Here we explored whether concomitant exposure to alcohol and 3,4-methylenedioxymethamphetamine (MDMA), two of the most frequently co-abused psychoactive agents, may affect the expression of these neuropeptidergic systems in the brain. To accomplish this objective, we used marchigian sardinian (msP) rats, an animal line genetically selected for excessive alcohol drinking that is voluntarily take in a binge like pattern.
Rats (n=6/group) were exposed to: a) chronic 10% alcohol for three weeks *ad libitum* (EtOH); b) MDMA (8 mg/kg for five days); c) chronic 10% alcohol for three weeks followed by five days of MDMA i.p. injection. Twenty-four hours after MDMA injection rats were sacrificed, then the amygdala (AM) was rapidly harvested and frozen.
Real-time qPCR analysis showed that three weeks of chronic EtOH intake caused an up-regulation of pDYN mRNA levels in the AM of msP rats (2.61 ± 0.20 vs. vehicle 1.00 ± 0.07, $p < 0.001$) and, similarly, MDMA treatment produced a significant pDYN gene expression increase (2.08 ± 0.22 vs. vehicle 1.00 ± 0.07, $p < 0.001$). Rats exposed to EtOH and subsequently treated with MDMA exhibited the pDYN gene expression up-regulation observed after each single drug exposure (2.22 ± 0.13 vs. vehicle 1.00 ± 0.07, $p < 0.001$).
The pN/OFQ gene expression analysis showed a significant down-regulation induced by EtOH alone (0.57 ± 0.09 vs. vehicle 1.00 ± 0.08, $p < 0.05$), whereas the MDMA exposure did not cause significant changes compared to vehicle. In contrast, rats exposed to EtOH and subsequently treated with MDMA exhibited a significant pN/OFQ mRNA up-regulation (1.57 ± 0.14 vs. vehicle 1.00 ± 0.08, $p < 0.01$).
The present findings reveal that EtOH and MDMA exert the same action on the pDYN gene expression regulation in the AM of msP rats, either alone or in combination. On the contrary, the effects on pN/OFQ gene regulation appear more complex, since EtOH and MDMA alone seem to cause opposite responses whereas their combination induce a significant up-regulation. The reversing rather than the potentiating effects of one drug on another are both likely and, in this regard, our data indicates that both pDYN and pN/OFQ may contribute to the peculiar effects elicited by multiple addictive drug exposure.

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