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CHRONIC ALCAR TREATMENT FAILS TO PREVENT COCAINE SENSITIZATION AND MODIFIES THE STATE OF PHOSPHORYLATION OF DARPP-32

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Acetyl-L-carnitine (ALCAR) is the acetyl ester of carnitine and both carnitine and ALCAR play an essential role in fatty acid oxidation. ALCAR has been reported to be effective in depression and Alzheimer's disease. In our previous study we have demonstrated that a 7-day treatment with ALCAR in rats increased dopamine and serotonin output in the Nucleus Accumbens (NAc). In view of the ability of ALCAR to modify dopaminergic transmission, we first studied whether chronic ALCAR administration was able to prevent the development of cocaine sensitization. This experiment was carried out using 4 groups of animals: 1) ALCAR (ALCAR 10 mg/kg b.i.d for 3 weeks); 2) COCA sensitized animals (cocaine 40 mg/kg every second day for 2 weeks); 3) ALCAR+COCA (a 7-day ALCAR pretreatment and then the concomitant administration of ALCAR and cocaine sensitization protocol); 4) CTR (saline administration). In order to assess the degree of cocaine sensitization, we measured the stereotypy score of rats of all experimental groups after a 10 mg/kg cocaine challenge. The CTR group did not show any stereotyped behavior, while the COCA group was sensitized to cocaine, as expected. The ALCAR+COCA group showed an intense degree of behavioral sensitization, indicating that ALCAR failed to prevent the development of coca sensitization. Moreover, the animals treated for 3 weeks with ALCAR when challenged with cocaine showed a stereotyped behavior and seemed to present a cross-sensitization to cocaine. In order to study whether the mechanisms underlying the effects of chronic ALCAR and cocaine sensitization were dependent on a similar activation of dopaminergic pathways, we measured the phosphorylation state of DARPP-32 (Dopamine and cAMP-regulated phosphoprotein of M_r 32,000), which is considered a regulator of efficacy of dopaminergic transmission. DARPP-32 is a phosphoprotein that can function either as a kinase- or a phosphatase-inhibitor, depending on the phosphorylation of specific amino acid residues. Recent studies showed that a single exposure to cocaine increases DARPP-32 phosphorylation at Thr 34, which is the protein phospatase-1 inhibitor (PP-1) species. When PP-1 is inhibited, the down-stream targets (such as voltage ion channels, NMDA and AMPA channels) are phosphorylated and so activated. After chronic cocaine treatment, DARPP-32 is predominantly phosphorylated at Thr 75, which is the PKA inhibitor species. In this state of phosphorylation, the down-stream effectors are dephosphorylated and so they are inactive. These adaptations seem to have the role to balance the stimulation of dopaminergic system induced by chronic cocaine. In this study we investigated by immunoblotting using phosphorylation-state specific antibodies whether the state of phosphorylation of DARPP-32 would be different in the ALCAR, COCA, ALCAR+COCA, and CTR groups. Preliminary results showed that in the ALCAR group there was a significant increase in phospho-Thr34-DARPP-32 and a decrease in phospho-Th75 species, while in cocaine sensitized animals there was an increase in phospho-Thr75-DARPP-32 and a reduction of phospho-Thr34-DARPP-32, both in the NAc and striatum. Moreover, in the COCA+ALCAR group the state of phosphorylation of DARPP-32 was similar to that observed in the CTR group, even though in the NAc there was a significant increase in phospho-Thr75-DARPP-32. These data could support the hypothesis that ALCAR modulates the dopaminergic transmission in a tonic way, while cocaine modifies it in a phasic mode.