Metabolic interactions between glutamatergic and dopaminergic neurotransmiter systems are mediated through D1, but not D2, dopamine receptors

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Introduction: Neurometabolic interactions between dopaminergic and glutamatergic neurotransmissions are known to play an important role in modulating information processing in the brain under normal conditions or during adaptive and plastic responses to pathophysiological states, such as Parkinson's disease, schizophrenia, drug abuse and personality disorders (Abekawa et al., Brain Res., 2000, 867: 250-4; David and Abraini, Eur. J. Neurosci., 2001, 13: 2157-64). However, even though the reciprocal modulations between glutamate-dopamine systems constitute a topic of clinical and scientific relevance, little is know about the mechanism of this interaction. High resolution ^{13}C NMR spectroscopy has been shown to provide a particularly useful tool to investigate glutamatergic neurotransmission and the glutamine-glutamate cycle activity (Cruz and Cerdán, NMR Biomed., 1999, 12: 451-62, García-Espinosa et al., Neurochem. Int., 2004, 45, 297-303). Results from our laboratory showed previously that D1 receptor suppression resulted in significant increases in the glutamine-glutamate cycle and associated glutamatergic neurotransmission. Here we report on the interactions between dopaminergic and glutamatergic neurotransmissions by estimating qualitatively the activity of the glutamine-glutamate cycle in transgenic mice knocked out in the D2 dopamine receptor. Our results disclose new evidences on the role of D2 receptors in the modulation of glutamate-dopamine interactions. In addition, we report here on the operation of the glutamine cycle in D1 (+/+) and D1 (-/-) mice treated with reserpine, a dopamine-depleting drug.

Methods: Gene targeting technology was used to generate D1 or D2 receptor knock-out mice: D1 (+/+), D1 (-/-), D2 (+/+) and D2 (-/-). D1 (+/+) and D1 (-/-) rats received an 18-hr pretreatment of reserpine (5 $mg.kg^{-1}$ body weight) before the ^{13}C labeled substrate infusion. Animals were anaesthetized with 1.5% isoflurane (in 95% O_2) and received a 60-min infusion of (1,2-$^{13}C_2$) acetate (24 $\mu mol.min^{-1}.100g^{-1}$ body weight) in the left jugular vein. The brains were funnel frozen and extracts were prepared and analyzed by ^{13}C NMR (125.13 MHz, pH 7.2; 25ºC) with proton-decoupling applied only during the acquisition. ^{13}C NMR spectra were simulated completely using the WINDAYSY program (Bruker Biospin, Rheinstetten, Germany), calculating the areas of every observed resonance relative to the unchanged inositol carbons.

Results: Figure 1A summarizes the relative ^{13}C incorporation in the doublet resonances of cerebral glutamate (GluC4d) and glutamine C4 (GlnC4d) in the different D1 (+/+) and D1 (-/-) reserpine-treated mice used in this study. Results obtained during our previous study are also presented as reference values for the comparisons. Figure 1B depicts the same relative patterns of ^{13}C incorporation for D2 (+/+) and D2 (-/-) mice. Treatment with reserpine increased significantly glutamine C4 labelling in D1 (+/+) and D1 (-/-), reaching in both cases a value similar to the one in the case of D1 -/- without reserpine treatment. These results reveal a similar increase in glutamine synthase activity in all reserpine-treated mice. However, D2 receptor expression did not affect significantly both glutamate C4 and glutamine C4 labelling, suggesting that D2 receptors are not involved in the interactions between dopaminergic and glutamatergic neurotransmissions.

Discussion and Conclusions: Our results confirm the important interaction between dopaminergic and glutaminergic neurotransmissions, modulated by D1 but not by D2 receptors. Removal of D1 receptors or reserpine treatment appears to induce a reactive increase in the glutamine cycle activity and glutamatergic neurotransmission. Nevertheless, removal of D2 receptors does not affect glutamine synthesis and thus, D2 receptors do not seem to participate in the modulation of the glutamatergic neurotransmission. Notably, our findings imply that neurological disturbances associated to drug addiction or Parkinson disease may be investigated in the animal or even in the human brain by ^{13}C NMR. Moreover, our results suggest that a successful treatment of these disorders should address both, glutamatergic and dopaminergic neurotransmissions.

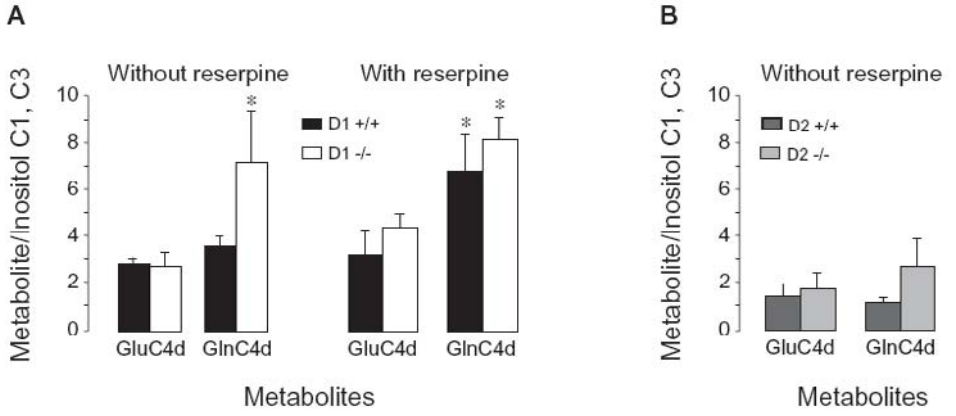

Fig.1: Relative ^{13}C incorporation in the doublet resonances of cerebral glutamate (GluC4d) and glutamine (GlnC4d) in the brain extracts of D1 (+/+) and D1 (-/-) mice with and without a pretreatment of reserpine (Fig.1A) and in D2 (+/+) and D1 (-/-) without reserpine (Fig.1B). Values are means ± SD. * Significantly different from GlnC4d in D1 (+/+) without reserpine (P<0.003).