Transgenic knock-out mice in D1 dopamine receptors have increased glutamine cycle activity as detected by 13C NMR

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Introduction.

Neurometabolic interactions between the glutamatergic and dopaminergic neurotransmissions are of vital importance for the integration of crucial cerebral functions. Dopaminergic neurotransmission is modulated by the primary stimulatory dopamine receptor-type D1 and plays an important role in motor control, cognition, motivation and emotional processing. Its abnormalities are associated to Parkinson's disease, schizophrenia, drug abuse and personality disorders. Previous studies reported evidences that dopaminergic hyperactivity may lead to hypofunction of glutamate system, suggesting a decrease in the glutamine cycle activity (Abekawa et al. Brain Res. 2000, 250-4). It was also shown that inhibition of the metabotropic glutamate receptor, modulates D1 receptor neurotransmission and locomotor activity (David and Abraini, Eur. J. Neurosci, 2001, 13, 2157-2164). These observations strongly supported the existence of interactions between dopaminergic and glutamatergic neurotransmissions, but still much controversy exists as if glutamate transmission stimulates or attenuates dopamine release and locomotor activity. ^{13}C NMR spectroscopy has been shown to be a particularly useful to investigate glutamatergic neurotransmission and the glutamine-glutamate cycle activity. Here we report on the interactions between dopaminergic and glutamatergic neurotransmissions by evaluating qualitatively the activity of the glutamine cycle in transgenic knock out mice lacking the D1 dopamine receptor.

Methods.

Gene targeting technology was used to generate D1 receptor knock-out mice: wild type mice (D1 +/+), heterozygous mutants (D1 +/-) or homozygous mutants (D1 -/-). All animals were anaesthetized with isoflurane, receiving a 60 minutes infusion of (1,2-$^{13}C_2$) acetate (24 $\mu mols.min^{-1}.100g^{-1}$) in the left jugular vein. The brains were then funnel frozen and extracts were prepared and analyzed by ^{13}C NMR (125.13 MHz, pH 7.2; 25ºC) with proton-decoupling only during the acquisition. ^{13}C NMR spectra were simulated completely using the WINDAYSY program (Bruker Biosopin, Rheinstetten, Germany), calculating the corresponding areas relative to the unchanged inositol carbons.

Results.

The Table summarizes the relative ^{13}C incorporation in the doublet resonances of cerebral glutamate (GluC4d) and glutamine C4 (GlnC4d) in the different mice used in this study.

Mice type	GluC4d	GlnC4d
D1 +/+	2.924 ± 0.313	3.631 ± 0.408
D1 +/-	2.527 ± 0.148	3.923 ± 0.759
D1 -/-	2.843 ± 0.529	7.203 ± 2.170

D1 receptor expression did not affect apparently glutamate C4 labelling. However, it increased significantly glutamine C4 labelling in (+,-) and (-/-) mice, revealing an increase in glutamine synthase activity in D1 defficient mice.

Discussion.

Using this experimental approach we are able to describe unambiguously an important interaction between dopaminergic and glutaminergic neurotransmissions. Removal of D1 receptors induces an increase in the glutamine cycle activity and glutamatergic neurotransmission. Present results suggest that a successful treatment of the disorders involving dopaminergic neurotransmission must address also the concomitant disturbances in glutamatergic neurotransmissions. The important neurometabolic interactions between these two neurotransmitter systems may be studied by ^{13}C NMR in animals and humans in vivo.

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