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Brief Communication

PET imaging for attention deficit preclinical drug testing in neurofibromatosis-1 mice

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ABSTRACT

Attention system abnormalities represent a significant barrier to scholastic achievement in children with neurofibromatosis-1 (NF1). Using a novel mouse model of NF1-associated attention deficit (ADD), we demonstrate a presynaptic defect in striatal dopaminergic homeostasis and leverage this finding to apply [^{11}C]-raclopride positron-emission tomography (PET) in the intact animal. While methylphenidate and L-Deprenyl correct both striatal dopamine levels on PET imaging and defective attention system function in *Nf1* mutant mice, pharmacologic agents that target de-regulated cyclic AMP and RAS signaling in these mice do not. These studies establish a robust preclinical model to evaluate promising agents for NF1-associated ADD.

Introduction

Children with the neurofibromatosis-1 (NF1) inherited cancer syndrome are prone to the development of benign and malignant tumors (Gutmann et al., 1997). However, the most prevalent neurologic problem in children reflects deficits in attention, such that 60–80% of affected individuals exhibit ADD symptomatology (Hyman et al., 2005). To define the neurochemical basis for NF1-associated attention deficits, we employed a unique *Nf1* genetically-engineered mouse (GEM) model (Brown et al., 2010a). These *Nf1* mutant mice demonstrate reduced exploratory behaviors, as well as selective and non-selective attention abnormalities, where the non-selective attention deficit was restored to wild-type levels following treatment with methylphenidate (MPH) or L-Dopa. Consistent with this pharmacologic correction, *Nf1* mutant mice have reduced striatal dopamine levels revealed by high-performance liquid chromatography (HPLC).

To translate these basic research findings to a preclinical therapeutic drug testing platform, we applied neurotransmitter imaging methods and behavioral analyses to monitor this dopaminergic deficit in the intact animal. In the current study, we establish that this dopaminergic defect in *Nf1* mutant mice is presynaptic, and can be quantified by [^{11}C]-raclopride PET imaging. We further demonstrate that correction of a non-selective attention deficit in *Nf1* mutant mice with MPH and L-Deprenyl correlates with normalization of raclopride binding *in vivo*, whereas therapies that target NF1-regulated RAS and cyclic AMP (cAMP) defects in these mice correct neither the behavioral nor the imaging abnormalities.

Materials and methods

Mice

Nf1+/−^{GFAP}CKO (CKO) and control littermate *Nf1*$^{flox/flox}$ (WT) mice were maintained on an inbred C57BL/6 background (Bajenaru et al., 2003; Brown et al., 2010a) with *ad libitum* access to food and water. *Nf1*+/−^{GFAP}CKO mice are *Nf1*+/− mice (reduced *Nf1* gene expression in all cells in the brain and body) which harbor complete *Nf1* gene loss in GFAP-expressing cells. All experiments were performed on 3–4 month old mice under active Animal Studies Committee protocols. Independently-generated groups of WT and CKO mice were used for the baseline PET imaging studies (Fig. 1), MPH and L-Deprenyl treatments (Fig. 2), and Lovastatin and Rolipram treatments (Fig. 3).

Abbreviations: ADD, Attention Deficit Disorder; cAMP, cyclic adenosine monophosphate; CKO, conditional knockout; CT, computerized tomography; DA, dopamine; GEM, genetically-engineered mouse; MPH, methylphenidate; PET, positron emission tomography.

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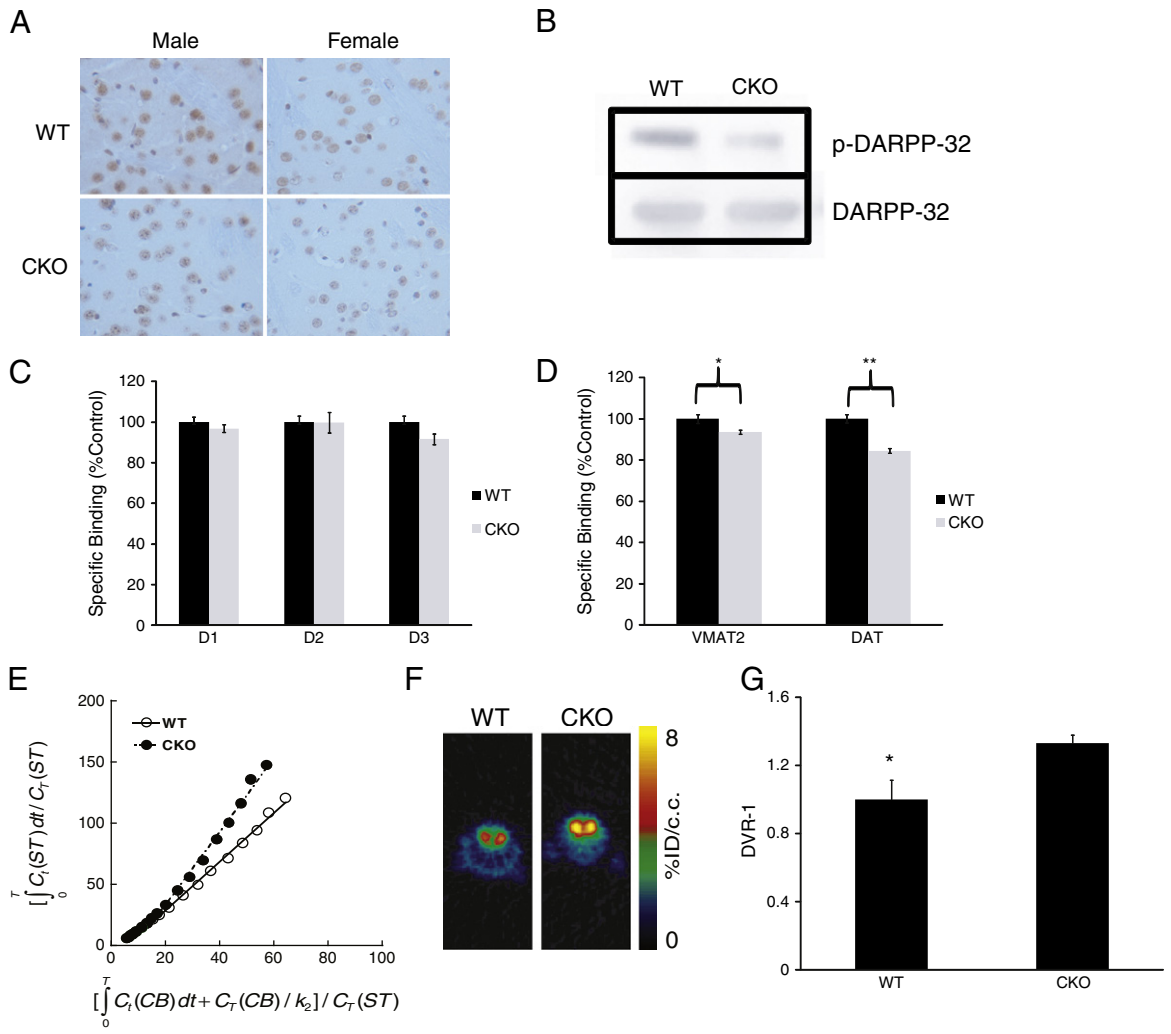

Fig. 1. *Nf1* CKO mice with attention system defects demonstrate a presynaptic DA defect, which can be visualized by PET imaging. (A) IHC reveals decreased DARPP32 phosphorylation (p-DARPP32) in the striatum of both male and female mice relative to WT littermates ($p = .01$; $N = 8$). (B) Western blot shows a 5.8-fold decrease in p-DARPP32 (following normalization to total DARPP32 levels) in CKO compared to WT mice. *In vitro* quantitative receptor autoradiography demonstrates no change in postsynaptic D1, D2 and D3 DA receptor expression in CKO mice relative to control WT littermates (C), whereas presynaptic VMAT2 and DAT expression is reduced (D; ~10%; $p = .03$, VMAT2, $p = .0004$, DAT; $N = 6$). (E) Representative Logan plots for WT and CKO mice (using the cerebellum as the reference region) are shown along with (F) representative [^{11}C]-raclopride transverse micro-PET images (summed across 5–60 min). The colorscale bar indicates the normalized peak uptake (percent injected dose per cubic centimeter tissue; %ID/cc). (G) In a cohort of WT and CKO mice, [^{11}C]-raclopride binding was increased in the striatum (Str) of CKO mice compared to control WT littermates on PET imaging ($p = .03$; $N = 4$ per genotype). C_t = tissue radioactivity at time t; T = time point of each frame of PET scanning course.

Radioligand and compound preparation

[^{3}H]-SCH23390, [^{3}H]-raclopride, [^{3}H]-Win 35428 (PerkinElmer Life and Analytical Sciences; Shelton, CT), [^{3}H]-WC-10, and [^{3}H]-a-Dihydrotetrabenazine ([^{3}H]-DTBZ; American Radiolabeled Chemicals; St. Louis, MO) were used as previously reported (Xu et al., 2009).

Quantitative receptor autoradiography

20-micron coronal sections were generated from flash-frozen brains and processed for autoradiography as previously described (Xu et al., 2009, 2010). Sections were incubated for 60 min at room temperature with [^{3}H]-SCH23390 (D1 receptors), [^{3}H]-raclopride (D2 receptors), [^{3}H]-WC-10 (D3 receptors), [^{3}H]-DTBZ (VMAT2), or [^{3}H]-Win 35428 (DAT) (Frey et al., 1997; Savasta et al., 1986; Xu et al., 2010). Non-specific binding was determined following the addition of 1 μM (+)-butaclamol, 1 μM eticlopride, 1 μM tetrabenazine, or 1 μM nomifensine, respectively. Quantitation was performed using the Beta Imager 2000Z Digital Beta Imaging System (Biospace, France) and the Beta-Vision Plus program (Xu et al., 2010).

Small animal PET analysis

Brain PET imaging was performed under isoflurane anesthetization. Mice were injected with ~200 μCi of [^{11}C]-raclopride (~2500 Ci/mmol specific activity) *via* the tail vein and data acquired as 1 h dynamic scans using the Siemens Focus F220 and Inveon scanners (Siemens Medical Solutions USA, Inc.). Acquired list mode data were converted into a 3D set of sinograms and binned into 5×60-sec, 5×2-min and 9×5-min time frames for processing using filter back projection algorithm with attenuation and scatter corrections. Using 5–60 min summarized PET images and co-registered CT images as references, regions of interest (ROI) for the striatum (ST) and cerebellum (CB) were manually drawn with the software Acquisition Sinogram Image PROcessing using IDL's Virtual Machine™ (ASIPro VM™) to obtain radioactivity uptake (nCi/c.c.) curves over time. Representative PET and CT co-registration images are shown in Supplementary Fig. 1A and B, respectively. Logan DVR-1 (binding potential) analyses were performed using the cerebellum as the reference region, with a K2 value of 0.2 (Logan, 2000).