Endocannabinoids Activate Transient Receptor Potential Vanilloid 1 Receptors to Reduce Hyperdopaminergia-Related Hyperactivity: Therapeutic Implications

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Background: *Knockout (KO) mice invalidated for the dopamine transporter (DAT) constitute a powerful animal model of neurobiological alterations associated with hyperdopaminergia relevant to schizophrenia and attention-deficit/hyperactivity disorder (ADHD).*

Methods: *Because of continuously increasing evidence for a neuromodulatory role of endocannabinoids in dopamine-related pathophysiological responses, we assessed endocannabinoid signaling in DAT KO mice and evaluated the ability of endocannabinoid ligands to normalize behavioral deficits, namely spontaneous hyperlocomotion in these mice.*

Results: *In DAT KO mice, we found markedly reduced anandamide levels, specifically in striatum, the dopamine nerve terminal region. Furthermore, three distinct indirect endocannabinoid agonists, the selective anandamide reuptake inhibitors AM404 and VDM11 and the fatty acid amidohydrolase inhibitor AA5HT, attenuated spontaneous hyperlocomotion in DAT KO mice. The hypolocomotor effects of AM404, VDM11, and AA5HT were significantly attenuated by co-administration of the transient receptor potential vanilloid 1 (TRPV1) antagonist capsazepine but not the selective cannabinoid type 1 (CB1)receptor antagonist AM251. Interestingly, TRPV1 binding was increased in the striatum of DAT KO mice, while CB1 receptor binding was unaffected.*

Conclusions: *These data indicate a dysregulated striatal endocannabinoid neurotransmission associated with hyperdopaminergic state. Restoring endocannabinoid homeostasis in active synapses might constitute an alternative therapeutic strategy for disorders associated with hyperdopaminergia. In this process, TRPV1 receptors seem to play a key role and represent a novel promising pharmacological target.*

Key Words: ADHD, psychosis, dopamine transporter, knockout mice, anandamide uptake inhibitors, FAAH inhibitors, CB1 receptor

In the central nervous system (CNS), endocannabinoids act as neuromodulators to fine-tune neuronal firing and neurotransmitter release in a dynamic, activity-dependent manner (Fowler and Jacobsson 2002; Giuffrida et al 2001; Goutopoulos and Makriyannis 2002). There is, in particular, ever increasing evidence for a dynamic multilevel dopaminergic (DAergic) and endocannabinoid interaction (reviewed in Rodriguez De Fonseca et al 2001; Fernandez-Ruiz et al 2002) critically implicated in neurophysiological, endocrine, and metabolic responses.

Elegant electrophysiological studies have shown that endocannabinoids can act as retrograde signaling molecules to modulate glutamate and gamma-aminobutyric acid (GABA) mediated regulation of the activity of mesotelencephalic dopaminergic neurons both at the somatic (substantia nigra/ventral tegmental area) (Melis et al 2004; Riegel and Lupica 2004) and the synaptic terminal (striatum/nucleus accumbens) (Robbe et al 2002) regions. Furthermore, studies either with mice invalidated for the cannabinoid type 1 (CB1) receptor (Di Marzo et al 2001b; Lichtman et al 2002), the predominant cannabinoid receptor in the brain, or with the specific CB1 receptor antagonists SR141716A (Rinaldi-Carmona et al 1994) and its analogues AM251 and AM281 (Gatley et al 1996; Gifford et al 1997) support a key role for the endocannabinoid system in dopamine (DA)-related adaptative processes that control feeding, affect, and learning and memory.

However, the way in which dopaminergic and endocannabinoid inputs are interregulated and the integrated role of the endocannabinoid-DA interface in the pathophysiology of CNS disease states still remain unknown. Notably, psychostimulants and dopaminergic agonists were shown to decrease the striatal levels of anandamide (Patel et al 2003), the principal CNS endocannabinoid, on acute systemic administration. In addition, experimental parkinsonism was shown to be associated with enhanced endocannabinoid signaling in the basal ganglia (Di Marzo et al 2000; Gubellini et al 2002). On the other hand, when dopaminergic agonists were applied locally in the striatum, they increased anandamide contents in this region (Giuffrida et al 1999; Centonze et al 2004). Seemingly contradictory hypotheses have been put forward also with regard to the pathogenic or protective role of cannabinoid signaling in CNS pathologies associated with forebrain DA dysfunction, such as psychosis and attention-deficit/hyperactivity disorder (ADHD). Thus, a putative antipsychotic pharmacological profile has been proposed for antagonists of the CB1 receptor (Poncelet et al 1999), suggesting the possibility of an increased endocannabinoid neurotransmission in psychosis. Conversely, a possible therapeutic potential for indirect cannabinoid agonists, i.e., ligands that increase the endocannabinoid tone by blocking the uptake and/or catabolism of cannabimimetic lipids, in DA-related disorders has also been advanced (Beltramo et al 2000). In view of the current state of the literature, further studies using

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animal models for DA-related pathologies are warranted to clarify the role of endocannabinoid signaling in the respective pathologies.

Genetically engineered mice invalidated for the dopamine transporter (DAT) have been previously generated (Giros et al 1996) and constitute a valuable model to study neurobiological alterations due to hyperdopaminergia that could be relevant to schizophrenia and ADHD (Gainetdinov et al 2002). Dopamine transporter knockout (KO) mice are hyperdopaminergic, hyperactive, and display perturbed sensorimotor gating, habituation, and cognitive performances. Strikingly, target validation and biochemical studies exploring this model have been limited to DAergic–and, to a lesser extent, to serotonergic/glutamatergic–systems (reviewed in Gainetdinov et al 2002).

Here, we used DAT KO mice 1) to investigate the consequences of constitutive hyperdopaminergia on endocannabinoid homeostasis, and 2) to evaluate the ability of ligands that target the endocannabinoid system to normalize behavioral deficits related to altered dopaminergic function.

Methods and Materials

Animals

Dopamine transporter wild-type (WT), heterozygous (HZ), and KO mice of the B6xD2F1 background were used in this study. Breeding, genotyping, and maintenance of the mice were as previously described (Morice et al 2005). All experiments were carried out in accordance with the European Communities Council Directive (86/809/EEC) regarding the care and use of animals for experimental procedures and approved by the local ethical committee.

Drugs

The AM251and AM404 (both from Tocris, Illkirch, France) and capsazepine (Sigma, L'Isle d'Abeau Chesnes, France), as well as VDM11 and AA5HT (synthesized in Dr. Di Marzo's laboratory, as previously described, Bisogno et al 1998; De Petrocellis et al 2000), were dissolved in 2% dimethyl sulfide (DMSO; Sigma), 2% Cremophor EL (Sigma), 96% saline immediately before injection at the appropriate doses.

Anandamide Measurements

Animals were decapitated and brains were quickly removed on ice. The striatum, hippocampus, cortex, and cerebellum were dissected, individually weighed, frozen rapidly, and stored to −80°C until use. Tissue anandamide contents were measured as described (Felder et al 1996). Results are expressed as ng anandamide per mg protein. Statistical analysis was performed with one-way analysis of variance (ANOVA) and Duncan's post hoc tests.

Locomotor Activity Measurements

Motor activity was analyzed in a computerized actimeter (Immetronic, Bordeaux, France). In a first series of experiments, animals were injected with either AM251 (1, 3 and 10 mg/kg) or vehicle, after a 20-minute habituation in the actimeter. Horizontal locomotor activity (measure of ambulatory behavior) was measured for 60 minutes after the injection. In parallel experiments, mice were injected with either AM404 (.3, 1, and 3 mg/kg), VDM11 (2 and 5 mg/kg), AA5HT (2 and 5 mg/kg), or vehicle under the same schedule. The range of doses studied for each compound were chosen from previous literature, as those being efficacious in animal disease models but displaying little or no activity in normal mice (Baker et al 2001; de Lago et al 2005). The 60-minute time period during which locomotor activity was monitored was chosen based on studies showing that AM404 elevation of plasma anandamide is significant within the first hour after its administration (Giuffrida et al 2000). A similar time effect was reported after repeated systemic administration of the other selective inhibitors of endocannabinoid administration (de Lago et al 2005). In a second series of experiments, mice were injected with AM251 (3 mg/kg) or vehicle co-administered with AM404 (3 mg/kg) or vehicle, after a 20-minute habituation in the actimeter. Horizontal locomotor activity (measure of ambulatory behavior) was measured for 60 minutes after the injection. In parallel experiments, mice were injected with capsazepine (5 mg/kg) or vehicle co-administered with AM404 (3 mg/kg) or vehicle under the same schedule. In the last series of experiments, mice were injected with either 1) vehicle; 2) VDM11 (5 mg/kg) or AA5HT (5 mg/kg); 3) VDM11 (5 mg/kg) in combination with AM251 (3 mg/kg) or AA5HT (5 mg/kg) in combination with AM251 (3 mg/kg); or 4) VDM11 (5 mg/kg) in combination with capsazepine (5 mg/kg) or AA5HT (5 mg/kg) in combination with capsazepine (5 mg/kg), again under the same time schedule. All injections were intraperitoneal (IP) at a volume of 10 mL/kg. Statistical analysis was performed with one, two-way (genotype × treatment or treatment 1 × treatment 2) or three-way (genotype × treatment 1 × treatment 2) ANOVA and Duncan's post hoc tests.

Quantitative Receptor Autoradiography

Dopamine transporter WT and KO mice were decapitated and brains were rapidly removed and stored at −80°C until use. Coronal slices 20 μm thick at the level of the striatal complex were cut on a cryostat and mounted on gelatin-coated slides. Receptor binding for CB1 receptors was performed as described previously (Adams et al 1998) using [3H]CP-55940 (NEN, Les Ulis, France). Nonspecific binding was determined by incubating [3H]CP-55940 in the presence of 1 μm WIN55,212. Receptor binding for vanilloid receptor 1 (VR1) receptors was performed as described previously (Roberts et al 2004) using [3H]resiniferatoxin (NEN). Nonspecific binding was determined by incubating

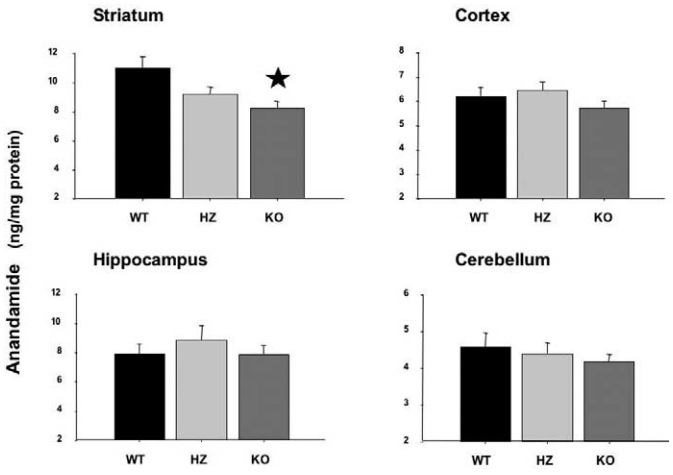

Figure 1. Region-specific alteration of endocannabinoid levels in DAT KO mice. Tissue levels of the endocannabinoid anandamide in the striatum, cortex, hippocampus, and cerebellum of DAT wild-type (WT), heterozygous (HZ), and knockout (KO) mice. Anandamide levels are reduced exclusively in the striatum of DAT KO mice as compared with WT littermates. Data represent mean ± SEM of $n = 8$ animals per group; stars indicate $p < .05$ as compared with WT (one-way ANOVA, Duncan's post hoc). DAT, dopamine transporter; KO, knockout; WT, wild-type; HZ, heterozygous; ANOVA, analysis of variance.