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Decreased vesicular monoamine transporter 2 (VMAT2) and dopamine transporter (DAT) function in knockout mice affects aging of dopaminergic systems

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ABSTRACT

Dopamine (DA) is accumulated and compartmentalized by the dopamine transporter (DAT; SLC3A6) and the vesicular monoamine transporter 2 (VMAT2; SLC18A2). These transporters work at the plasma and vesicular membranes of dopaminergic neurons, respectively, and thus regulate levels of DA in neuronal compartments that include the extravesicular cytoplasmic compartment. DA in this compartment has been hypothesized to contribute to oxidative damage that can reduce the function of dopaminergic neurons in aging brains and may contribute to reductions in dopaminergic neurochemical markers, locomotor behavior and responses to dopaminergic drugs that are found in aged animals. The studies reported here examined aged mice with heterozygous deletions of VMAT2 or of DAT, which each reduce transporter expression to about 50% of levels found in wild-type (WT) mice. Aged mice displayed reduced locomotor responses under a variety of circumstances, including in response to locomotor stimulants, as well as changes in monoamine levels and metabolites in a regionally dependent manner. Several effects of aging were more pronounced in heterozygous VMAT2 knockout (KO) mice, including aging induced reductions in locomotion and reduced locomotor responses to cocaine. By contrast, some effects of aging were reduced or not observed in heterozygous DAT KO mice. These findings support the idea that altered DAT and VMAT2 expression affect age-related changes in dopaminergic function. These effects are most likely mediated by alterations in DA compartmentalization, and might be hypothesized to be exacerbated by other factors that affect the metabolism of cytosolic DA.

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1. Introduction

The neurotransmitter dopamine (DA) is located in neurotransmitter vesicles, extracellular spaces, and extravesicular cytoplasmic compartments. In the extravesicular cytoplasmic compartments of dopaminergic neurons, DA can produce free radicals and/or form adducts with important cellular proteins that can contribute to cellular stress and damage (Uhl, 1998). Extravesicular cytoplasmic DA concentrations are regulated by the serial actions of two transporters: the plasma membrane dopamine transporter (DAT), which mediates neuronal uptake of DA from extracellular spaces into this compartment (Giros et al., 1992; Kilty et al., 1991; Nirenberg et al., 1996b; Shimada et al., 1991; Usdin et al., 1991), and the vesicular monoamine transporter 2 (VMAT2), which translocates DA from this extravesicular cytoplasmic compartment into synaptic vesicles (Erickson et al., 1992; Gonzalez et al., 1994; Liu et al., 1994, 1996, 1992; Merickel et al., 1995; Nirenberg et al., 1996a; Peter et al., 1996, 1995; Roghani et al., 1996; Surratt et al., 1993; Takahashi and Uhl, 1997). Amphetamine-like compounds block VMAT2-mediated transport of DA into synaptic vesicles, elevating extravesicular cytoplasmic DA levels and releasing DA via mechanisms that include DAT-mediated reverse transport. Elevated intracellular levels of DA may contribute to the toxic effects of amphetamine derivatives on DA neurons (Larsen et al., 2002; Lotharius and O'Malley, 2001; Seiden and Ricaurte, 1987).

We and others have cloned the genes that encode these transporters (Giros et al., 1992; Kilty et al., 1991; Shimada et al., 1991;

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Usdin et al., 1991) and produced KO mice with reduced levels of DAT and VMAT2 expression (Donovan et al., 1999; Fon et al., 1997; Giros et al., 1996; Mooslehner et al., 2001; Sora et al., 1998; Takahashi et al., 1997; Wang et al., 1997). Homozygous VMAT2 KO mice die in the first few postnatal days (Takahashi et al., 1997), but heterozygous +/− mice are viable, express about one-half WT levels of VMAT2 mRNA and protein, display relatively modest changes in brain monoamine levels and in a number of motor and behavioral tests (Takahashi et al., 1997). Homozygous DAT KO mice are small and display striking hyperlocomotion (Giros et al., 1996; Sora et al., 1998). However, heterozygous DAT KO mice, expressing one-half of WT levels of DAT mRNA and protein, display weight and locomotion that are nearly identical to those found in WT mice.

Heterozygous mice with one-half of WT levels of VMAT2 and DAT mRNA and protein expression also display differential sensitivities to the dopaminergic toxin MPTP. Dopaminergic toxicity induced by MPTP is profoundly enhanced in VMAT2 +/− mice (Takahashi et al., 1997) and reduced in DAT +/− mice (Fumagalli et al., 1998; Gainetdinov et al., 1997; Takahashi et al., 1997). When DAT is over-expressed by about one-third in transgenic mice, MPTP toxicity is enhanced (Donovan et al., 1999). These data suggest that DAT and VMAT2 expression influences DA compartmentalization of MPP+, the active metabolite of MPTP, which greatly affects the toxicity of this compound (Javitch et al., 1985, 1984). These results also validate these KO strains as reasonable *in vivo* model systems for dopaminergic neuronal toxicity that might depend on mechanisms that regulate DA compartmentalization.

Dopaminergic neurons are especially vulnerable to the effects of aging, as manifested in biochemical, behavioral and pharmacological assessments of many mammalian species (see Morgan and Finch, 1988, for review). DA compartmentalization has been suggested to be an important mediator of the potential toxicity of DA involved in these functional and neurochemical impairments (Hastings et al., 1996a; Lotharius and Brundin, 2002; Rabinovic et al., 2000; Uhl, 1998). Indeed, a human α-synuclein mutant cell line with reduced VMAT2 expression has been shown to have higher cytoplasmic DA and superoxide levels (Lotharius et al., 2002). Mice with reduced levels of expression of DAT and VMAT2 are thus especially good candidates to display altered availability of DA in the extravesicular cytoplasmic neuronal compartments in which oxidation and/or adduct formation could contribute to aging effects on dopaminergic systems. The alterations in transporter expression found in heterozygous KO mice (Sora et al., 1998; Takahashi et al., 1997) lie near the reported ranges of human individual variation in levels of expression of these proteins (Wilson et al., 1996a, 1996b). These mice thus provide models for the effects of common human allelic variations on aging of dopaminergic brain systems. We now report assessments of DA-related behavioral and neurochemical function in aged DAT +/− and VMAT2 +/− mice in comparison to WT littermates. These studies provide data that is consistent with the hypothesis that age-related declines in DA function, and associated behavioral responses, are affected by the levels of expression of genes that influence the cellular compartmentalization of DA.

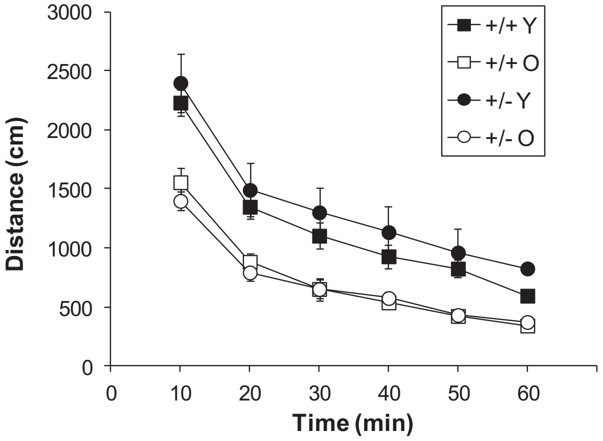
2. Materials and methods

2.1. Animals

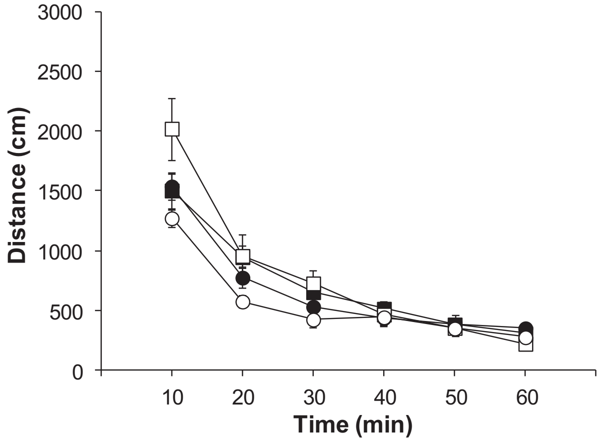
DAT +/− and VMAT2 +/− KO mice and WT littermates were bred from heterozygote–heterozygote crosses of KO mice from mixed C57BL6J-129Sv genetic backgrounds (Sora et al., 1998; Takahashi et al., 1997). Genotypes were confirmed by PCR as previously described in those publications. Mice were group-housed in standard ventilated housing chambers at 24 °C in 50% relative humidity on a 12/12 h light/dark cycle with lights on at 7:00 A.M. and off at 7:00 P.M., with *ad libitum* access to standard mouse chow and water. All experiments were conducted in accordance with American Association for Laboratory Animal Care and all applicable NIH guidelines. Separate groups of mice were examined from 3 to 6 months of age (young) and from 18 to 24 months of age (old) for each experiment. Each study compared heterozygote KO mice to WT littermates.

2.2. Behavioral testing procedures

Locomotor activity was assessed as total distance traveled when mice were placed individually in 46 × 25 × 19 cm clear plastic cages in Optovarimax activity monitors (Columbus Instruments, Columbus, OH), under dark, sound-attenuated conditions. Young (3–5 months) and aged (18–24 months) DAT KO and VMAT KO mice, and WT littermates, were tested for locomotor activity twice. In the first session, mice were habituated to the apparatus for a period of time, 1 h for VMAT2 KO mice ($N = 54–87$/group) and 3 h for DAT KO mice ($N = 30–68$/group), injected with saline and locomotor activity was measured for two more hours. At least 48 h after this initial test session mice were tested again, but divided into separate drug testing groups. In the second session mice were placed in the apparatus to habituate for the same period of time as the first session (1 h for VMAT2 KO mice and 3 h for DAT KO mice), and then injected with amphetamine or cocaine. For drug testing a between-subjects design was used so that each mouse was tested under only one drug (data was combined for the analysis of locomotion after the initial saline injections). Two doses of amphetamine were tested in VMAT2 KO mice (0.5 mg/kg IP; $N = 13–27$/group; or 1 mg/kg IP; $N = 40–71$/group) and one dose of cocaine (20 mg/kg SC; $N = 19–21$/group). DAT KO mice were injected with amphetamine (1 mg/kg IP; $N = 14–18$/group) or cocaine (10 mg/kg SC; $N = 16–52$/group). After injections locomotor activity was monitored for 2 more hours.



A. Session 1 habituation



B. Session 2 habituation

Fig. 1. Effects of aging on the time-course (10 min time bins) of locomotor activity in VMAT2 +/+ and VMAT +/− mice ($N = 54–87$/group) during (A) initial locomotor habituation (novel, session 1) prior to saline injection, and (B) subsequent locomotor habituation (familiar, session 2) prior to drug injection. Data are expressed as mean ± the standard error of the mean (SEM).