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Genetic alteration in the dopamine transporter differentially affects male and female nigrostriatal transporter systems

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ABSTRACT

Female mice with a heterozygous mutation of their dopamine transporter (+/− DAT) showed relatively robust reductions in striatal DAT specific binding (38–50%), while +/− DAT males showed modest reductions (24–32%). Significant decreases in substantia nigra DAT specific binding (42%) and mRNA (24%) were obtained in +/− DAT females, but not +/− DAT males (19% and 5%, respectively). The effects of this DAT perturbation upon vesicular monoamine transporter-2 (VMAT-2) function revealed significantly greater reserpine-evoked DA output from +/+ and +/− DAT female as compared to male mice and the DA output profile differed markedly between +/+ and +/− DAT females, but not males. No changes in VMAT-2 protein or mRNA levels were present among these conditions. On the basis of these data, we propose: (1) a genetic mutation of the DAT does not exert equivalent effects upon the DAT in female and male mice, with females being more affected; (2) an alteration in the DAT may also affect VMAT-2 function; (3) this interaction between DAT and VMAT-2 function is more prevalent in female mice; and (4) the +/− DAT mutation affects VMAT-2 function through an indirect mechanism, that does not involve an alteration in VMAT-2 protein or mRNA. Such DAT/VMAT-2 interactions can be of significance to the gender differences observed in drug addiction and Parkinson's disease.

1. Introduction

The dopamine transporter (DAT) plays an important role in calibrating the duration and intensity of DA neurotransmission in the central nervous system. The DAT accomplishes this goal by rapid reuptake of dopamine (DA) from the synaptic cleft into presynaptic terminals, and thereby controls the intensity and duration of dopaminergic neurotransmission by setting the concentration of DA in the extracellular space [1]. In addition, the DAT is the site through which amphetamine-like drugs result in reverse transport of DA [2,3] and the site of entry for neurotoxins that are relatively selective for dopaminergic neurons (e.g. MPP+ and 6-hydroxydopamine) [4–7]. Accordingly, the DAT can exert a significant influence upon dopaminergic function in both healthy and diseased conditions.

There exist significant differences in the DAT between females and males, which can contribute to gender differences in conditions ranging from drug addiction to Parkinson's disease [8,9]. The numbers/densities of DATs are greater in female versus male rats [10,11] and assays of DAT function indicate that a more active DAT is present within females versus males [12,13]. These findings of a sex difference in the DAT are not limited to animal studies, as clinical reports have shown greater numbers/densities of DATs within healthy adult women versus men [14–16].

One approach to study DAT function is with the use of mice possessing a mutation in the DAT [2]. However, given the predominant sex difference in DATs, an important issue to address is whether this mutation is processed equally in females and males. There are data which show that striatal DA concentrations of heterozygous mutant DAT (+/− DAT) female mice are significantly decreased as compared with +/− DAT males. No such difference is present between wild type (+/+ DAT) control female and male mice [17]. Accordingly, there exits some basis for the proposal that dopaminergic function is not affected equally in +/− DAT female and male mice, suggesting the existence of an important interaction between sex and a mutation of the DAT.

Abbreviations: ANOVA, analysis of variance; DA, dopamine; DAT, dopamine transporter; EDTA, ethylenediaminetetraacetic acid; KRP, kreb's ringer phosphate; MPP+, 1-methyl-4-phenylpyridinium; 6-OHDA, 6-hydroxydopamine; VMAT-2, vesicular monoamine transporter-2; WT, wild type.

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Additional considerations of significance regarding DAT mutations is that of the extent that other dopaminergic processes are affected, whether these represent direct or indirect effects of the mutation and, again, the potential for differential modulation within females and males. A precedent for this proposition can be traced back to data showing marked dissimilarities in dopaminergic function among different mouse strains. For example, the significant differences in striatal D2 receptors observed among different strains of mice can secondarily alter a variety of responses like neuroleptic-induced catalepsy [18] and amphetamine-induced locomotion [19]. Moreover, strain differences in striatal calmodulin can affect a number of striatal functions, and do so in an unpredictable manner, due to its generalized actions in regulating brain calcium concentrations [20]. Therefore, the importance of considering not only the primary, but also secondary, effects resulting from a targeted mutation can be appreciated. An example of a related dopaminergic function that could be affected by a DAT mutation is that of the vesicular monoamine transporter-2 (VMAT-2). The VMAT-2 sequesters cytoplasmic DA. This function prevents the oxidation of DA in the cytoplasm and thus contributes to the DA available for release. In this way, during normal dopaminergic neurotransmission, the DAT regulates extracellular concentrations of DA, while VMAT-2 regulates cytosolic concentrations of DA and indirectly regulates extracellular DA by affecting the amount of DA available for release from vesicular stores. Therefore, these two transporters play a critical role in dopaminergic function by regulating availability and access of intracellular and extracellular DA. It has been reported that the function of these two transporters may be linked [21,22]. To the best of our knowledge the potential for sex-dependent interactions between the DAT and VMAT-2 have not been investigated.

The information provided from this brief review highlight a key role for the DAT in dopaminergic functioning and the presence of sex-differences in this function. What is not clear is the extent to which a genetic DAT perturbation may be revealed in females and males and the potential for sex-dependent alterations in other dopaminergic functions. The purpose of this report is to evaluate the relationship among these variables. In specific, heterozygous mutant DAT (+/−DAT) and wild type control (+/+ DAT) male and female mice are compared in two series of experiments. First, sex differences in DAT specific binding within various regions of the striatum and substantia nigra and DAT mRNA within the substantia nigra are compared among +/+ and +/− DAT male and female mice. Since it has been observed that striatal DA concentrations and function are more severely affected within +/− DAT female versus male mice [17], in this initial series of experiments we determine whether the protein and message levels of DATs show a corresponding sex differences between +/+ and +/− DAT mice. In a second series of experiments we utilize these +/+ and +/− DAT mice to assess the possibility for a sex-dependent interaction between DAT and VMAT-2 function. To accomplish this goal responses of superfused striatal tissue to an infusion of the VMAT-2 blocker, reserpine, was evaluated within +/+ and +/− DAT male and female mice. As reserpine binds to the VMAT-2 to impair vesicular uptake and storage of DA within nerve terminals [23], it has been used as an agent to assess VMAT-2 function in various animal models [24–26]. To establish whether any potential differences in response to reserpine are attributable to a direct effect of DAT perturbation on the VMAT-2, VMAT-2 specific binding within the striatum and substantia nigra and VMAT-2 mRNA within the substantia nigra was measured among +/+ and +/− DAT male and female mice. With the performance of these experiments within male and female mice where a normal or perturbed DAT function is present, it will be possible to determine whether this DAT mutation differentially affects male and female mice as well as gain an understanding of the sex-dependent interaction that may exist between the DAT and VMAT-2.

2. Materials and methods

2.1. Animals

The mice (3–7 months of age) were male and female littermate wild type controls (+/+ DAT) and heterozygous (+/− DAT) DAT mutant mice derived from the C57BL/6 strain [2] supplied through the generosity of Dr. Marc Caron of Duke University. In these experiments, comparisons between +/+ versus +/− DAT male and female mice were used to evaluate the impact of gender in a condition of a DAT deficit, as opposed to the complete absence of the DAT that would characterize the null mutant (−/− DAT) DAT mice. The use of +/− DAT for comparison with wild type mice was performed for three reasons. First, a DAT deficit, as opposed to a DAT absence (−/− DAT), provides for a more realistic condition that would be applicable and relevant to the clinical setting [27,28]. Second, −/− DAT mice show a marked hypopituitarism with glands being about half the size of that in normal animals [29]. Such secondary endocrine effects may confound some of the sex/hormonal parameters to be evaluated within the proposed experiments. Finally, the interpretations of results derived from −/− DAT mice can sometimes be problematic due to the extreme nature of the deficit and accompanying developmental adaptations that can result from the absence of the DAT [30]. While estrous cycle stage may influence the parameters to be measured, basic sex difference in nigrostriatal dopaminergic function remain present [31], and it is the evaluation of these basic sex differences that serve as the purpose of this report. Mice were genotyped by polymerase chain reaction and 1% agarose gel of electrophoresis of tail DNA. Primers for genotyping DAT mice were Neo2 5′ tga ccg ctt cct cgt gc 3′; JAH1 5′ ccc gtc tac cca tga gta aaa 3′; and JAH2 5′ ctc cac ctt cct agc act aac 3′ used in 1:1:1 ratio. The mutant fragment (900 kb) is larger than the wild type fragment (600 kb) and can be used to genotype mice as +/+ or +/−. All mice were housed in the vivarium under a 12 h light/dark cycle (lights on at 06:00 h) with food and water available *ad libitum*. The Animal Care and Use Committee at NEOUCOM in accordance with NIH guidelines approved all experimental procedures. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. General procedure

Two series of experiments were performed within this report. In the first series both protein (striatum and substantia nigra) and message levels (substantia nigra) of DAT were determined within +/+ and +/− DAT male and female mice. In the second series of experiments, the sex-dependent effects of this DAT perturbation upon the VMAT-2 were assessed. In these latter experiments two different approaches were used to evaluate the VMAT-2. These included measures of: (1) reserpine-evoked striatal DA output and (2) protein (striatum and substantia nigra) and message levels (substantia nigra) of VMAT-2. In this way, both a static and dynamic assessment of the VMAT-2 was achieved. To perform these assays, mice were euthanized by rapid decapitation, the brain removed and bisected. One half of the bisected brain was immediately frozen and stored at −80 °C until assay for DAT and VMAT-2 specific binding protein (striatum and substantia nigra) and mRNA (substantia nigra) levels. The striatum was dissected out from the contralateral half and prepared for measurement of reserpine-evoked striatal DA responses as determined using *in vitro* superfusion.

2.3. In vitro superfusion

Following a midline bisection, the ventricles on the medial side of the brain were pried open and the cortex cut away revealing