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Research Report

Norepinephrine transporter knock-out alters expression of the genes connected with antidepressant drugs action

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ABSTRACT

Norepinephrine transporter knock-out mice (NET-KO) exhibit depression-resistant phenotypes. They manifest significantly shorter immobility times in both the forced swim test and the tail suspension test. Moreover, biochemical studies have revealed the up-regulation of other monoamine transporters (dopamine and serotonin) in the brains of NET-KO mice, similar to the phenomenon observed after the chronic pharmacological blockade of norepinephrine transporter by desipramine in wild-type (WT) animals. NET-KO mice are also resistant to stress, as we demonstrated previously by measuring plasma corticosterone concentration. In the present study, we used a microdissection technique to separate target brain regions and the TaqMan Low Density Array approach to test the expression of a group of genes in the NET-KO mice compared with WT animals. A group of genes with altered expression were identified in four brain structures (frontal and cingulate cortices, dentate gyrus of hippocampus and basal-lateral amygdala) of NET-KO mice compared with WT mice. These genes are known to be altered by antidepressant drugs administration. The most interesting gene is *Crh-bp*, which modulates the activity of corticotrophin – releasing hormone (CRH) and several CRH-family members. Generally, genetic disturbances within noradrenergic neurons result in biological changes, such as in signal transduction and intercellular communication, and may be linked to changes in noradrenaline levels in the brains of NET-KO mice.

1. Introduction

The molecular mechanisms of antidepressant drugs (ADs) have been studied for years, but they are still far from being elucidated. The role of transporters responsible for the reuptake of biogenic amines is well established because they are inhibited by ADs. One of the most important transporter, in addition to serotonin and dopamine, is the norepinephrine

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transporter (NET). ADs, such as reboxetine, desipramine, amitriptyline and nortriptyline, administered repeatedly regulate the level of NET (Benmansour et al., 2004; Hébert et al., 2001), e.g., chronic reboxetine treatment elevates NET mRNA expression (Zhao et al., 2009). Conversely, repeated treatment with desipramine reduced the expression of NET in the cerebral cortex and hippocampus (Zhao et al., 2008), similar to the effect induced by knock-out of the norepinephrine transporter. The level of NET is reduced in the brains of norepinephrine transporter knock-out mice (NET-KO), as indicated by [3H]nisoxetine binding and NET mRNA expression, as shown by in situ hybridization studies, is not observed in the brains of NET-KO mice (Solich et al., 2011). In addition, NET-KO mice exhibit many other features of a depression-resistant phenotype because they manifest significantly shorter immobility times in both the forced swim test and the tail suspension test (Dziedzicka-Wasylewska et al., 2006). They are also resistant to stress, as shown by the plasma corticosterone concentrations (Solich et al., 2008). We have also shown that a life-long deletion of the NET-encoding gene induces the up-regulation of other monoamine transporters, dopamine and serotonin transporters (DAT and SERT respectively), similar to the effect of chronic pharmacological blockade of this transporter by desipramine in wild-type (WT) animals (Solich et al., 2011).

Considering this evidence, it is interesting to study whether the same genes are changed in NET-KO mice as with repeated AD treatment, which alters the expression of genes in the periphery (e.g., blood) and in the brain. These genes are connected with the monoaminergic system, the stress hormone system/HPA axis, inflammation and neuroplasticity (Menke, 2013). However, a significant part of studies concern the serotonin reuptake inhibitors.

The differential gene expression profile of NET-KO mice has been studied by Hu et al. (2009). However, that study was performed in neural crest stem cells and in the locus coeruleus of the mouse brain, but not in the brain regions that are rich in noradrenergic nerve endings. This analysis demonstrated differential expression of noradrenergic biosynthetic enzymes (dopamine-β-hydroxylase, tyrosine hydroxylase and monoamine oxidase-A); genes that are likely to contribute to the NET-KO phenotype (cocaine and amphetamine regulated transcript; the serotonin receptor subunit and the T-cell leukemia homeobox 2); members of the Notch pathway (Numb-like, beta amyloid precursor protein); and deregulation of the TGF-beta and BMP signaling pathways (neurodegeneration associated protein 1).

The present study determined the gene expression in various brain regions that are rich in norepinephrine nerve endings, such as the frontal cortex, cingular cortex, dentate gyrus of the hippocampus and basal–lateral amygdala, in adult NET-KO and WT mice (Schroeter et al., 2000). The goal of this study was to examine the changes in gene expression due to the effect of ADs (Böhm et al., 2006; Conti et al., 2007; Drigues et al., 2003; Orsetti et al., 2008; Urani et al., 2005; Urigüen et al., 2008). We used a microdissection technique (Fig. 1), which resulted in a very precise localization of the target brain regions and a TaqMan Low Density Array approach to evaluate the expression of 44 genes chosen based on previous reports indicating their involvement in the action of ADs.

2. Results

2.1. *TaqMan low density array studies*

We performed the TaqMan low density array to characterize the expression of various genes in four brain regions (frontal – FrA – and cingulate – Cg – cortex; dentate gyrus of the hippocampus – DG; and basal–lateral amygdala – BL) of NET-KO mice compared with WT brains. A Venn diagram (Fig. 2A) indicates the differentially expressed genes in the four brain regions depending on the genotype. The expression of some genes were increased or decreased in more than one tested brain structure of the NET-KO mice. Some genes, such as *Adra2a*, *Caly* and *Fos*, were differentially expressed in the FrA, and *Hdac5* and *Pax6* were differentially expressed in the Cg of NET-KO mice compared with WT mice. The largest number of genes was altered in the DG of NET-KO animals (*Adra1d*, *Cacna1a*, *Ntrk2*, *Egr1*, *Gria1*, *Gria3*, *Ntf3*, *Scn2a1*, *Il1r1*). There were groups of genes that changed both in the FrA and Cg (*Dbp*, *Ndrg2*), in the FrA and DG (*Nr2f2*, *Htr2c*, *Sst*) or in the Cg and DG (*Penk1*). The genes *Crhbp*, *Npy* and *Nr4a1* were differentially expressed in the FrA, Cg and DG of NET-KO mice compared with WT animals. The direction of gene expression alterations in the brains of NET-KO mice compared with that of WT mice is presented in the graphs (Fig. 2B–E). All of the studied genes that were significantly differentiated in NET-KO compared with WT animals were increased in both cortices and the hippocampus of NET-KO mice. The expression of *Htr2c* (serotonin receptor 2C) and *Ntf3* (neurotrophin 3) were lower in these animals. In the basal–lateral amygdala, the tested genes did not change.

Full analysis of altered genes has been added as supplementary materials (Table 2).

Analyses of these genes identified the connected paths and defined their molecular functions and biological processes (supplementary materials – Table 3). The genes that were different in NET-KO compared with WT animals indicated the importance of five interesting biological processes, which are particularly significant in the dentate gyrus of the hippocampus. Among them were cell communication, cellular processes, system process, neurological system processes, and signal transduction. “Cell communication” includes interactions such as signaling between two cells (*Adra1d*, *Il1r1*, *Penk1*, *Ntrk2*, *Nr4a1*, *Cacna1a*, *Nr2f2*, *Npy*, *Sst*, *Htr2c*, *Ntf3*, *Gria1*, *Gria3*, *Crhbp*). The group of genes linked to “cellular processes”, which assume communication between more than two cells, were the following: *Adra1d*, *Il1r1*, *Penk1*, *Ntrk2*, *Nr4a1*, *Cacna1a*, *Nr2f2*, *Npy*, *Sst*, *Htr2c*, *Ntf3*, *Gria1*, *Gria3*, *Crhbp*, *Egr1*. Processes in multicellular organisms performed by the organs or tissues are called “system processes”. This function included the following genes: *Penk1*, *Adra1d*, *Ntrk2*, *Cacna1a*, *Nr2f2*, *Sst*, *Htr2c*, *Crhbp*, *Gria1*, *Gria3*. “Neurological system processes” are related to the nervous system. Cascade processes in which the ligand interacts with the receptor leading to changes in cell function and changes in the activity of second messengers is called “signal transduction”. *Adra1d*, *Il1r1*, *Penk1*, *Ntrk2*, *Nr4a1*, *Nr2f2*, *Npy*, *Sst*, *Htr2c*, *Ntf3*, *Gria1*, *Crhbp*, *Gria3* are connected to signal transduction.