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DESIPRAMINE INDUCED CHANGES IN THE NOREPINEPRHINE TRANSPORTER, α - AND γ -SYNUCLEIN IN THE HIPPOCAMPUS, AMYGDALA AND STRIATUM

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Abstract

The high incidence of depression in Parkinson's Disease (PD) has been well-documented in the clinic; however, the underlying molecular mechanisms of these overlapping pathologies remain elusive. Using a rodent model of depression, the Wistar-Kyoto (WKY) rat, we previously demonstrated that in the frontal cortex the altered expression and protein interactions of alpha- and gamma-synuclein (α -Syn, γ -Syn) were associated with dysregulated trafficking of the norepinephrine transporter (NET). Chronic treatment with Desipramine (DMI), a NET-selective antidepressant, caused a disappearance of depressive-like behavior that was accompanied by a change in α -Syn and γ -Syn expression and their trafficking of NET. Using this same model, we examined the expression of NET, α -Syn and γ -Syn in the hippocampus, amygdala, brainstem, and striatum, all regions implicated in the development or maintenance of depression or PD pathology. Following chronic treatment with DMI, we observed a significant decrease in NET in the hippocampus, amygdala, and brainstem; decrease in γ -Syn in the hippocampus and amygdala; and, increase in α -Syn in the hippocampus and amygdala. Unexpectedly, we observed a significant decrease in α -Syn expression in the striatum of the WKY following chronic DMI treatment. The altered expression of NET, α -Syn and γ -Syn in different brain suggest that DMI's ability to improve depressive-like behavior in a rodent is associated with region-specific changes in the regulation of NET by α - and γ -Syn.

Keywords

α -synuclein; γ -synuclein; desipramine; norepinephrine transporter; Parkinson's disease; depression

INTRODUCTION

The circuitry involved in depression pathology is complex and likely encompasses many nuclei and neurotransmitter systems, which may be differentially affected in different patient

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populations. It is known that in some patient populations successful treatment of symptoms involves pharmacologically blocking the activity of the Norepinephrine transporter (NET), the primary mechanism for removing norepinephrine from the synaptic cleft. Recently, we have found that following chronic treatment with desipramine (DMI), a NET-selective tricyclic antidepressant, there was a reduction of NET levels in the frontal cortex of the Wistar-Kyoto (WKY) rat [1]. Accompanying this was a reduction in γ -synuclein (γ -Syn) levels, but an increase in α -synuclein (α -Syn) protein expression. These neurochemical findings were accompanied by an improvement in depressive-like behavior innately displayed by the WKY rodent [1].

The modulation of synuclein levels, changes in protein interactions, and enhanced trafficking of NET by the synucleins between cellular compartments that were found in this report further strengthened evidence for a role of synucleins as protein traffickers that has been hypothesized by us and others [2–4]. Importantly, the studies of Jeannotte et al. [1] described a novel role for both α -Syn and γ -Syn in the genesis and maintenance of depression. While α -Syn mutations and aggregation are associated with Parkinson's Disease [PD; 5–8] and γ -Syn in cancer [9–12], these proteins have not previously been implicated in depression or other mood disorders.

We sought to assess changes in the expression of NET, α -Syn and γ -Syn in response to chronic DMI, and to determine if these were similar to those we had seen in the frontal cortices of the WKY and Wistar, the control comparison strain to WKY. We chose to examine the hippocampus, amygdala, brainstem, and striatum, regions of the brain known to be involved in depression or PD pathology [13–22] where we would expect to see changes in the expression of some or all of these proteins during disease progression. If modulations of NET, α -Syn or γ -Syn proteins in these regions are similar to those seen in the frontal cortices, this would suggest that underlying the pathology of depression and behavioral improvements in response to chronic antidepressants is a common mechanism involving the trafficking of monoamine transporters via their interactions with the synuclein family of proteins.

MATERIALS AND METHODS

Animals and Tissue

The ethical use of animals was approved by the Georgetown University Animal Care and Use Committee. Animals were housed 2–3 per cage in the Georgetown University animal care facility, fed *ad libitum*, and kept in a 24 hour light/dark cycle. Male Wistar and Wistar-Kyoto (WKY) rats were obtained from Harlan (Indianapolis, IN). All adult animals were sacrificed at 16–20 weeks of age. Animals were treated starting at 16 weeks. Animals were either used for behavioral testing or sacrificed 24 hours following the last treatment. Animals used for acute studies of antidepressant treatment were sacrificed 24 hours after the first treatment. WKY and Wistar animals were given once daily subcutaneous injections of either DMI (10 mg/kg/day) or saline. Animals were treated for 14 consecutive days and then sacrificed to collect and use the brain tissue. Following decapitation, the brain tissue was dissected on ice and used immediately for biochemical analysis.

Immunoblotting

Proteins from brain tissue were isolated in homogenization buffer (20 mM Tris pH 7.5, 5 mM KCl, 200 μ M sodium orthovanadate, protease inhibitor cocktail [Roche]) in a Dounce homogenizer before being sonicated and spun (1,850 x g for 10 minutes at 4°C), and the supernatant was used for analysis. The protein in all samples was determined, diluted in dilution buffer (20 mM Tris pH 7.4, 200 μ M sodium orthovanadate, 1 mM EDTA, 1 mM EGTA, 1 tablet/10 ml protease inhibitors) and Laemmli buffer (BioRad) with 10% β -mercaptoethanol, heated at 65°C for 30 minutes or heated in boiling water for 5 minutes to resolve α -Syn protein.

Samples are electrophoresed either on SDS-polyacrylamide gels or on Nu-PAGE™ 4–12% Bis-Tris polyacrylamide gradient gels as described before [23] and transferred onto PVDF membranes (Millipore). Blots are blocked with 20 mM Tris-buffered saline, pH 7.6 containing 0.1% Tween 20 (TBST) and 5% (wt/vol) non-fat dry milk for 1 hour at room temperature. Blots are subsequently incubated overnight at 4°C with anti-NET mAb (1:1000; Mab Technologies), anti-SERT mAb (1:100; Mab Technologies), anti-DAT mAb (1:500, Santa Cruz), anti- α -synuclein mAb (1:500; BD Transduction Labs), anti- γ -synuclein pAb (1:2000; AbCam), anti- α - or β - tubulin mAb (1:1000; Santa Cruz); anti- β -actin mAb (1:500; Santa Cruz). After incubation for 2 hours at room temperature with HRP-conjugated secondary antibodies (1:5000; Santa Cruz), proteins are revealed by enhanced chemiluminescence (Perkin Elmer). Images were scanned and then quantified using ScionImage.

Data Analysis

All data is represented as the average $\pm$ standard error of the mean. Student's *t* test was used to compare DMI treated samples to saline strain comparisons, **p*<0.05. All expression levels were normalized to β -actin expression and expressed as arbitrary units (A.U.) of band luminosity as measured by ScionImage. GraphPad Prism 4.0 (GraphPad Software, San Diego, CA) software was used to graph results.

RESULTS

The expression of NET, but not DAT or SERT, are significantly reduced in the hippocampus, amygdala, and brainstem following chronic DMI treatment

Following chronic treatment of WKY and Wistar rodents with DMI (14 days; 1 daily subcutaneous injection, 10 mg/kg), the expression of the monoamine transporters in the hippocampus (Hippo), amygdala, brainstem (BS) and striatum were examined. NET expression across most brain regions and in both strains decreased following DMI treatment (Fig. 1-A). In the hippocampus, NET expression was significantly decreased in both the WKY and Wistar (44%, **p*<0.05, *n*=6). In the amygdala and brainstem, there was a reduction of NET in the WKY (28%, 42%, respectively, **p*<0.05, *n*=6), but not the Wistar. There was only a trend for decreased NET expression in both strains in the striatum. These changes are in agreement with decreased NET binding in the hippocampus, amygdala, and brainstem following chronic antidepressant treatment using similar administration protocols [13,18,19]. There were no significant changes following DMI treatment in the expression of either SERT or DAT in any of the tested brain regions in either strain treated (Fig. 1-B and Fig. 1-C, respectively; *n*=6), indicating that the effects of DMI were highly specific for NET.

The expression of α -Syn and γ -Syn is differentially altered following chronic DMI treatment

Our previous work has shown the levels of α -Syn and γ -Syn, but not β -synuclein, are significantly altered in the frontal cortex of both WKY and Wistar rats following chronic DMI treatment. We tested if similar alterations occurred in α -Syn and γ -Syn in other brain regions following treatment with DMI. α -Syn protein levels were increased in both the hippocampus and amygdala of the WKY (Fig. 2A, 48%, 54%, respectively, **p*<0.05, *n*=6), which is similar to our earlier findings in frontal cortex of these animals after DMI treatment. Interestingly, however, we also found that α -Syn levels were significantly decreased in the WKY's striatum following DMI treatment (47%, **p*<0.05, *n*=6).

By contrast to α -Syn, γ -Syn levels were decreased in hippocampus and amygdala after DMI treatment (Fig. 2B, 51%, 39%, respectively, **p*<0.05, *n*=6). These results are even greater than the decreases seen in the frontal cortex, where a decrease of 20% in the expression of γ -Syn protein was found [1]. No other significant changes in γ -Syn protein levels were seen in either the brain stem or in striatum.

The changes observed in either NET or α -Syn and γ -Syn were not due to changes in morphology, since expression levels of α -tubulin and γ -tubulin remained unchanged in both WKY and Wistar rats upon chronic DMI treatment (data not shown).

DISCUSSION

These studies demonstrate that following chronic DMI treatment of the WKY rat, there is a significant decrease in NET in the hippocampus, amygdala, and brain stem, concomitant with a decrease in γ -Syn and an increase in α -Syn in the hippocampus and amygdala. These changes in NET, α -Syn and γ -Syn are more robust than changes previously observed in the frontal cortex, which were shown to be associated with an improved behavioral response in WKY rats that were chronically administered DMI [1]. In the brain stem, only a decrease in the expression of NET was observed in the WKY following DMI treatment, without alterations in α -Syn or γ -Syn. In the striatum, by contrast, there were no changes in either NET or γ -Syn, but a significant decrease in α -Syn was seen. This is the first time a change in α -Syn and γ -Syn levels in response to DMI treatment has been quantified in the hippocampus, amygdala, and striatum. The hippocampus, amygdala and striatum are thought to be part of a circuit involved in depression pathology [16,20,21] and our findings lend further support to this hypothesis while also identifying a potential molecular mechanism. Thus, as shown previously in frontal cortex, these results suggest that changes in α -Syn and γ -Syn expression levels in brain regions linked to depression, may aid the dysregulation of NET function and trafficking in these regions, which contributes to the depressive-like behaviors observed in the WKY rat. Upon DMI treatment, appropriate α -Syn and γ -Syn levels are restored, via their increased or decreased expression, respectively, such that the normative functional activity of the transporter is restored.

Additional experiments should be performed to determine the subcellular expression levels of NET, α -, and γ -Syn proteins. In Jeannotte et al. (2009) we demonstrated that total NET expression in the frontal cortex did not change in response to DMI treatment in the WKY, but subsequent analysis revealed a decrease in NET within membrane fractions and an increase in the cytosolic fractions. Thus, DMI treatment caused a shift in the cellular localization of the transporter that was attributed to a greater trafficking via interactions with α -Syn. In the current study we have shown a significant decrease in total NET protein levels in the hippocampus of both the WKY and the Wistar. In the WKY the changes in synuclein levels in this brain region were similar to those observed in the frontal cortex, yet changes in Wistar synuclein levels were not similar. Additionally, in the amygdala a significant decrease in NET expression was observed in the WKY, but not the Wistar. This change was in parallel with changes in synuclein expression levels that were similar to those in the frontal cortex. Such diverse changes reinforce the necessity of performing studies to determine the relative expression and interactions of these proteins in the membrane and cytosolic fractions in response to DMI in a variety of brain regions and strains. This type of analysis will aid in characterizing the differential positive or negative impacts of DMI across brain regions that are involved in different sensory, cognitive, or behavioral circuitry.

Our earlier studies have shown that the dynamic associations between NET and α -Syn allows for increased trafficking of the transporter away from the cell surface, mediated through interactions with microtubules [1,24–26]. Such trafficking acts to regulate NET levels at the plasma membrane, leading to modulation of its re-uptake activity. By contrast, γ -Syn forms strong associations with NET that prevent appropriate trafficking of the transporter causing its retention at the cell surface leading to excessive removal of NE from the synaptic cleft [1]. The reduction of γ -Syn expression permits α -Syn to override the effects of γ -Syn on NET, leading to restoration of appropriate trafficking and expression levels of NET at the plasma membrane.

Similar studies should be undertaken to examine the interactions of the microtubules, NET, α -Syn and γ -Syn in the hippocampus, amygdala, brainstem, and striatum.

The overexpression or aggregation of α -Syn in dopaminergic neurons of the striatum has been linked with PD pathology. Therefore, an antidepressant-induced decrease in its expression in this region may represent the initial stages of altered cellular dynamics that could reduce or prolong the initiation of degeneration in this brain region. Additionally, these studies highlight DMI as a potential therapeutic option that should be explored for PD, as early and ongoing reduction in overexpressed and aggregated α -Syn may prevent or slow the onset of the disease. However, similar changes in α -Syn expression should be tested with other antidepressants, such as the selective reuptake inhibitors, that are generally better tolerated with less side effects.

Given the ability of α -Syn to modulate monoamine transporters, particularly NET and SERT, combined with its known role in the genesis of PD, we have hypothesized that depression and mood disorders are co-morbid with PD. Indeed, clinical studies have demonstrated variable rates, up to 74%, of PD patients are diagnosed with depression [27,28]. Thus, treatment of one disease could potentially result in the worsening or improvement of either or both conditions upon receiving treatments specific to either one. The results presented here suggest that DMI treatment may actually slow PD pathogenesis by reducing the protein expression of α -Syn in the striatum, leading to less potentially toxic effects of α -Syn aggregate, while additionally improving depressive symptoms. Additionally, imaging or post-mortem research should examine changes in the hippocampus, amygdala, and brainstem in patients with depression that are later diagnosed with PD. These types of studies may more accurately identify the role of these other brain regions in disease pathology, potentially identify other proteins involved in their overlapping etiologies, and identify new therapeutic options.

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Abbreviations

α -Syn, α -synuclein
 γ -Syn, γ -synuclein
 DMI, desipramine
 NET, Norepinephrine transporter
 DAT, Dopamine transporter
 SERT, Serotonin transporter
 NE, norepinephrine
 MT, microtubule
 mAb, monoclonal antibody
 pAb, polyclonal antibody
 WKY, Wistar-Kyoto
 Wis, Wistar

REFERENCES

1. Jeannotte AM, McCarthy JG, Redei EE, Sidhu A. Desipramine modulation of alpha-, gamma-synuclein, and the norepinephrine transporter in an animal model of depression. *Neuropsychopharm* 2009;34:987–998.
2. Clayton DF, George JM. Synucleins in synaptic plasticity and neurodegenerative disorders. *J Neurosci Res* 1999;58:120–129. [PubMed: 10491577]

3. Ostrerova N, Petrucelli L, Farrer M, Mehta N, Choi P, Hardy J, Wolozin B. Alpha-Synuclein shares physical and functional homology with 14-3-3 proteins. *J Neurosci* 1999;19:5782–5791. [PubMed: 10407019]
4. Sidhu A, Wersinger C, Vernier P. Does alpha-synuclein modulate dopaminergic synaptic content and tone at the synapse? *Faseb J* 2004;18:637–647. [PubMed: 15054086]
5. Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L, Reiss O. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet* 1998;18:106–108. [PubMed: 9462735]
6. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 1997;276:2045–2047. [PubMed: 9197268]
7. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muentert M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K. Alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 2003;302:841. [PubMed: 14593171]
8. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature* 1997;388:839–840. [PubMed: 9278044]
9. Ji H, Liu YE, Jia T, Wang M, Liu J, Xiao G, Joseph BK, Rosen C, Shi YE. Identification of a breast cancer-specific gene, BCSG1, by direct differential cDNA sequencing. *Cancer Res* 1997;57:759–764. [PubMed: 9044857]
10. Jia T, Liu YE, Liu J, Shi YE. Stimulation of breast cancer invasion and metastasis by synuclein gamma. *Cancer Res* 1999;59:742–747. [PubMed: 9973226]
11. Lavedan C, Leroy E, Dehejia A, Buchholtz S, Dutra A, Nussbaum RL, Polymeropoulos MH. Identification, localization and characterization of the human gamma-synuclein gene. *Hum Genet* 1998;103:106–112. [PubMed: 9737786]
12. Liu H, Liu W, Wu Y, Zhou Y, Xue R, Luo C, Wang L, Zhao W, Jiang JD, Liu J. Loss of epigenetic control of synuclein-gamma gene as a molecular indicator of metastasis in a wide range of human cancers. *Cancer Res* 2005;65:7635–7643. [PubMed: 16140929]
13. Bauer ME, Tejani-Butt SM. Effects of repeated administration of desipramine or electroconvulsive shock on norepinephrine uptake sites measured by [3H]-nisoxetine autoradiography. *Brain Res* 1992;582:208–214. [PubMed: 1327403]
14. Braak E, Sandmann-Keil D, Rub U, Gai WP, de Vos RA, Steur EN, Arai K, Braak H. Alpha-synuclein immunopositive Parkinson's disease-related inclusion bodies in lower brain stem nuclei. *Acta Neuropathol (Berl)* 2001;101:195–201. [PubMed: 11307617]
15. Braak H, Del Tredici K, Rub U, de Vos RA, Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 2003;24:197–211. [PubMed: 12498954]
16. Folstein, SE.; Peyser, CE.; Starkstein, SE.; Folstein, MF. Subcortical triad of Huntington's disease—a model for a neuropathology of depression, dementia, and dyskinesia. In: Carrel, BJ.; Barrett, JE., editors. *Psychopathology and the Brain*. New York: Raven; 1991. p. 65-75.
17. Gould GG, Pardon MC, Morilak DA, Frazer A. Regulatory effects of reboxetine treatment alone, or following paroxetine treatment, on brain noradrenergic and serotonergic systems. *Neuropsychopharmacology* 2003;28:1633–1641. [PubMed: 12825093]
18. Hebert C, Habimana A, Elie R, Reader TA. Effects of chronic antidepressant treatments on 5-HT and NA transporters in rat brain: an autoradiographic study. *Neurochem Int* 2001;38:63–74. [PubMed: 10913689]
19. Klimek V, Stockmeier C, Overholser J, Meltzer HY, Kalka S, Dilley G, Ordway GA. Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. *J Neurosci* 1997;17:8451–8458. [PubMed: 9334417]
20. McHugh PR. The neuropsychiatry of basal ganglia disorders. *Neuropsychiatr Neuropsychol Behav Neural* 1989;2:239–247.
21. Swerdlow NR, Koob GF. Dopamine, schizophrenia, mania and depression: toward a unified hypothesis of cortico-striato-pallido-thalamic function. *Behav Brain Sci* 1987;10:197–245.

22. Videbech P, Ravnkilde B. Hippocampal volume and depression: a meta-analysis of MRI studies. *Am J Psychiatry* 2004;161:1957–1966. [PubMed: 15514393]
23. Moussa CE, Wersinger C, Rusnak M, Tomita Y, Sidhu A. Abnormal migration of human wild-type alpha-synuclein upon gel electrophoresis. *Neurosci Lett* 2004;371:239–243. [PubMed: 15519765]
24. Wersinger C, Jeannotte A, Sidhu A. Attenuation of the norepinephrine transporter activity and trafficking via interactions with alpha-synuclein. *Eur J Neuro* 2006;24:3141–3152.
25. Jeannotte AM, Sidhu A. Regulated interactions of the norepinephrine transporter by the actin and microtubule cytoskeletons. *J Neurochem* 2008;105:1668–1682. [PubMed: 18331289]
26. Jeannotte AM, Sidhu A. Regulation of the norepinephrine transporter by alpha-synuclein-mediated interactions with microtubules. *Eur J Neuro* 2007;26:1509–1520.
27. Allain H, Schuck S, Mauduit N. Depression in Parkinson's disease. *Bmj* 2000;320:1287–1288. [PubMed: 10807601]
28. Veazey C, Aki SO, Cook KF, Lai EC, Kunik ME. Prevalence and treatment of depression in Parkinson's disease. *J Neuropsychiatry Clin Neurosci* 2005;17:310–323. [PubMed: 16179652]

Figure 1. Chronic DMI treatment alters the expression of NET, but not SERT or DAT, across several brain regions in the WKY

Tissue from the hippocampus (Hippo), amygdala, brainstem (BS), and striatum were removed from WKY and Wistar animals that were chronically treated with either saline or DMI. Tissue was prepared as described in Materials and Methods and probing was done using antibodies that detected multiple bands of each transporter to NET (A), SERT (B), and DAT (C).

Representative western blots of transporters and respective β -actin are shown. Bands were detected at 80 and 55, 62 and 50, and 85 and 58 kDa, for NET, SERT, and DAT, respectively, these are consistent with reported molecular weights of these proteins in rat brain homogenates. Data was collected and analyzed from 6 animals in each group and reported as the mean $\pm$ SE.

SEM, * $p < 0.05$, Student's t test. Expression levels are expressed as Arbitrary Units (A.U.) or protein, normalized to A.U. of β -actin.

Figure 2. Chronic DMI treatment modulates the expression of α - and γ -Syn in the hippocampus, amygdala, and striatum of the WKY rat

Tissue from DMI-treated WKY and Wistar animals were removed and prepared as described in Figure 1. Resolution of α -Syn (A) and γ -Syn (B) was performed using monoclonal antibodies. Data was collected, analyzed, and represented as described in Figure 1, * $p < 0.05$, Student's t test. Representative western blots of synucleins and respective β -actin are shown. α - and γ -Syn were detected at 19 and 13 kDa, respectively.