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Effects of Manipulation of Noradrenergic Activities on the Expression of Dopaminergic Phenotypes in Aged Rat Brains

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Abstract

This study investigated the effects of the pharmacological manipulation of noradrenergic activities on dopaminergic phenotypes in aged rats. Results showed that the administration of L-threo-3,4-dihydroxyphenylserine (L-DOPS) for 21 days significantly increased the expression of tyrosine hydroxylase (TH) and dopamine transporter (DAT) in the striatum and substantia nigra (SN) of 23-month-old rats. Furthermore, this treatment significantly increased norepinephrine/DA concentrations in the striatum and caused a deficit of sensorimotor gating as measured by prepulse inhibition (PPI). Next, old rats were injected with the α2-adrenoceptor antagonist 2-methoxy idazoxan or β2-adrenoceptor agonist salmeterol for 21 days. Both drugs produced similar changes of TH and DAT in the striatum and SN. Moreover, treatments with L-DOPS, 2-methoxy idazoxan, or salmeterol significantly increased the protein levels of phosphorylated Akt in rat striatum and SN. However, although a combination of 2-methoxy idazoxan and salmeterol resulted in a deficit of PPI in these rats, the administration of 2-methoxy idazoxan alone showed an opposite behavioral change. The *in vitro* experiments revealed that treatments with norepinephrine markedly increased mRNAs and proteins of ATF2 and CBP/p300 and reduced mRNA and proteins of HDAC2 and HDAC5 in MN9D cells. A ChIP assay showed that norepinephrine significantly increased CBP/p300 binding or reduced HDAC2 and HDAC5 binding on the TH promoter. The present results indicate that facilitating noradrenergic activity in the brain can improve the functions of dopaminergic neurons in aged animals. While this improvement may have biochemically therapeutic indication for the status involving the degeneration of dopaminergic neurons, it may not definitely include behavioral improvements, as indicated by using 2-methoxy idazoxan only.

Keywords

Tyrosine hydroxylase dopamine transporter, norepinephrine, L-DOPS, adrenal receptors, prepulse inhibition

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Abbreviation

ARs: adrenoceptors; BDNF: brain-derived neurotrophic factor; CBP/p300: cAMP-responsive element (CRE)-binding protein-binding protein/p300; ChIP: chromatin immunoprecipitation assay; DA: dopamine; DAT: dopamine transporter; DBH: dopamine β-hydroxylase; ECL: enhanced chemiluminescence; FBS: fetal bovine serum; FC: frontal cortex; HATs: histone acetyl transferases; HDAC2: histone deacetylases 2; HDAC5: histone deacetylases 5; HPLC: high-performance liquid chromatography; HP: hippocampus; i.p.: intraperitoneal injection; LC: locus coeruleus; L-DOPS: L-threo-3,4-dihydroxyphenyl serine; NE: norepinephrine; p-Akt: phospho-RAC-alpha serine kinases; PBS: phosphate-buffer saline; PD: Parkinson's disease; PPI: prepulse inhibition; SDS: sodium lauryl sulfate; SN: substantia nigra; TH: tyrosine hydroxylase; VTA: ventral tegmental area.

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Summary Statement

The present study employs pharmacological manipulations to enhance noradrenergic activities in aged rats to observe their effects on dopaminergic phenotypes. The results demonstrated that these treatments significantly increased the expression of these phenotypes, possibly through an epigenetic mechanism.

Introduction

In the brain, the noradrenergic and dopaminergic cell bodies are mainly located in the locus coeruleus (LC) and substantia nigra (SN)/ventral tegmental area, respectively. These constitute the principal source of the noradrenergic and dopaminergic neuronal projection in the brain. It has been reported that aging causes a structural and functional decline in both central noradrenergic and dopaminergic systems. It is logical that degeneration of one would affect the other, because both neurotransmitters are on the same biosynthesis pathway. For example, human postmortem analyses showed an age-related reduction of LC neurons (Chan-Palay & Asan, 1989; Lohr & Jeste, 1988; Manaye et al., 1995; Tomonaga, 1983; Vijayashankar & Brody, 1979). Animal studies also showed that age-associated LC neuronal loss was observed in mice (Leslie et al., 1985; Sturrock & Rao, 1985; Tatton et al., 1991). Similarly, in human postmortem brains, nigral dopaminergic neurons were lost at a rate of 5%–10% per decade (Fearnley & Lees, 1991; Ma et al., 1999), culminating in deficits of 30%–80% at advanced ages (Haycock et al., 2003; Rudow et al., 2008). Animal studies have also showed an age-related decline of dopaminergic cell bodies in the SN and striatal dopaminergic terminals in rats (Felten et al., 1992; Himi et al., 1995; Sabel & Stein, 1981). As a consequence of age-related biochemical and functional decline in both systems, the norepinephrine (NE) and dopamine (DA) deficiency in the aged brain has been used as an index for aging. In addition, there is a reduced expression of dopamine β-hydroxylase (DBH), tyrosine hydroxylase (TH) and DA transporter (DAT) in the aged brain (Chan-Palay & Asan, 1989; Cruz-Muros et al., 2009; van Dyck et al., 2002).

It is now well accepted that there is a biochemical and functional correlation between noradrenergic and dopaminergic neuronal systems. While an integrity of the noradrenergic neuronal system would provide neuronal protection on dopaminergic neurons such as facilitating the survival of dopaminergic neurons, a declined noradrenergic system would attenuate or weaken the functional processing of the dopaminergic nigro-striatal tract (Delaville et al., 2011; Hassani et al., 2020; Isaias et al., 2011). For instance, in animals treated with the dopaminergic toxin MPTP, damaged LC caused by lesion or pretreated with the neurotoxin resulted in a more severe loss of dopaminergic neurons in the SN compared to controls, which was accompanied by prominent motor deficits (Fornai et al., 1996; Mavridis et al., 1991; Srinivasan & Schmidt, 2003; Yao et al., 2015). On the other hand, if the synthesis of NE is augmented, or NE reuptake inhibitors were used, MPTP-induced damage in dopaminergic neurons was reduced (Kilbourn et al., 1998; Rommelfanger et al., 2004), accompanied by an alteration in motor performance (Kreiner et al., 2019). Therefore, it seems to be feasible that if the noradrenergic system is augmented in aged brain, it may be neuroprotective toward the degeneration of the dopaminergic system.

It is well known that NE plays a prominent role in neuronal survival, differentiation, and plasticity and participates in brain repair mechanisms. There has been converging evidence showing that the manipulation of the LC-NE system through increasing central NE levels, or reducing NE metabolism, is beneficial in the control of the progression of aging in the NE and DA systems. For example, the administration of L-threo-3,4-dihydroxyphenylserine (L-DOPS), a NE precursor, to animals protects neurons against cell damage (Biaggioni & Robertson, 1987; Lee et al., 1994; Yamagami et al., 1998) and facilitates behavioral recovery (Kato et al., 1987b; Kikuchi et al., 2000). Alpha-2-adrenoceptors (ARs) exert a tonic inhibitory control on adrenergic transmission (Kable et al., 2000; Trendelenburg et al., 1999). Their blockade can lead to the activation of LC-derived adrenergic projections to facilitate noradrenergic modulatory effects over dopaminergic neurons (Donaldson et al., 1975; Nutt et al., 1994; Srinivasan & Schmidt, 2004). This manipulation exerts a protective role upon dopaminergic neurons via the noradrenergic network innervating the SN (Gobert et al., 2004; Martel et al., 1998; Srinivasan & Schmidt, 2004). Similarly, β2-AR agonists that directly or indirectly interact with the receptor to induce the release of endogenous catecholamine including NE (Peterson et al., 2014). Therefore, these pharmacological manipulations can be a possible way to enhance the physiological and functional ability of the central noradrenergic neurons.

In the present study, several pharmacological manipulations on the noradrenergic system were used to explore their potentially beneficial roles for the dopaminergic system in old animals. These treatments include the administration of L-DOPS, α2-AR antagonist 2-methoxy idazoxan, and a long-acting β2-AR agonist salmeterol to aging (18 months) as well as aged (23 months) rats for the goal of whether they increase the expression of dopaminergic phenotypes in the brain and improve behavior related to dopaminergic activity. All of these pharmacological manipulations were also analyzed on auditory sensorimotor gating as measured by prepulse inhibition (PPI), which is a behavioral task with a strong relationship to integrity of the dopaminergic system (Geyer et al., 2001; Mansbach et al., 1988; Swerdlow et al., 1994). Furthermore, *in vitro* experiments were performed to explore the potential epigenetic mechanisms underlying effects of NE on the expression of dopaminergic phenotypes. The present study suggests that pharmacological manipulations strengthening noradrenergic activity can successfully protect against dopaminergic neurodegeneration in aged brains.

Materials and Methods

Animals and Treatments

Experiments were carried out in male and female Fischer 344 rats (RRID: RGD_10395235) at the age of 18 or 23 months. These rats were purchased from Envigo Inc. (Indianapolis, IN, USA) and maintained on a 12-h on-and-off lighting schedule with access to standard rodent chow and water *ad libitum*. All experimental procedures were performed in accordance with the East Tennessee State University Animal Care and Use Committee guide established by the NIH Guide for the Care and Use of Laboratory Animals (Council, 2011). After an acclimation period of seven days, all rats were randomly divided into different experimental groups.

One set of experiments focused on L-DOPS treatment. Considering that L-DOPS has been shown to effectively restore NE deficiency in $DBH^{-/-}$ mice (Thomas et al., 1998; Thomas & Palmiter, 1997), it is also likely effective in restoring NE deficiency in aged rats. In this experiment, a dose of 200 mg/kg L-DOPS was injected daily (i.p.) to 23-month-old rats for 21 days, followed by behavioral and biochemical analyses. Each rat was simultaneously administered with benserazide (10 mg/kg, i.p.), an L-aromatic amino acid decarboxylase inhibitor to reduce peripheral conversion of L-DOPS to NE, as it cannot pass through the blood–brain barrier (Kato et al., 1987b; Lee et al., 1994) for the same period. A separate group of 23-month-old rats was treated with vehicle + similar doses of benserazide as the control.

In a different set of experiments, α2-AR antagonist 2-methoxy idazoxan or β2-AR agonist salmeterol was administered to both 18 (representing aging) and 23-month-old (representing aged) rats. Generally, the blockade of α2-ARs leads to the activation of LC-derived adrenergic projections. Similarly, long-acting β2-AR agonists can either directly or indirectly interact with the receptor to induce the release of endogenous catecholamine including NE (Peterson et al., 2014). The α2-AR antagonist 2-methoxy idazoxan was selected based on the fact that it is five times more selective for α2-ARs, lacks binding to imidazoline receptors, and has considerably less intrinsic activity at α2-ARs than most of the putative α2-AR antagonists (Clarke & Harris, 2002). In addition, it was reported that the chronic administration of salmeterol resulted in neuroprotective effects on dopaminergic neurons (Maris et al., 2004; Qian et al., 2011; Semkova et al., 1996). Therefore, 2-methoxy idazoxan (2.5 mg/kg, daily, i.p.) and salmeterol (1 mg/kg, daily, i.p.) were injected to 18- and 23-month-old rats for 21 days in the present study. The control (18- or 23-month-old rats) received an equivalent amount of the vehicle for the same period.

Cell Cultures

MN9D cells (RRID: CVCL_M067) are a dopaminergic cell line (Choi et al., 1991). They were grown in Dulbecco's modified Eagle's medium, which is supplemented with heat inactivated 10% fetal bovine serum, plus antibiotics penicillin (100 U/ml) and streptomycin (100 µg/ml) (all from Gibco-Invitrogen, Carlsbad, CA, USA). These cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 as previously reported by our laboratory (Zhu et al., 2019). These cells were used for quantitative real-time polymerase chain reaction (qPCR), western blotting, and chromatin immunoprecipitation assay (ChIP) assays. For all experimental groups after cell harvesting, trypan blue exclusion was used to measure the cell viability, which was > 95% for all experimental cells.

RNA Isolation and qPCR Analysis for mRNA of TH and DAT

The method is similar to those reported previously (Deng et al., 2016; Huang et al., 2015). Briefly, RNAzol reagent (Molecular Research Center, Inc., Carlsbad, CA) was used to extract total RNAs from dissected brain tissues or cultured cells, followed by using the superscript III First-Strand Synthesis Kit (Applied Biosystems/Life technologies, Forster City, CA, USA) to convert cDNAs based on the manufacturer's instruction. The SYBR green Platinum Quantitative PCR supermix (Invitrogen, Carlsbad, CA, USA) in Stratagene Mx3000P (Agilent Technologies, Santa Clara, CA, USA) was used to conduct qPCR. The primers for q-PCR were as follows: rat TH: 5′- GCAGCCCTACCAAGATCAAACC-3′ and 5′-CG CTGGATACGAGAGGCATAGT-3′; rat DAT: 5′-GCTGCG TCACTGGCTGTTGC-3′ and 5′-CTGTCCCCGCTGTTGTG AGGT; rat β-*actin*: 5′-AGATTACTGCCCTGGCTCCTA-3′ and 5′- AGGATAGAGCCACCAATCCAC-3′; mouse cAMP-responsive element (CRE)-binding protein-binding protein/p300 (CBP/p300): 5′- TGGAAGAACTGCACACGACA-3′ and 5′-GAGTCCTCATCTGCTGGTGG-3′; mouse ATF-2: 5′-TCCTCCGGGGCTAGTTTGTA-3′ and 5′-CTCGTTGGT AAAACGCTGGC-3′; mouse histone deacetylases 2 (HDAC2): 5′-CTATCCCGCTCTGTGCCCTA-3′ and 5′-TCGAGGATG GCAAGCACAAT-3′; mouse HDAC5: 5′- CCGGGAACCA TCCTTGGAAA-3′ and 5′-GTGGGAGGGAATGGTTGAG G-3′. All measurements were performed in triplicate. The $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001) was used to calculate the relative amount of mRNA expression levels of TH/DAT by normalization and comparison to those of β-actin (ΔCt).

Western Blotting Analysis

Western blotting was used to measure the protein levels of TH, DAT, and p-Akt in brain striatum, SN, and hippocampus (HP) of rats, as well as those of ATF-2, CBP/p300, HDAC2, and HDAC5 in cultured cells. All measurements were performed in triplicate. Briefly, the sample buffer containing sodium lauryl sulfate (SDS), β-mercaptoethanol, and protease inhibitors was used to lyse the dissected tissues or cultured cells, which were then briefly homogenized and centrifuged

(1,000*g*). Supernatants were transferred to new tubes, and protein assay was performed. Equal amounts of sample proteins were loaded on 10% or 15% SDS-polyacrylamide gels, followed by electrophoresis and transfer processing. Samples were transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA) and incubated to primary antibodies after blocking in TBS/0.1% Tween-20/5% milk. The primary antibodies are listed as follows: anti-DAT from rabbit (1:400 dilution, Cat# ab5990, RRID: AB_305226, Abcam, Cambridge, MA, USA); anti-TH from mouse (H-16, 1:1,000 dilution, Cat# AMAb91112, RRID: AB_2665805, Sigma-Aldrich, Saint Louis, MO, USA); anti-phosphor-RAC-alpha serine kinases (p-Akt) (ser473) from rabbit (1: 2,000 dilution, Cat# S473, ab47261, RRID: AB_867561, Abcam, Cambridge, MA, USA); anti-Akt from rabbit (Cat# 9272, 1: 1,000 dilution, RRID: AB_329827); anti-ATF-2 from rabbit (1: 1,000 dilution, D4L2X, Cat# 350331, RRID: AB_2799069); anti-CBP/p300 from mouse (1: 1,000 dilution, D8Z4E, Cat# 86377, RRID: AB_2800077); anti-HDAC2 from rabbit (1: 1,000 dilution, D6S5P, Cat# 57156, RRID: AB_2756828); and anti-HDAC5 from rabbit (1:1,000 dilution, D1J7V, Cat# 20458, RRID: AB_2713973). All antibodies against Akt, ATF-2, CBP/P300, HDAC2, and HDAC5 were purchased from the Cell Signaling Technology, Danvers, MA, USA. The secondary antibodies against rabbit or mouse were then probed to these membranes after washing. Enhanced chemiluminescence (ECL, Amersham; Piscataway, NJ, USA) was applied to visualize the protein bands and detected by G: Box Imaging (Fyederick, MD, USA). The same blot was stripped and re-exposed to anti-β-actin antibody by similar steps. All signals of the protein of interest and β-actin were analyzed by Image J (RRID: SCR_003070).

Measurement of NE/DA by High-Performance Liquid Chromatography (HPLC)

NE/DA levels were measured by HPLC as has been reported previously (Fan et al., 2020). The dissected brain striatum was sonicated, followed by homogenization in a buffer that included perchloric acid (0.2 M), ascorbic acid (1×10^{-7} M). A 2 µg/ml concentration of dihydroxybenzylamine was added to the buffer as the internal standard for catecholamine. After centrifugation (10,000*g*, 4 °C, 5 min), the sample supernatants were further filtered through nylon disposable syringe filters (0.2 µm) and stored at −80 °C until measured on HPLC. The protein assay was also performed in this preparation with the goal being to define the concentration of NE/DA in this preparation of the striatum tissue.

To assay the NE/DA levels in tissue preparation through HPLC, we followed the procedures as reported before (Fan et al., 2020). Briefly, the HPLC was equipped with an Ultrasphere ODS reverse-phase column (Beckman). The mobile phase consisted of 4% acetonitrile, 0.1 M sodium nitrate, 0.08 M sodium dihydrogen phosphate, 0.2 mM sodium octyl sulfate, and 0.1 mM EDTA at pH 2.7. A 20-µl supernatant aliquot was injected directly into the HPLC column. The samples were eluted isocratically and detected using a Hitachi D-2500 Chromato-Integrator. The NE/DA in the supernatants was identified by matching their elution times and sensor ration measures to those of the internal standard, and their concentrations were calculated by comparing peak areas to those of known standards. Resulted amounts are presented as pg/mg proteins, because this preparation was from the tissue homologies from brain tissues and their amounts should be defined in whole preparation.

Sensorimotor Gating Apparatus

PPI is a neurological phenomenon in which an animal acquires the ability to inhibit a startle response through the temporal contiguous presentation of a weak auditory prepulse (73, 76, and 82 dB) presented immediately before a startling pulse stimulus (120 dB). The procedures used for this task are based on previous work from a collaborator's laboratory (Gill et al., 2020). For all sensorimotor gating behavioral testing, the Startle Monitor II apparatus and software was used (Kinder Scientific, Poway, CA). Rats were placed in a stainless-steel dome (height = 8 cm) that was attached to a platform (11 cm wide × 15 cm long) mounted on a stainless-steel ellipse in a sound-attenuating chamber (28 cm high × 30 cm wide × 36 cm depth). The animal response was recorded and measured (in Newtons, N) within a 250-millisecond (ms) window immediately following stimulus presentation through a computer interface. On each day of testing, all chambers were calibrated according to the instructions provided with the software. Calibration was based on the animal's weight once the animal was placed into the enclosure. Each animal was behaviorally tested in the same apparatus over the three days of PPI testing. If calibration needed to be performed, an error message appeared in the software that does not allow the animal to be tested until calibration was completed.

PPI methods: PPI was administered for three consecutive days, which started one day after 21-day drug treatment. After being placed into a stainless dome on each daily session, rats were given a 5-min habituation period with only the background noise (70 dB) presented, followed by the pulse, prepulse, and no stimulus trials. A *pulse* trial was a 120 dB startle pulse administered alone. A *prepulse* trial was an auditory stimulus that was 3, 6, or 12 dB above the 70 dB background noise (i.e., 73, 76, and 82 dB) followed 250 ms later by the 120-dB startle pulse. A *no stimulus* trial was a trial in which no stimulus is given. All animals underwent 60 randomized trials that included a total of 20 *pulse*, 10 *no stimulus*, and 30 *prepulse* trials (10 trials of each 73, 76, and 82 dB). For all prepulse trials, percent PPI was calculated with the following equation: 100 − ([mean prepulse response / mean pulse response] × 100). A mean of performance on the prepulse trials over these consecutive three days was statistically analyzed. A functional improvement in

behavior was interpreted as a statistically significant increase in PPI percentage on the three prepulse auditory intensities analyzed as compared to control groups. The response on pulse trials, when the startle stimulus was presented alone, was also analyzed.

ChIP

The previous studies from this laboratory reported that in dopaminergic MN9D cells treated with NE, an increased H4 acetylation (Zhu et al., 2019) and the methylation of H3 in the TH promoter measured by ChIP assays (Fan et al., 2020). Although a significant alteration in H3 acetylation was not found, a tendency toward an increase was observed (unpublished data). These results indicated that NE-induced increases in TH expression are related to chromatin remodeling on the TH promoter. As an extension of those studies, more *in vitro* experiments were performed to further investigate the details related to NE-induced changes in histone acetylation of the TH promotor. A ChIP assay was performed as described previously (Zhu et al., 2019) using an EZ-Magna ChIP™ A kit (Millipore Biotechnology, Billerica, MA, USA) based on the manufacturer's instructions. Briefly, lysed cells were processed for sonication at 4 °C to get approximately 200–500 bp size chromatins. After centrifugation, supernatants in sheared chromatin were immunoprecipitated, respectively, using rabbit monoclonal antibodies anti-CBP/p300 (1:250 dilution, Cat# MA1-16622, RRID: AB_568668), polyclonal anti-HDAC2 (1:250 dilution, Cat# PA1-861, RRID: AB_2118520), or polyclonal anti-HDAC5 (1:250 dilution, Cat# PA1-41117, RRID: AB_2116776) (all from ThermoFisher, Grand Island, USA), or normal rabbit IgG provided in the kit. DNA amounts in different samples were measured before immunoprecipitation. Then, PCR amplification was performed from purified DNA of both the immunoprecipitated and pre-immune (pre) samples using the following primers F: 5′-CCAGTGAGA-GGGCTTCTA-3′ and R: 5′-CACCTGCCTCTGAATCAC −3′, which recognize the −340 to +1 bp region of the TH promoter. ChIP-PCR-derived DNA was also electrophoresed through 2% agarose gels and stained with ethidium bromide.

Statistics

All experimental values were shown as the mean ± SEM. The number of replicates is enumerated in the figure legends ($N = x$/group). While the number indicates the replicates of cultured cells separated for each group, it also shows the number of animals in a given group in the animal experiment. Statistical significance in the experiments related to administration of 2-methoxy idazoxan or salmeterol and *in vitro* investigations (Figures 8 and 10) was determined using one-way analysis of variance (ANOVA, SigmaStat, Systat Software Inc., Richmond, USA), followed by Student–Newman–Keuls' multiple comparisons *post hoc* test. In experiments using L-DOPS (Figures 1 and 2), mRNA assay for ATF-2, CBP/p300, HDAC2 and HDAC5 in MN9D cells treated with NE (Figure 8A), and ChIP assays (Figure 9) where treatments were compared to a single control group, student *t*-test was used. For the behavioral tests of PPI, a two-way repeated measure ANOVA was performed, with drug treatment group and levels of prepulse auditory intensity as the two factors.

Results

Effects of Administration of L-DOPS on Dopaminergic Phenotypes in Aged Rat Brains

To examine the effects of L-DOPS on the expression of the dopaminergic phenotype in the brain, L-DOPS (200 mg/kg) was administered (daily, i.p.) to rats at the age of 23 months for 21 days. Rats were immediately sacrificed after behavior tests and brains were taken for biochemical analysis. Each rat was also simultaneously administered with benserazide for the same period (10 mg/kg, i.p.). As shown in Figure 1, administration of L-DOPS significantly increased mRNA and protein levels of TH (Figure 1A, $t_8 = 2.78$, $p < .05$; Figure 1C, $t_8 = 5.21$, $p < .01$) and DAT (Figure 1B, $t_8 = 3.56$, $p < .05$; Figure 1D, $t_8 = 2.98$, $p < .05$) in the SN region of aged rats. However, in the striatum there was only an increased protein level of TH (Figure 1C, $t_8 = 2.79$, $p < .05$), and there were no significant alterations for mRNA of TH and DAT (Figure 1A and B, all $p > .05$), as well as protein levels of DAT (Figure 1D, $p > .05$). Furthermore, the administration of L-DOPS markedly enhanced protein levels of TH in the frontal cortex and HP (Figure 1E, FC: $t_8 = 3.37$, $p < .05$; HP: $t_8 = 4.99$, $p < .01$).

In a separate experiment, all groups including controls and treated rats were sacrificed on the 22nd day after drug treatment and the striatum was dissected for further analyses of NE and DA concentrations through HPLC. As shown in Figure 2A, NE and DA levels in the striatum were significantly increased (for NE: $t_8 = 5.07$, $p < .01$; for DA: $t_8 = 3.16$, $p < .05$) as compared to corresponding controls. PPI is presented as a function of decibel level of the control and L-DOPS groups in Figure 2B. A two-way ANOVA revealed a significant main effect of drug ($F_{1,12} = 10.81$, $p < .017$), and a significant drug × prepulse interaction ($F_{1,12} = 4.39$, $p < .037$). Post hoc analyses revealed that treatment with L-DOPS resulted in a deficit in PPI on both 73 and 76 dB prepulse trials, although this effect was not present on 82 dB prepulse trials (Figure 2B).

Effects of Administration of 2-Methoxy Idazoxan and Salmeterol on the Expression of Dopaminergic Phenotypes in Old Rat Brains

First, the mRNAs of TH and DAT in the SN from rats at the age of 18 and 23 months were measured. As in the aged rats

injected with L-DOPS there was no a significant alteration for the mRNAs of TH and DAT in the striatum (Figure 1A and B), therefore, in this experiment using 2-methoxy idazoxan and salmeterol, mRNAs of TH and DAT in the striatum were not measured. In Figure 3, fold difference is presented as a function of 18- and 23-month-old rats. A one-way ANOVA analysis revealed that administration of 2-methoxy idazoxan and salmeterol significantly enhanced the mRNAs of TH and DAT in the SN (for 18 months: $F_{3,23} = 3.67$, $p < .05$ for TH; $F_{3,23} = 3.99$, $p < .05$ for DAT; for 23 months: $F_{3,23} = 3.91$, $p < .05$ for TH; $F_{3,23} = 4.01$, $p < .05$ for DAT). However, there was no additive effect when rats were injected with both drugs together. We did not analyze the difference between 18- and 23-month-old rats.

The results of TH and DAT protein levels for the SN, striatum, and HP are presented in Figure 4, with the band density presented as a function of treatment groups for 18-month-old rats. TH and DAT protein levels in the SN are presented in Figure 4A and B, respectively. A one-way ANOVA revealed a significant main effect of group for TH ($F_{3,23} = 4.25$, $p < .05$) and DAT ($F_{3,23} = 3.87$, $p < .05$) in the SN. *Post hoc* analyses revealed that the administration of salmeterol or 2-methoxy idazoxan plus salmeterol significantly increased TH in the SN. However, regarding DAT, all three groups, treatments with 2-methoxy idazoxan, salmeterol, and their combination, markedly increased DAT in the SN. The protein levels of TH and DAT in the striatum are presented in Figure 4C and D, respectively. A one-way ANOVA revealed a significant main effect of group for TH in striatum ($F_{3,23} = 4.12$, $p < .05$), as well as DAT in the striatum ($F_{3,23} = 5.23$, $p < .01$). All three groups, 2-methoxy idazoxan, salmeterol, and their combination, demonstrated significant increases of TH as well as DAT in the striatum. The protein levels of TH and DAT in the HP are presented in Figure 4E and F, respectively. A one-way ANOVA revealed a significant main effect of group for TH ($F_{3,23} = 3.54$, $p < .05$) and DAT ($F_{3,23} = 4.89$, $p < .05$) in the HP. Post hoc analyses revealed that treatment with 2-methoxy idazoxan or salmeterol alone increased TH in the HP, but their combination was not significantly changed as compared to the controls. Treatments with salmeterol alone or the combination of 2-methoxy idazoxan + salmeterol significantly increased DAT protein levels, as compared to controls.

The effects of these two drugs on protein levels of TH and DAT in 23-month-old rats are presented in Figure 5. Generally, the administration of 2-methoxy idazoxan or salmeterol to 23-month-old rats produced resembling effects on TH and DAT in the SN and striatum as in 18-month-old rats (TH in the SN: $F_{3,23} = 4.14$, $p < .05$; TH in the striatum: $F_{3,23} = 5.12$, $p < .05$, Figure 5C; DAT in the SN: $F_{3,23} = 4.85$, $p < .05$, Figure 5B; DAT in the striatum: $F_{3,23} = 3.78$, $p < .05$, Figure 5D), except for that in 23-month-old rats treatment with salmeterol did not increase TH protein levels in the SN (Figure 5A). However, in the HP, there was an enhancement of TH protein levels caused by these two drugs ($F_{3,23} = 3.69$, $p < .05$), but there were no significant changes in DAT. Similarly, no additive effect was observed when 2-methoxy idazoxan combined with salmeterol (Figure 5E).

In addition, PPI tests were performed in 18- and 23-month-old rats. As shown in Figure 6A, in 18-month-old rats, the treatment with 2-methoxy idazoxan produced an improvement in PPI at 73 and 76 dB prepulse intensities as compared to controls. Nevertheless, the treatment of salmeterol alone and combination of 2-methoxy idazoxan and salmeterol produced a significant deficit in PPI across 73, 76, and 82 dB prepulse levels. A two-way repeated measures ANOVA revealed a significant main effect of group ($F_{3,32} = 6.11$, $p < .002$). In 23-month-old rats, the treatment of salmeterol only produced a deficit in PPI at the 73-dB prepulse trial. Treatment with 2-methoxy idazoxan produced an improvement at the 73 and 82 dB prepulse intensity in PPI compared to controls. In addition, similar to 18-month-old rats, the combined treatment of 2-methoxy idazoxan and salmeterol demonstrated significant PPI deficits compared to controls at all 73, 76 and 82 dB prepulse intensity. A two-way repeated measures ANOVA revealed a significant main effect of group ($F_{3,15} = 4.17$, $p < .02$, Figure 6B).

Effects of Administration of L-DOPS, 2-Methoxy Idazoxan, and Salmeterol on Phosphorylated-Akt (p-Akt) Protein Levels in Old Rat Brains

Proteins levels of p-Akt and Akt were measured in these samples by western blotting. P-Akt and Akt densitometry results were normalized to corresponding loading control β-actin, and the ratio of p-Akt/Akt was used as the final results. Figure 7A presents p-Akt as a function of brain area of 23-month-old rats after treatment with L-DOPS. A *t*-test revealed that L-DOPS treatments produced a significant increase of p-Akt in both the striatum and SN after 21 days of treatment (both $p < .05$). Figure 7B and C presents p-Akt levels in both the striatum and SN for 18 months as a function of treatment groups. A one-way ANOVA on p-Akt in the striatum revealed a significant main effect of group ($F_{3,23} = 3.85$, $p < .05$) and SN ($F_{3,23} = 4.14$, $p < .05$). *Post hoc* analysis showed that 2-methoxy idazoxan and salmeterol alone, as well as their combination produced significant increases of p-Akt in the striatum and SN in 18-month-old rats, except for 2-methoxy idazoxan in the striatum. Figure 7D and E presents p-Akt levels in the striatum and SN for 23-month-old rats as a function of group. There was a significant main effect for both the striatum ($F_{3,23} = 3.45$, $p < .05$) and SN ($F_{3,23} = 3.08$, $p < .05$). *Post hoc* tests revealed that only the combination of 2-methoxy idazoxan + salmeterol increased p-Akt in the striatum for 23-month-old rats, whereas all three drug groups (alone and combination) produced increases in p-Akt in the SN of rats at 23 months of age.

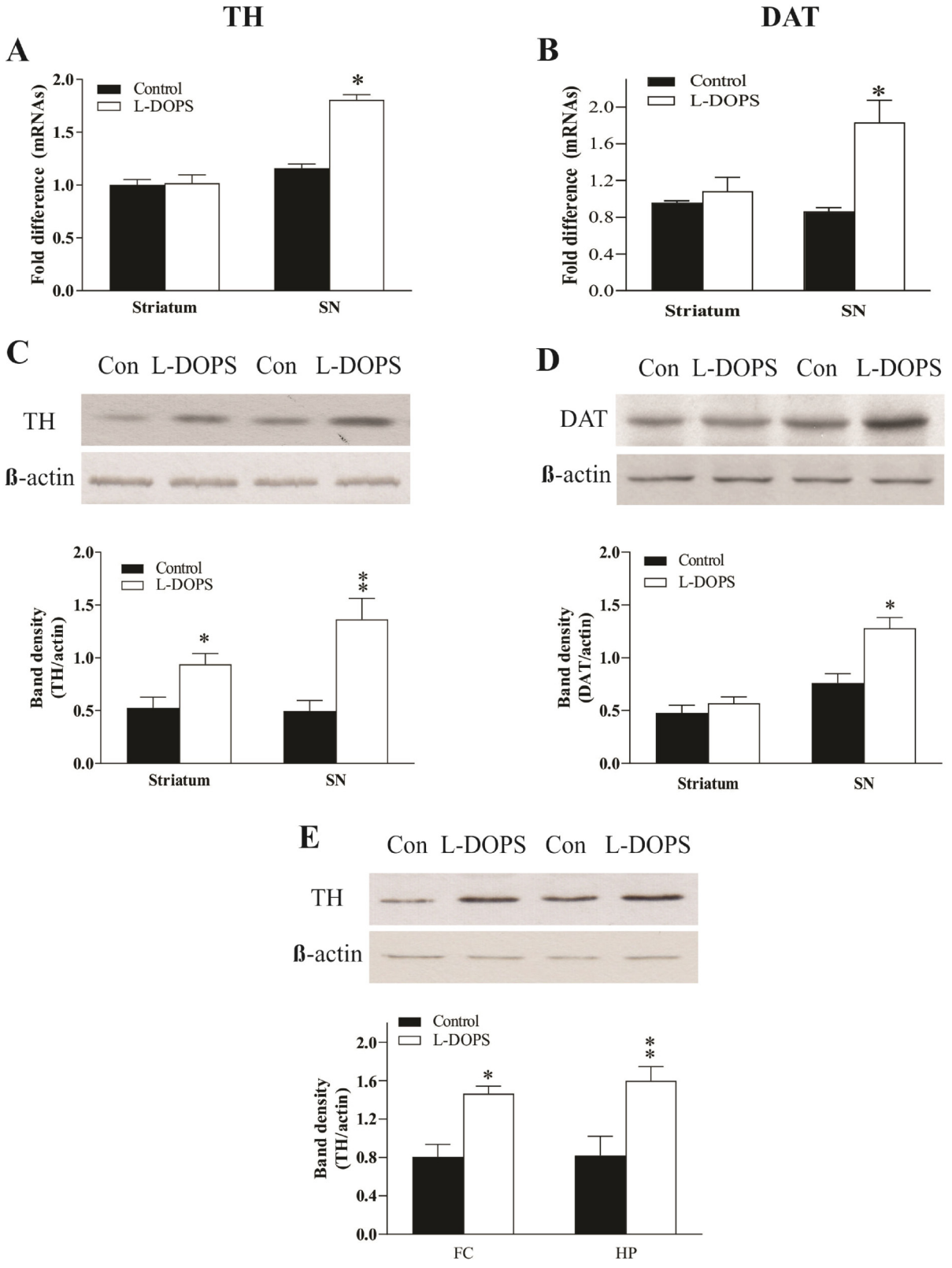

Figure 1. Expression of TH and DAT in brain regions of aged rats after injection of L-DOPS. A and B: mRNAs of TH and DAT in the striatum and SN measured by qPCR ($N = 5$/group). C and D: protein levels of TH and DAT in the striatum and SN measured by western blotting ($N = 5$/group). E: TH protein levels in the FC and HP measured by western blotting ($N = 5$/group). Upper panels in C, D, and E are representative micrographs of western blotting. Lower panels in C, D, and E are quantitative analysis of their protein levels in these regions of aged rats. * $p < .05$, ** $p < .01$, compared to the control. Abbreviations: Con: controls; FC: frontal cortex; HP: hippocampus; L-DOPS: injection with L-DOPS; SN: substantia nigra; TH: tyrosine hydroxylase.

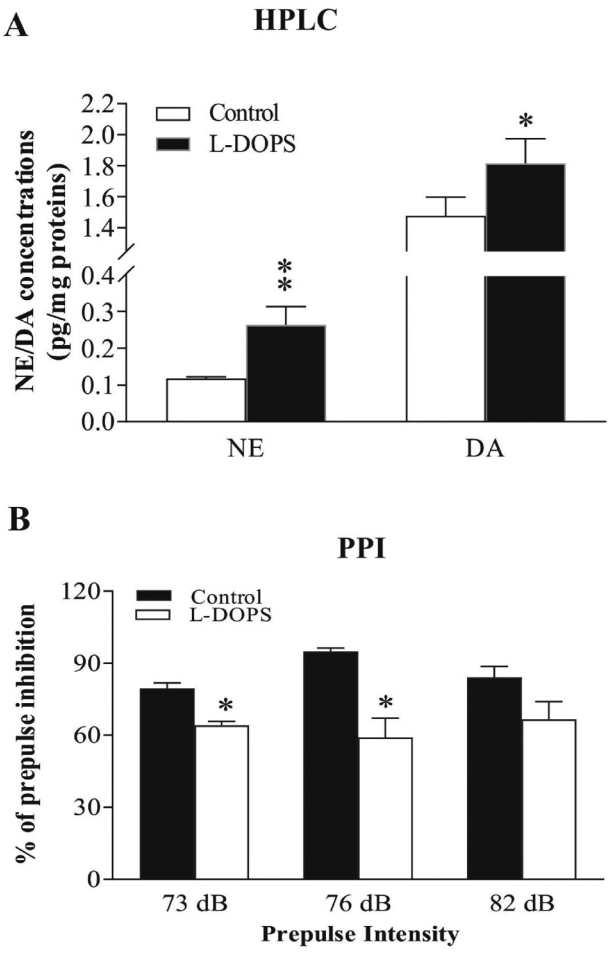

Figure 2. Effects of treatment with L-DOPS on NE/DA levels in the striatum (A) and behavior in aged rats (B). A: Injection of L-DOPS increased NE/DA concentrations in the striatum measured by HPLC ($n = 5$/group). B: Injection of L-DOPS resulted in a deficit in PPI compared to controls at 73 and 76 dB prepulse intensities ($N = 6$/group). * $p < .05$, ** $p < .05$, compared to the control. Abbreviations: DA: dopamine; L-DOPS: L-threo-3,4-dihydroxyphenylserine; NE: norepinephrine; PPI: prepulse inhibition; 73, 76, and 82 dB: prepulse trials.

NE-Induced Increases in TH Expression May Be Mediated Through the Epigenetic Mechanisms

It has been reported that histone acetylation is regulated by the activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). In addition, CBP/p300 as a HAT is related to gene transcription (Colvis et al., 2005; Korzus et al., 2004; Peixoto & Abel, 2013; Yuan & Gambee, 2001). ATF-2 is also an intrinsic HAT (Kawasaki et al., 2000). Therefore, CBP/p300 and ATF-2, representatives of HATs, as well as HDAC2 and HDAC5, representatives of HADCs, were measured in this experiment. First, MN9D cells were exposed to 300 nM NE for 2 days and mRNA levels were measured by qPCR. Figure 8A depicts fold change in mRNAs as a function of HATs (ATF-2 and CBP/p300) and histone deacetylases HDAC2 and HDAC5. A student *t*-test analysis showed that mRNA levels of ATF-2, CBP/P300, HDAC2, and HDAC5 in the cells exposed to NE revealed a significant group main effect with NE, demonstrating higher levels of ATF-2 ($t_8 = 3.72$, $p < .05$) and CBP/p300 ($t_8 = 5.72$, $p < .01$), as well as lower levels of HDAC2 ($t_8 = 3.48$, $p < .05$) and HDAC5 ($t_8 = 6.14$, $p < .01$), as compared to controls, respectively. For measuring protein levels of these HATs and HDACs, MN9D cells were exposed to 100–500 nM NE for two days. Western blot analysis (one-way ANOVA) revealed that while 300 nM NE significantly increased protein levels of ATF-2 ($F_{3,219} = 3.78$, $p < .05$, Figure 8B), 300 and 500 nM NE increased CBP/p300 protein levels ($F_{3,19} = 4.32$, $p < .05$, Figure 8C). In contrast, 300 or 500 nM NE markedly reduced protein levels of HDAC2 and HDAC5 ($F_{3,19} = 4.52$, $p < .05$ for HDAC2; $F_{3,19} = 3.57$, $p < .05$ for HDAC5; Figure 8D and E).

Next, ChIP assays were performed using antibodies against CBP/p300, HDAC2, and HDAC5. As shown in Figure 9, *t*-test revealed that treatments with 300 nM NE for two days caused an increased binding of CBP/300 antibody to the TH promoter ($p < .01$) and a reduced binding of HDAC2 ($p < .01$) and HDAC5 ($p < .01$) antibodies to the TH promoter (Figure 9A). With normal rabbit IgG, there was no binding of the TH promoter. In addition, quantitative real-PCR confirms these reductions of levels of HDAC2 and HDAC5 in NE-treated samples over the control cells (lower panels in Figure 9B and C).

One more experiment was carried out to identify which isoforms of HDACs affecting the bind to the TH promoter after NE treatment. Based on the above results, MN9D cells were exposed to vehicle, 300 nM NE, and NE plus the HDAC inhibitors (1 mM sodium butyrate; this dose has been reported to completely block HDACs) (Gao et al., 2013; Yuan et al., 2013) for two days, and ChIP assays was performed thereafter. As shown in Figure 10, NE-induced H3 or H4 acetylation in the TH promoter was significantly enhanced by treatment with HDAC inhibitor sodium butyrate (for H3: $F_{2,11} = 6.39$, $p < .01$; for H4: $F_{2,11} = 5.31$, $p < .01$), as compared to the NE only group, confirming the HDAC inhibitor role on NE-induced histone acetylation.

Discussion

This study examined the effects of pharmacological manipulations of noradrenergic activities on the expression of dopaminergic phenotypes in the brain. Aging and aged rats were administered with either the NE precursor L-DOPS, a α2-AR antagonist, or β2-AR agonist for 21 days. Neurochemical measurements showed that the administration of L-DOPS significantly increased mRNA and protein levels of TH and DAT in the SN, as well as DAT protein in the striatum, and markedly enhanced NE and DA concentrations in the striatum. The injection of the α2-AR antagonist 2-methoxy idazoxan and β2-AR agonist salmeterol similarly enhanced the expression of TH

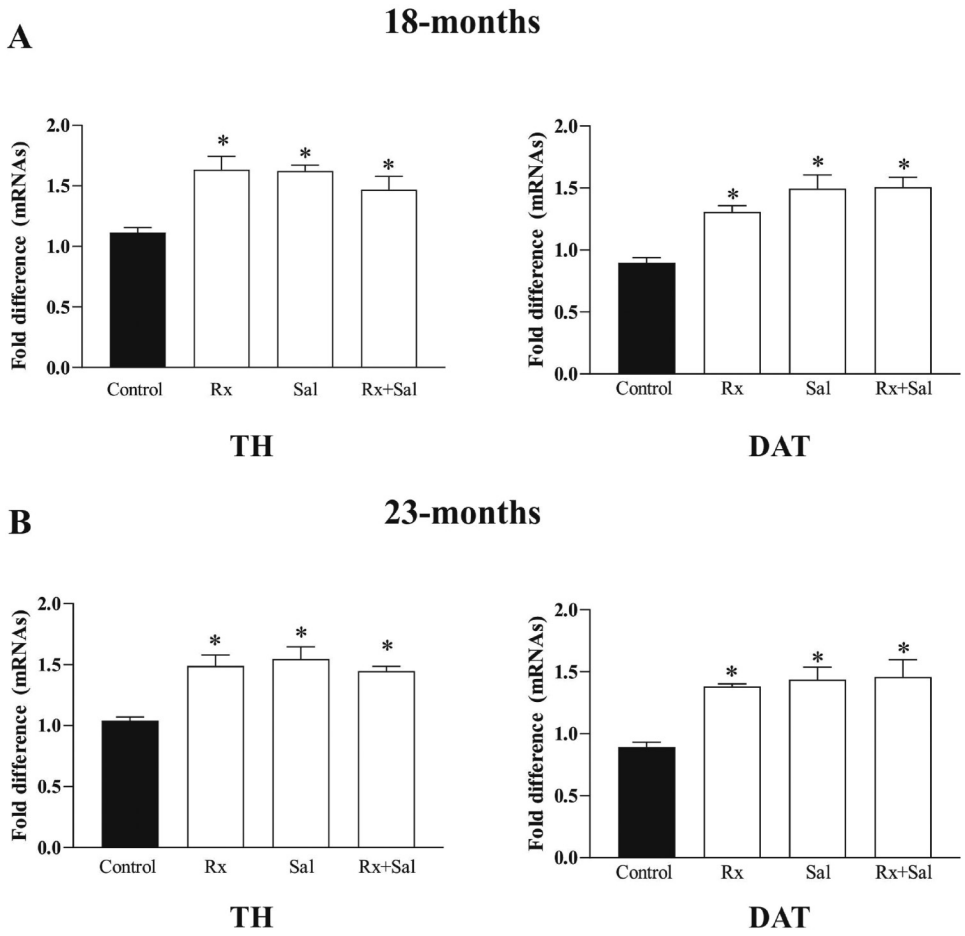

Figure 3. Effects of treatments with 2-methoxy idazoxan and salmeterol on mRNAs of TH and DAT in the SN from rats at ages of 18 (A) and 23 (B) months ($N = 6$/group). * $p < .05$, compared to the control. Abbreviations: DAT: dopamine transporter; Rx: 2-methoxy idazoxan, Sal: salmeterol; SN: substantia nigra; TH: tyrosine hydroxylase.

and DAT in these brain regions of 18- and 23-month-old rats. Behaviorally, the NE precursor L-DOPS, which results in increases of dopaminergic tone, resulted in PPI deficits at both 73 and 76 dB in 23-month-old rats, which is sensible, because PPI deficits typically result when dopaminergic tone is increased (Swerdlow et al., 2000). In addition, different AR-related NE drugs generated different behavioral effects on the PPI tasks. While the α2-AR antagonist 2-methoxy idazoxan significantly increased PPI levels, β2-AR agonist salmeterol alone or a combination of both drugs resulted in a deficit in PPI at 18 and 23 months of age. Moreover, there was a markedly enhanced protein level of p-Akt, an important protein kinase to mediate the neurotrophic effects of NE in the brain. *In vitro* experiments further confirmed NE-induced histone acetylation on the TH promoter to facilitate the transactivation of TH gene. The present study extends our previous reports that an improvement of noradrenergic activity has a beneficial effect on the dopaminergic activity and function, which expands our understanding of the link between the LC-NE and DA systems during the progression of aging.

L-DOPS is an artificial amino acid and has been clinically used for the treatment of orthostatic hypotension (Suzuki et al., 1981). In the present study, L-DOPS was administered to aged rats for 21 days. The results showed that this treatment dramatically increased mRNA or protein levels of dopaminergic phenotypes in the striatum or SN, and improved sensorimotor gating, which is related to dopaminergic signaling. Several explanations can be postulated for L-DOPS effects observed here. First, the effect of L-DOPS on dopaminergic phenotypes can be a result of the pharmacological effects of NE, as L-DOPS can convert to NE both peripherally and centrally through decarboxylation by the aromatic L-amino acid decarboxylase (Bartholini et al., 1975; Inagaki & Tanaka, 1978). It was reported that the systemic administration of L-DOPS produced a prolonged increase in extracellular levels of NE or its metabolite, 3-methoxy-4-hydroxy-phenylglycol levels in the brain (Brannan et al., 1990; Kato et al., 1987a; Kikuchi et al., 2000; Tohgi et al., 1993) or in the cerebrospinal fluid (by 5- to 75-fold) (Tohgi et al., 1990, 1993). In $DBH^{-/-}$ mice, after multiple injections of L-DOPS, NE levels were increased to

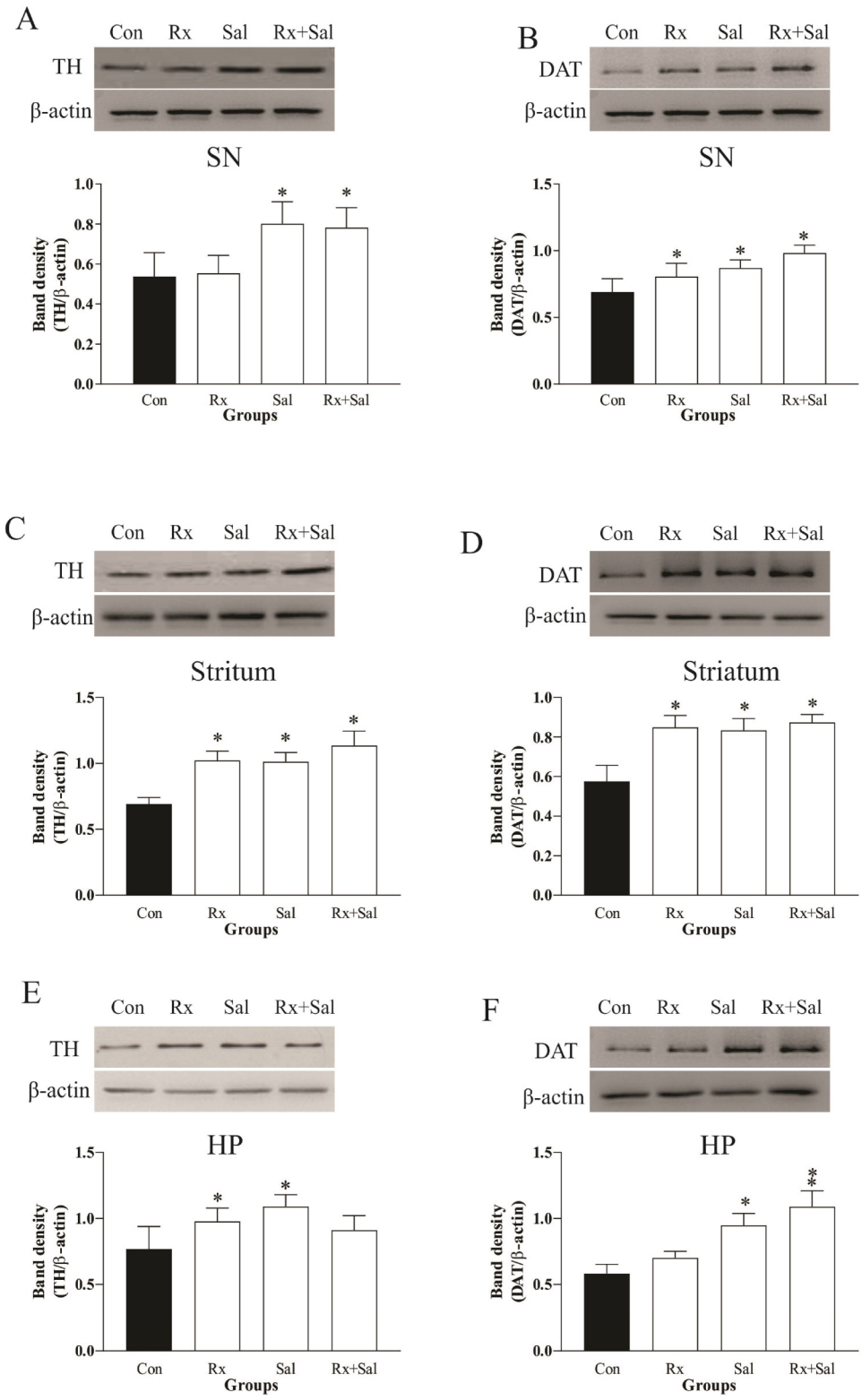

Figure 4. Effects of treatments with 2-methoxy idazoxan and salmeterol on protein levels of TH and DAT in the brain regions of 18-month-old rats. The administration of 2-methoxy idazoxan and salmeterol increased these protein levels in the SN (A and B), striatum (C and D), and HP (E and F) of rats measured by western blotting (all $N = 6$/group). The upper panels in each figure show autoradiographs obtained by western blotting. The lower panels in each figure show the quantitative analysis of band densities. * $p < .05$, ** $p < .01$, compared to the control. See legends of Figures 1 and 3 for abbreviations.

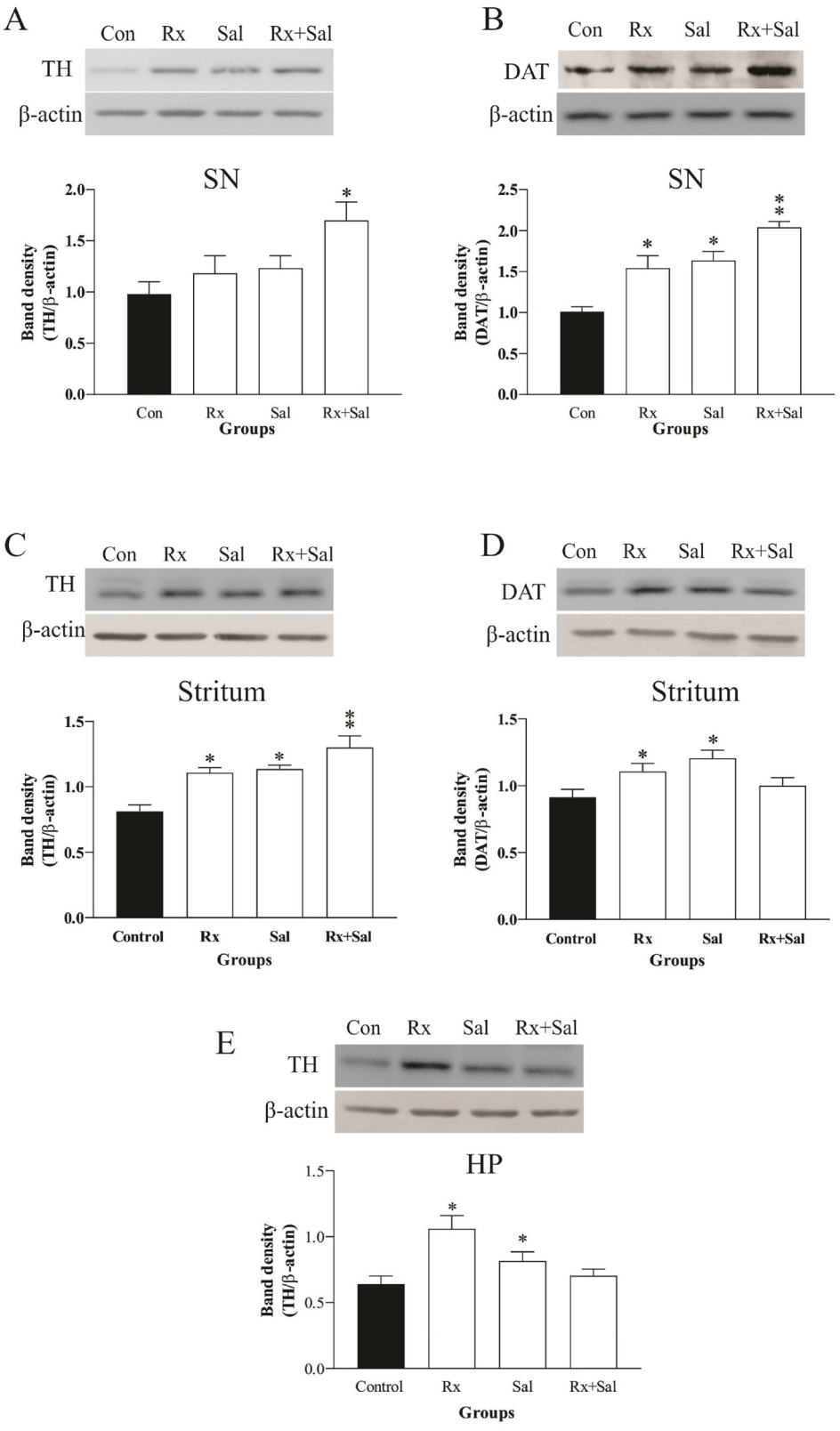

Figure 5. Effects of treatments with 2-methoxy idazoxan and salmeterol on protein levels of TH and DAT proteins in brain regions of 23-month-old rats. The administration of 2-methoxy idazoxan and salmeterol increased these protein levels in the SN (A and B), striatum (C and D), and HP (E) of rats measured by western blotting (all $N = 6$/group). The upper panels in each figure show autoradiographs obtained by western blotting. The lower panels in each figure show quantitative analysis of band densities. * $p < .05$, ** $p < .01$, compared to the control. See legends of Figures 1 and 3 for abbreviations.

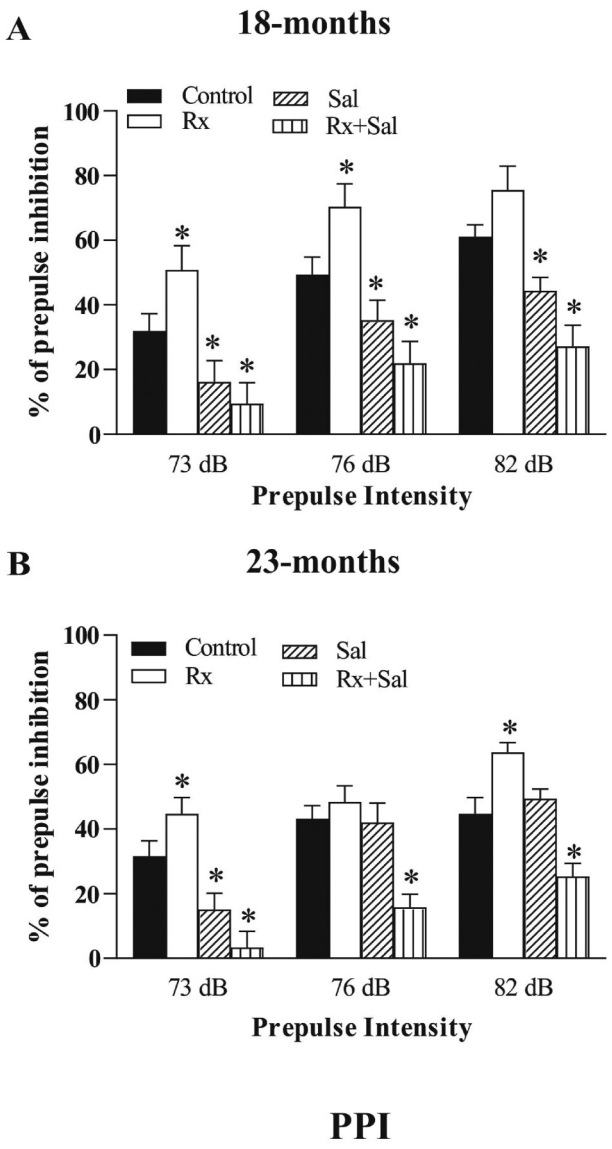

Figure 6. Effects of treatments with 2-methoxy idazoxan and salmeterol on the PPI performance of rats at ages of 18 (A, $N = 8$/group) and 23 months (B, $N = 9$/group). Behavior test was measured by the PPI. * $p < .05$, compared to the control. See legend of Figure 3 for abbreviations.

not be significantly different from normal controls in the frontal cortex and brain stem regions (Thomas et al., 1998). In addition, the injection of L-DOPS to animals protects neurons against cell damage (Biaggioni & Robertson, 1987; Lee et al., 1994; Yamagami et al., 1998) and facilitates behavioral recovery (Kato et al., 1987b; Kikuchi et al., 2000). Clinical trials revealed that L-DOPS effectively facilitates motor recovery and restores neurological deficits (Fukada et al., 2013; Ogawa et al., 1985; Tohgi et al., 1993; Yoshida et al., 1989). Second, identical to the previous report that L-DOPS increased extracellular levels of DA and 5-hydroxytryptamine (5-HT) (Maruyama et al., 1994), the present study demonstrated increased DA levels in the striatum. Third, the results observed in the present study may also indicate the involvement of brain-derived neurotrophic factor (BDNF), as BDNF can be a main mediator for the trophic signal derived from noradrenergic afferents (Fawcett et al., 1998). It is well known that L-DOPS increased nerve growth factor synthesis (Lee et al., 1994) and increased BDNF protein and mRNA expression in the cortex and HP (Kalinin et al., 2012), suggesting that an increased BDNF could provide trophic support for LC neurons, which also is related to its mechanism of neuroprotection. Thus, the present study provides the evidence that L-DOPS can be potentially useful to improve aging-related noradrenergic declines, which is coincident with the fact that L-DOPS has been applied to the patients of Parkinson's disease and alleviated L-DOPA-refractory symptoms (Kondo, 1993; Tohgi et al., 1993).

In the present study, α2-AR antagonist and β2-AR agonist were used as one method to manipulate noradrenergic activity. Alpha-2-ARs exert a tonic inhibitory control on adrenergic transmission (Kable et al., 2000; Trendelenburg et al., 1999). The blockade of these α2-ARs leads to the activation of LC-derived adrenergic projections (Millan et al., 2001; van Veldhuizen et al., 1993). Thus, it facilitates noradrenergic modulatory effects over dopaminergic neurons (Donaldson et al., 1975; Nutt et al., 1994; Srinivasan & Schmidt, 2004) and exerts a protective role upon dopaminergic neurons via the noradrenergic network innervating the SN (Gobert et al., 2004; Martel et al., 1998; Srinivasan & Schmidt, 2004). It was reported that 2-methoxy idazoxan could increase NE levels in the brain (Mandel et al., 2007; Millan, 2010; Millan et al., 2001; Qian et al., 2011; Sagi et al., 2007; Shenoy et al., 2006; Srinivasan & Schmidt, 2004; van Veldhuizen et al., 1993). Similarly, β2-AR agonists (long acting) can either directly or indirectly interact with the receptors to induce the release of endogenous catecholamine including NE (Peterson et al., 2014). Therefore, an increased expression level of dopaminergic phenotypes in the brain observed in the present study can be considered as the results of activation of noradrenergic systems. Besides, the mechanisms underlying the gene regulation contributed by α2-AR antagonists and β-AR agonists may similarly be related to their ability to increase neurotrophic factors including BDNF released from adrenergic neurons (Debeir et al., 2004; Weinreb et al., 2007). For example, salmeterol can significantly increase BDNF concentrations in serum and platelets when administered to patients, which was confirmed by *in vitro* study that salmeterol increased the release of BDNF from mononuclear cells (Lommatzsch et al., 2009). In addition, 2-methoxy idazoxan has been reported to increase DA levels in the striatum and SN (Maruyama et al., 1994; Srinivasan & Schmidt, 2004).

Akt, also known as protein kinase B, is a crucial mediator of various cellular process. It is well known that the phosphoatidylinositol-3-kinase (PI-3K)/Akt pathway is a major mediator of cell survival signaling leading to the transcription of many genes in pro-survival signal pathways

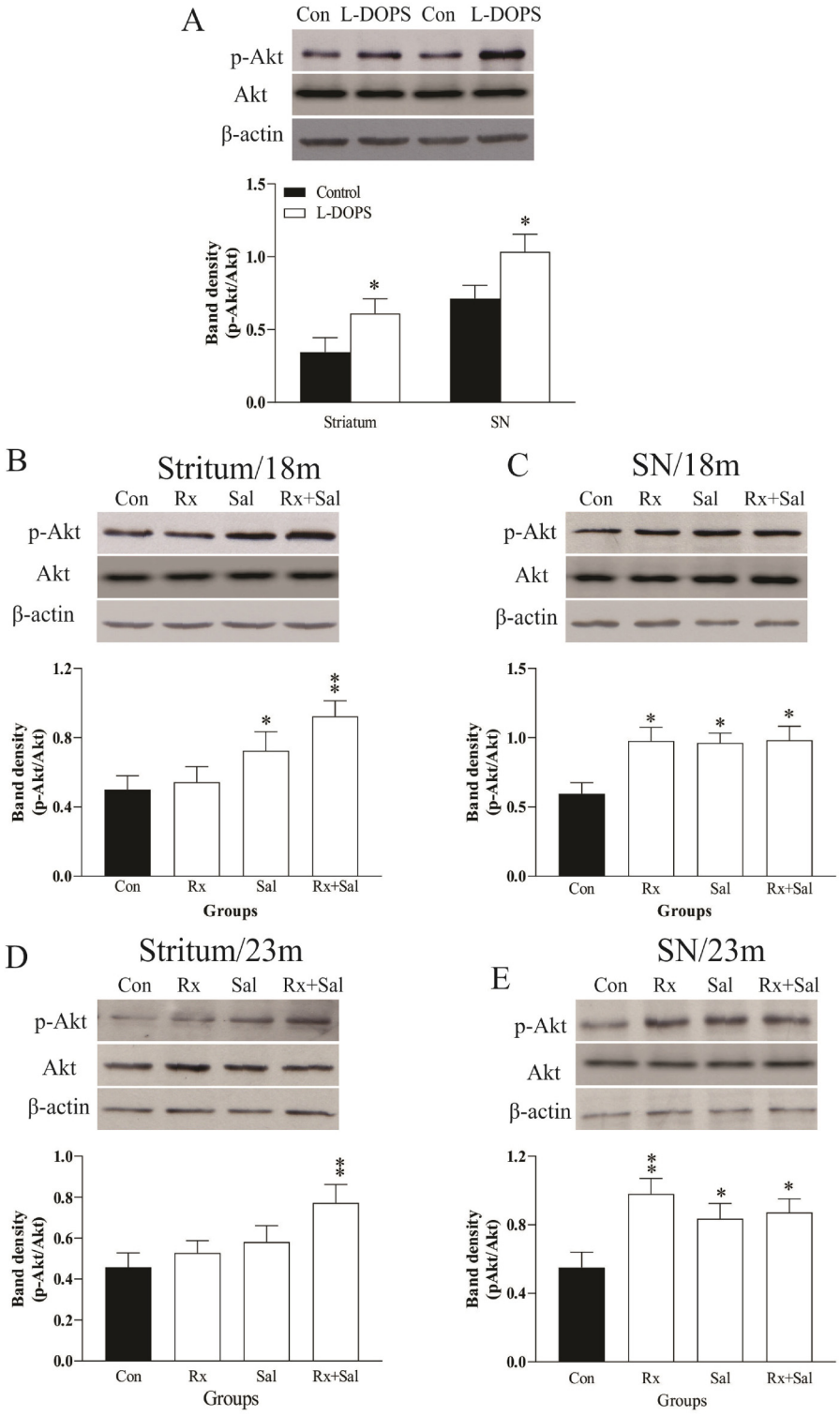

Figure 7. Effects of treatments with L-DOPS, 2-methoxy idazoxan and salmeterol on protein levels of p-Akt in the brain regions of old rats measured by western blotting (all $N = 6$/group). P-Akt and Akt densitometry results were respectively normalized to corresponding β-actin and the ratio of p-Akt/Akt scores was used as the final result in each case. A: Administration of L-DOPS significantly increased p-Akt in rat striatum and SN of 23-month-old rats. C and E: Injection of 2-methoxy idazoxan and salmeterol alone, or combination of two drugs markedly increased p-Akt protein levels in the SN of 18- and 23-month-old rats. However, only salmeterol alone (B) or combination of two drugs significantly increased p-Akt protein levels in the striatum of 18- or 23-month-old rats (B and D). The upper panels in each figure show autoradiographs obtained by western blotting. The lower panels in each figure show quantitative analysis of p-Akt/Akt band densities. * $p < .05$, ** $p < .01$, compared to the control. See legend of Figure 3 for abbreviations.

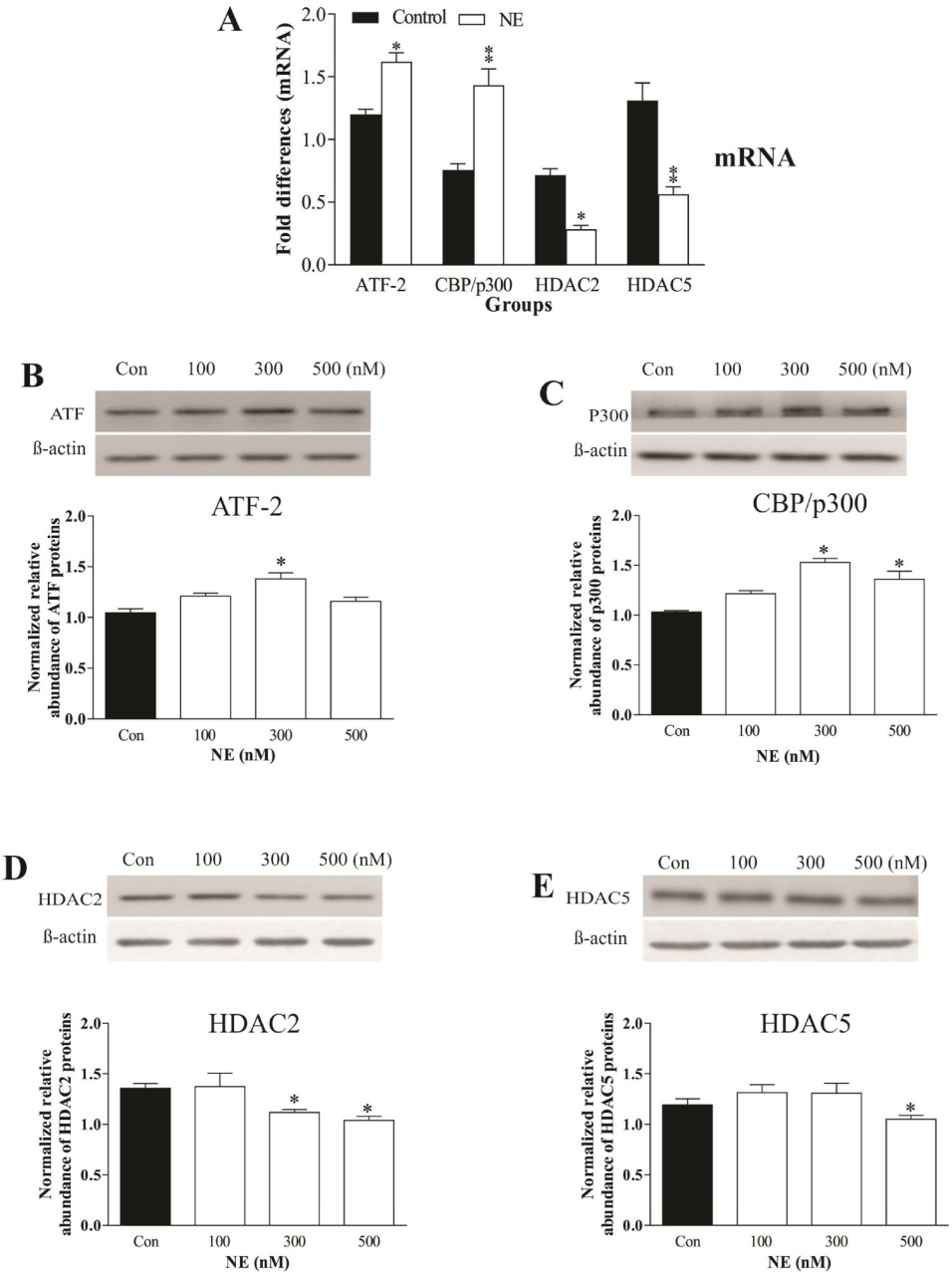

Figure 8. Effects of administration of NE on expression of ATF-2, CBP/p300, HDAC2, and HDAC5 in MN9D cells (all $N = 5$/group). A: Exposure of MN9D cell to 300 nM NE for two days significantly increased mRNAs of ATF-2 and CBP/300 and reduced mRNAs of HDAC2 and HDAC5. Exposure of MN9D cells to different concentrations of NE significantly increased protein levels of ATF-2 (B), CBP/p300 (C), as well as reduced protein levels of HDAC2 (D) and HDAC5 (E). * $p < .05$, ** $p < .01$, compared to the control. Abbreviations: Con: controls; NE: norepinephrine.

(Brunet et al., 2001), which also mediates the neurotrophic effects of NE (Patel et al., 2010). In the present study, the administration of L-DOPS, 2-methoxy idazoxan, and salmeterol significantly increased p-Akt protein levels in the striatum and SN, indicating that this protein kinase is possibly involved in the NE-induced upregulation of dopaminergic phenotypes of old rats. Studies have shown that age-related alterations in neuronal systems involve the modification of signaling pathways including those associated with Akt (Smith et al., 2005). For example, aged human tissue analyses revealed a decreased activation of PI-3K pathway in dendritic cells (Agrawal et al., 2008). A senescence mouse model exhibited a greater decrease of phosphorylation of Akt in the HP, as compared to controls (Nie et al., 2009). Therefore, in the present study, these reduced p-Akt expression levels may also present in the brain of aging and aged

rats, although we did not compare them to those of normal adult rats. Furthermore, it has been reported that treatment with NE significantly increased phosphorylation of Akt and activating Akt pathway in a dose- and time-dependent manner in cultured hippocampal or cortex cells (Jiang et al., 2014; Jiao et al., 2016; Patel et al., 2010). Akt plays a pivotal role in transducing a variety of extracellular stimuli into a wide range of cellular processes, including metabolisms, cell proliferating, and gene regulations (Sale & Sale, 2008). Increases in p-Akt resulted from those pharmacological manipulations can be considered as an exacerbated cell survival via the augmentation of PI-3K/Akt pathways activity caused by these treatments (Chen & Russo-Neustadt, 2005, 2007).

Our previous studies demonstrated that treatment with NE to MN9D cells increased H4 acetylation and H3 methylation in the TH promoter (Fan et al., 2020; Zhu et al., 2019), indicating that epigenetic histone modification plays an important role in the NE-induced upregulation of dopaminergic phenotypes. Although a significant alteration in H3 acetylation was not found, a tendency toward an increase was observed (unpublished data). Taking advantage of previous studies using ChIP, more experiments regarding the histone acetylation were performed. These experiments showed that the administration of NE to MN9D cells significantly increased mRNA and protein levels of ATF-2 and CBP/p300, two representatives of HATs, as well as reduced mRNA and protein levels of HDAC2 and HDAC5, two representatives of HDACs (Figure 8). Furthermore, ChIP assays demonstrate that while CBP/p300 enhanced NE-induced binding in the TH promoter, HDAC2 and HDAC5 significantly reduced NE-induced binding in the TH promoter. The involvement of HDACs in this regulation was confirmed by use of HDAC inhibitor, which accelerated NE-induced binding to the TH promoter. It has been reported that aging leads to the transcriptional downregulation of genes involved in brain functions such as cognition and synaptic plasticity (Lee et al., 2000; Lu et al., 2004), which can be counteracted by modulating the activities of histone-modifying enzymes (Peleg et al., 2016). On the other hand, aging was accompanied by a reduced histone acetylation at the promoter regions of genes involved in neurotransmission (Peleg et al., 2010; Pina et al., 1988), and an increased expression of HDAC (Chouliaras et al., 2013). Obviously, any factors to activating chromatin remodeling with increasing histone acetylation or inhibiting HADAC would result in enhanced transcriptions of related genes to interfere aging. Therefore, the present experiments may provide some information to reveal the involvement of chromatin remodeling in the transcriptional activation of TH gene by NE. These data may provide some useful evidence supporting the use of epigenetic therapy for the pharmacological interventions of aging (Khan et al., 2016), as HDAC inhibitors have shown promise as a treatment to combat the cognitive decline associated with aging (Penney & Tsai, 2014).

In the present study, PPI was performed to test the possible role of the manipulation of noradrenergic activities on a behavioral task highly related to dopaminergic activity. Generally, PPI is an index of attentional processes, which operationally measures sensorimotor gating (Graham, 1975). While this task has been reported to be related to several psychiatric disorders (Braff et al., 2001), it is an excellent behavioral measure for older rats, because of their inability to swim and thermos-regulate in the Morris water maze, and many other cognitively related tasks which can be affected by performance variables. Generally, PPI has been reported to be closely linked to increases in DA and/or NE activity. For example, animal studies showed that the direct infusion of DA into the nucleus accumbens in rats (Swerdlow et al., 1990) or the administration of agents that facilitate DA neurotransmission can reliably lead to PPI disruption (Geyer et al., 2001; Mansbach et al., 1988; Swerdlow et al., 1994). Similarly, the stimulation of cholinergic or glutamatergic receptors in the LC, which activates LC neuronal firing and elevates NE release in LC terminal regions, has also been shown to disrupt PPI performance (Alsene & Bakshi, 2011). PPI has been reported to be regulated by systemic manipulations of NE, stimulation α1/β-ARs, depleting of α2-ARs, and presumed augmentation of NE release (Alsene et al., 2006; Carasso et al., 1998; Lahdesmaki et al., 2004; Swerdlow et al., 2006). In the present study, the administration of L-DOPS or the combination of α2-AR antagonist and β-AR agonist significantly disrupted PPI performance. It is consistent to the notion that an increased noradrenergic tone, either through increased release of NE, or direct action at postsynaptic adrenergic receptors, plays a crucial modulatory role for PPI (Pudovkina et al., 2001).

In the present study, the administration of the α2-AR antagonist 2-methoxy idazoxan caused an increase in the startle response magnitude of rats at the age of 18 or 23 months, an effect contrary to those from injections of salmeterol alone or a combination of 2-methoxy idazoxan and salmeterol on the PPI. Interestingly, our present results are somewhat in accordance with those reported previously. For example, the administration of 2-methoxy idazoxan increased (Larrauri & Levin, 2012) or had a tendency to increase (Ozcetin et al., 2016) the prepulse intensity. Furthermore, this drug effectively counteracted disruption of PPI caused by amphetamine, dizocilpine or clonidine (Larrauri & Levin, 2012; Ozcetin et al., 2016), showing as the increased mean PPI values. The comparable results also came from the studies that used other α2-AR antagonist in which the administration of atipamezole weakly affected PPI (Lahdesmaki et al., 2004). Collectively, these data reveal that there is a difference between biochemical and behavioral effects regarding the administration of 2-methoxy idazoxan, considering that 2-methoxy idazoxan did increase brain DA levels (Grenhoff et al., 1993; Hertel et al., 1999) and elevated DA levels in the brain have been shown to play an essential role in PPI deficits in rats (Powell et al., 2003). Currently, we do not have a satisfactory explanation for this difference. However, the difference in biochemical and behavioral effects of some α2-AR antagonists including 2-methoxy

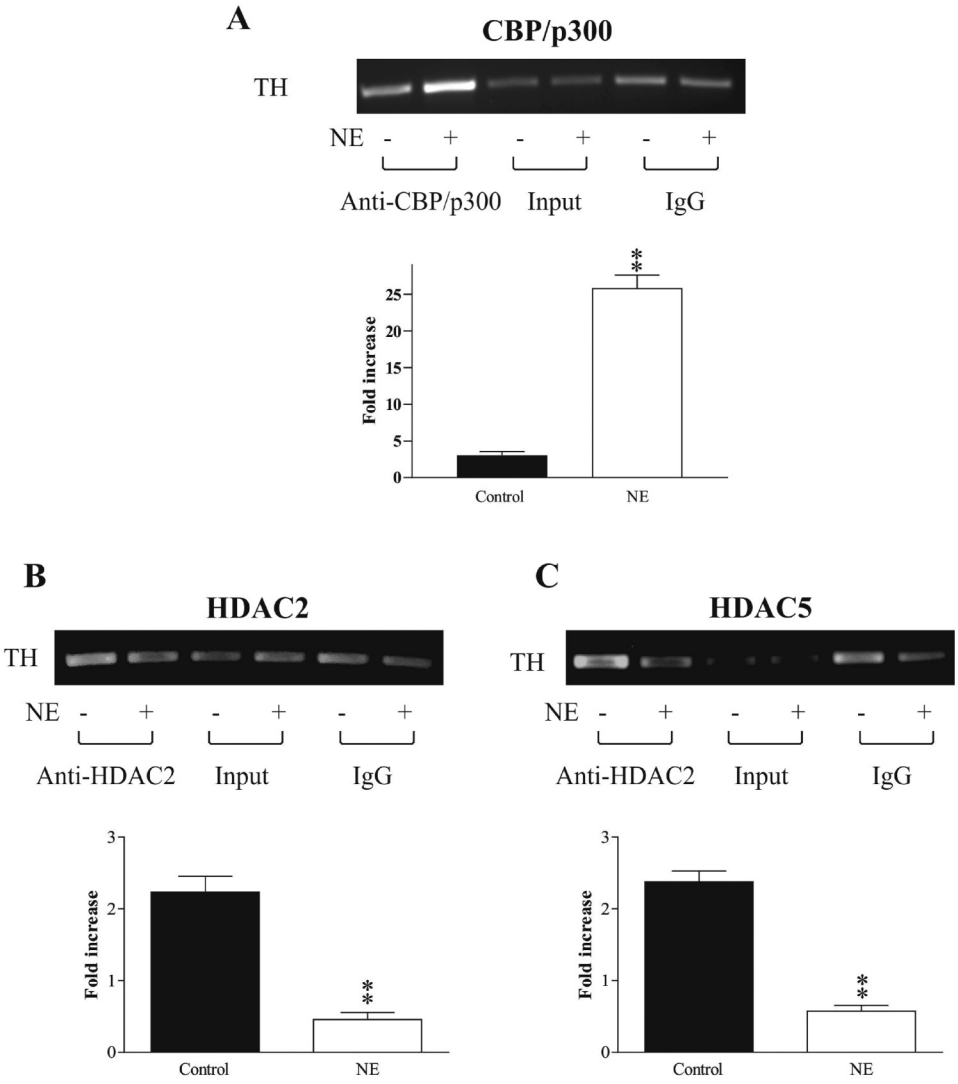

Figure 9. Identify the binding of the HAT & HDAC to the TH promoter by ChIP assay (all $N = 4$/group). Exposure of MN9D cells to NE for two days increased CBP/p300 binding to TH promoter (A) and reduced the binding of HDAC2 (B) and HDAC5 (C) to the TH promoter. "Input" serves as a loading control and rabbit IgG immunoprecipitation serves as a negative control. Top panel in each figures showed the binding to the TH promoter in response to NE treatment. Low panel in each figure showed quantitative real-time PCR of the TH promoter regions from immunoprecipitation with antibody against CBP/p300, HDAC2, and HDAC5. The fold enrichment value is shown as the normalized ChIP signals divided by the normalized input signal. Each bar from both pictures represent data obtained from four separate experiments ($N = 4$/group). ** $p < .01$, compared to the control. NE: treatment with 300 nM NE. Abbreviations: ChIP: chromatin immunoprecipitation assay; HAT: histone acetyl transferase; HDAC: histone deacetylases; NE: norepinephrine; PCR: polymerase chain reaction; TH: tyrosine hydroxylase.

idazoxan has consequently led to a suggestion that the antagonisms of the α2-ARs may not contribute to the regulation of the PPI (Uys et al., 2017). Moreover, multiple receptors have been shown to modulate PPI of startles, including 5-HT_{1A} (Hertel et al., 1999; Powell et al., 2005), 5-$HT_{2A/2c}$ (Larrauri & Levin, 2010), and H1 (Roegge et al., 2007), besides the receptors of DA and NE. The influence from these different receptors may also be one possible explanation for our results.

Some limitations to the present study must be acknowledged. Firstly, only 2-methoxy idazoxan and salmeterol were tested to represent α2-AR antagonists and β2-AR agonists. The α2-AR antagonists piribedil (Millan et al., 2001) and atipamezole (Gobert et al., 2004), as well as β2-AR agonist formoterol (Dang et al., 2014), have a similar mechanism as both 2-methoxy idazoxan and salmeterol. More examination will be performed in the near future. Secondly, all these pharmacologic treatments here are the monotherapy. Alternatively, a combination trial may be performed. For example, L-DOPS can be administered with α2-AR antagonist 2-methoxy idazoxan, or β2-AR agonist salmeterol. These combination treatments may increase their synergic effects on the DA system, although there is not any study reported so far. Finally, in the present

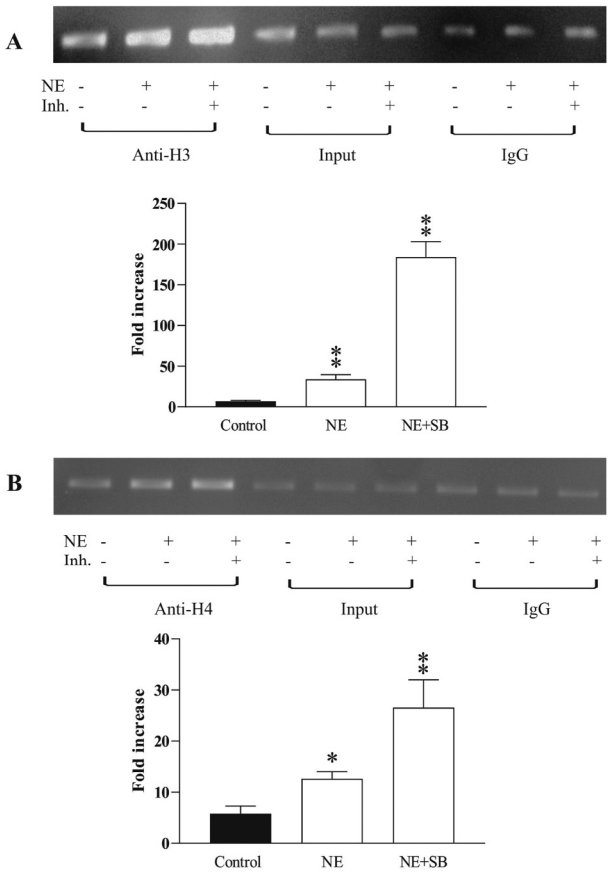

Figure 10. Effects of HDAC inhibitor on H3/H4 acetylation on the TH promoter. MN9D cells were exposed to 300 nM NE or 300 nM NE + 1 mM sodium butyrate for 2 days ($N = 4$/group). ChIP assay showed an increased H3 (A) and H4 (B) acetylations on the TH promoter regions ($N = 4$/group). * $p < .05$, ** $p < .01$, compared to the control. Abbreviations: ChIP: chromatin immunoprecipitation assay; HDAC: histone deacetylases; NE + SB: treatment with 300 nM NE plus 1 mM sodium butyrate; NE: treatment with 300 nM NE; TH: tyrosine hydroxylase.

study, effects of HATs and HDACs on NE-induced histone modification to effectively regulate TH transcription were investigated as a potential mechanism. However, this represents only a part of the entire epigenetic regulation. Owing to scope limitations, only limited HATs and HDACs were selected to be tested. In addition, compared to HATs, HDACs are more important, as chromatin remodeling mediated by HDACs is closely involved in the regulation of gene transcription, and HDAC inhibitors have shown promise as a treatment to combat the cognitive decline associated with aging (Penney & Tsai, 2014). Other HDACs and their possible roles in NE-induced gene regulation may also be examined in the future.

In conclusion, the present study demonstrated that the treatment of old rats with L-DOPS, α2-AR antagonist, or β-AR agonist significantly enhanced the expression of dopaminergic phenotypes in the striatum, SN, and HP. Coincident with their upregulations, these pharmacological manipulations mostly resulted in a deficit in PPI compared to controls. Furthermore, these treatments markedly increased protein levels of p-Akt, a signaling kinase downstream of the PI-3K/Akt pathway, indicating the involvement of PI-3K/Akt pathways in NE-induced upregulation of dopaminergic phenotypes. The *in vitro* experiments extend our previous observations that the histone acetylation plays a role in these regulations. Therefore, the results from the present study would provide important information regarding the usefulness of these pharmacologic interferences as potential disease-modifying therapeutic agents against aging.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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