

Original Article

# Physical Exercise Improves Aging-Related Changes in Angiotensin, IGF-1, SIRT1, SIRT3, and VEGF in the Substantia Nigra

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## Abstract

Dysregulation of tissue renin–angiotensin system (RAS) is involved in oxidative and inflammatory processes observed in major aging-related diseases, including neurodegenerative diseases such as Parkinson’s disease (PD). Physical exercise has beneficial effects against aging-related changes, dopaminergic neuron vulnerability, and PD progression. The present study indicates that sedentary aged rats have an increase in activity of the nigral angiotensin (Ang) II/Ang type 1 receptor (AT1) axis (ie, the pro-oxidative pro-inflammatory arm), and a decrease in the activity of the RAS protective arm (ie, Ang II/AT2 and Ang 1–7/Mas receptor axis) in comparison with young rats. In addition, sedentary aged rats showed a decrease in levels of nigral IGF-1, SIRT1, SIRT3, and VEGF. Treadmill running induced a significant increase in levels of IGF-1, SIRT1, SIRT3, and VEGF, as well as an increase in expression of the protective Ang 1–7/Mas axis and inhibition of the Ang II/AT1 axis. The exercise-induced increase in IGF-1 and sirtuins may mediate the effects of exercise on the nigral RAS. However, exercise may induce the increase in VEGF and modulation of RAS activity by different pathways. Exercise, via RAS, contributes to inhibition of the pro-oxidative and proinflammatory state that increase dopaminergic neuron vulnerability and risk of PD with aging.

**Keywords:** Aged, Neuroinflammation, Neuroprotection, Parkinson, Insulin-like growth factor

Aged brains show a pro-oxidative and pro-inflammatory state and a decrease in levels of several neurotrophic factors that increase vulnerability of neurons to neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD). Aging-related decreases in insulin-like growth factor-1 (IGF-1) (1,2), sirtuin 1 (SIRT1) and SIRT3 (3,4), and vascular endothelial growth factor (VEGF) (5) are major factors involved in aging and neurodegeneration. The humoral or circulating renin–angiotensin system (RAS) has initially been related to control of blood pressure and water homeostasis. However, it is now known that there are local RAS in many tissues and that dysregulation of tissue RAS is involved in oxidative

and inflammatory processes observed in major aging-related diseases such as hypertension, diabetes, atherosclerosis, and neurodegeneration (6–8). Angiotensin II (Ang II) is the most effective peptide of the system and it is formed by the action of renin and angiotensin converting enzyme (ACE) on the precursor glycoprotein angiotensinogen. Ang II, via its type 1 receptor (AT1), is a major activator of the NADPH–oxidase complex, leading to pro-oxidative and pro-inflammatory effects. However, these effects are counteracted by a RAS opposite arm constituted by Ang II/AT2 receptor signaling and Ang 1–7/Mas receptor signaling. Ang 1–7 is formed from angiotensin I (Ang I) or Ang II by angiotensin-converting enzyme 2 (ACE2).

Disruption of AT1 receptors promotes longevity in mice (9), and ACE and AT1 polymorphisms have been related to human longevity (10,11). Dysregulation of the local brain RAS has been involved in progression of major neurodegenerative diseases such as AD and PD (12,13).

Physical exercise has beneficial effects against aging-related changes observed in different tissues and organs (14,15), including brain (16–18). Aging is the major risk factor for PD, and beneficial effects of exercise against nigral dopaminergic neuron vulnerability and PD progression have been shown in several studies (19,20). A number of possible mechanisms have been suggested (21–23). In several previous studies in different PD models, we have shown that overactivation of the nigral Ang II/AT1 axis promotes progression of dopaminergic degeneration, which can be counteracted by AT1 receptor antagonists (reviewed in refs. 13,24). However, it is not known whether RAS modulation may mediate the effects of exercise against neurodegeneration, and against dopaminergic degeneration and PD in particular.

In several tissues, aging-related dysregulations of IGF-1, SIRT1, and SIRT3 were improved by exercise (14,25–27). Interestingly, we have recently observed a reciprocal regulation between the nigral RAS and IGF-1 (28) and sirtuins (29). This suggests that the nigral RAS may be modulated by exercise *via* IGF-1 and/or sirtuins. In several tissues, an aging-related decrease in angiogenesis has been related to decrease in VEGF levels, which was improved by exercise (17,30). However, it is not known if there exists mutual regulation between VEGF and RAS in the aged brain, and particularly in the substantia nigra, and whether this may be modulated by physical exercise. In this study, we used young and aged rats and mice to investigate whether physical exercise may affect the nigral RAS, and whether IGF-1, SIRT1, SIRT3, and VEGF are also affected by exercise and may mediate RAS changes.

## Methods

### Experimental Design

All experiments were carried out in accordance with the European Communities Council Directive 2010/63/ EU, Directive 86/609/EEC, and Spanish RD 526/2014, and were approved by the corresponding committee at the University of Santiago de Compostela. Young (3 months old) and aged (18–20 months) male rats and mice were housed under a 12 hours light/dark cycle and with *ad libitum* access to food and water. In a first series of experiments (group-A animals), we used young (3 months old) and aged (18–20 months) male Sprague-Dawley adult rats to investigate the effect of aging and exercise on several RAS components and several factors that may mediate changes in RAS activity (IGF-1, SIRT1, SIRT3, VEGF). Young ( $n = 14$ ) and aged ( $n = 14$ ) rats were assigned to exercised (treadmill running for a 4-week period;  $n = 14$ ) or no exercised (sedentary;  $n = 14$ ) subgroups. Non-exercised rats were subjected to the same handling than exercised rats except running. In recent studies, we have shown a reciprocal regulation between IGF-1 (28), sirtuins (29), and RAS in the substantia nigra. However, possible VEGF/RAS interactions were not studied. Therefore, we investigated possible mutual regulation between VEGF and RAS in the substantia nigra in additional series of experiments as follows.

A second series of experiments (group-B animals) were used to investigate whether VEGF may mediate the exercise-induced changes in RAS observed in group-A animals. Aged male adult Sprague-Dawley rats (18–20 months;  $n = 6$ ) were subjected to

exercise (treadmill running) as above and treated with vandetanib (ie, a tyrosine kinase inhibitor that targets the VEGF receptor inhibiting VEGF signaling *in vivo*; 30 mg/kg/day during 2 weeks before sacrifice; TINIB TOOLS). Vandetanib was administered orally mixed in very little portions of “Nocilla” hazelnut-cream spread (Nutrexpa, Barcelona, Spain). Control aged male adult Sprague-Dawley rats (18–20 months;  $n = 6$ ) were subjected to exercise and treated with vehicle only (Nocilla).

A third series of experiments (group-C animals) was used to study the possible regulation of VEGF by RAS. We compared young (3 months old) and aged (18–20 months) male mice deficient for AT1 or AT2 receptors with the corresponding wild-type (wt) controls: C57BL-6 wild-type ( $n = 12$ ) (Charles River, L ‘Arbresle, France), and homozygous C57BL-6 male mice deficient for AT1a ( $n = 12$ ) (the major mouse AT1 isoform, and the closest murine homolog to the single human AT1; Jackson Laboratory, Bar Harbor, ME), and young (3 months old) and aged (18–20 months) homozygous C57BL-6 male mice deficient for AT2 receptors ( $n = 12$ ) (gift of Dr. Daniel Henrion).

Forty-eight hours after the last exercise session (ie, at the beginning of the dark period), exercised and non-exercised animals were euthanized. Animals were stunned, decapitated, and the area of the substantia nigra in the right and left ventral mesencephalon was rapidly dissected on an ice bath, frozen in dry ice, and processed for western blot (wb) and real-time quantitative reverse-transcription polymerase chain reaction (RT-PCR).

### Treadmill Running

Rats in exercised group were trained on a treadmill system (CT-2 treadmill system, Columbus Instruments) with two lanes connected to a computer for system control and data management. Electrical stimulus to force the animals to run was not used. A 2-day pre-training period (30 min/day at 6–10 m/min at 0 degree of inclination) was adopted. Rats that failed to complete the pre-training sessions (ie, non-runners) were excluded. In order to provide a similar environment, animals from the sedentary group were placed on the stationary treadmill (31). Animals from exercised group were trained to run on the treadmill over four consecutive weeks (one 30-min session per day at 17 m/min, 5 days a week, at 0 degree of inclination). The training period was always performed at the beginning of the dark period.

### Real-Time Quantitative PCR Analysis

Total ribonucleic acid (RNA) from the nigral region was extracted with Trizol method according to the manufacturer’s instructions. The RNA concentration was estimated using a NanoQuant plate and an Infinite M200 multiwell plate reader (TECAN, Salzburg, Austria). Total RNA (2  $\mu$ g) was reverse-transcribed to complementary DNA (cDNA) with deoxynucleotide triphosphate (dNTP), random primers, and Moloney murine leukemia virus reverse transcriptase (M-MLV; 200 U, Invitrogen).

Real-time PCR was used to examine the relative levels of AT1, AT2, ACE2, and Mas receptors and VEGF, IGF-1, SIRT1, and SIRT3. Primers were designed for each gene by using Beacon Designer software (Premier Biosoft, Palo Alto, CA) and primer sequences used are shown in [Supplementary Table 1](#). A real-time iCycler PCR platform (BioRad, Hercules, CA) was used to perform the experiments (BioRad) using the IQ SYBR Green Supermix kit (BioRad).  $\beta$ -Actin was used as housekeeping gene in all cases and was amplified in parallel with the genes of interest. The data were evaluated by the

delta-delta Ct method ( $2^{-\Delta\Delta Ct}$ ) where Ct is the cycle threshold. The expression of each gene was obtained as relative to the housekeeping transcripts. The data were then normalized to the values of the control group and the results were expressed as mean  $\pm$  standard error of the mean (SEM).

### Western Blot Analysis

Rat or mouse ventral mesencephalon was homogenized in RIPA buffer containing protease inhibitor cocktail (P8340, Sigma). Tissue lysates were centrifuged, and the protein concentrations were determined by the Pierce BCA protein assay (Thermo Scientific, Fremont, CA). Equal amounts of protein were separated by 5–10% bis-Tris polyacrylamide gel and transferred to nitrocellulose membrane. The membranes were incubated overnight with the following primary antibodies: goat anti-AT1 (sc-31181), rabbit anti-AT2 (sc-9040), goat anti-IGF-1 (sc7144), rabbit anti-VEGF (sc-152), mouse anti-SIRT-1 (1:200, sc74465), goat anti-SIRT-3 (sc49744), and goat anti-CD31 (PECAM-1 sc-1506), all 1:200 from Santa Cruz Biotechnology, rabbit anti-ACE 2 (Ab108252) from Alomone and rabbit anti-Mas receptor (AAR-013) 1:1,000 from Abcam. The specificity of the antibodies (AT1 sc-31181; AT2 sc-9040) was confirmed in our laboratory by pre-adsorption with the corresponding synthetic peptide antigen (see ref. 32 for details). We also used western blot analysis of lysates from HEK293 cells transfected with AT1 or AT2 tagged to fusion tail DDK (TA50011 from Origene, Rockville, MD; DDK tag: DYKDDDDK). Using HEK293 cells, the specificity of the antibodies was confirmed by the presence of a predominant immunoreactive band in lysates from positively transfected cells and also by the absence of this band in negative controls, which consisted of lysates of cells transfected with empty vectors (see ref. 33 for details). The membranes were incubated with the following HRP-conjugated secondary antibodies: donkey anti-goat (1:2,500), goat anti-rabbit (1:2,500), and goat anti-mouse (1:2,500) from Santa Cruz Biotechnology. Immunoreactive bands were detected with an Immun-Star HRP Chemiluminescent Kit (170–5044; Bio-Rad) and visualized with a chemiluminescence detection system (Molecular Imager ChemiDoc XRS System; Bio-Rad). Blots were stripped and reprobed for anti-GADPH (glyceraldehyde 3-phosphate dehydrogenase) (1:25,000, Sigma) as loading control. For each animal, protein expression was measured by densitometry of the corresponding band and expressed relative to the GADPH band value. As an increase in brain mitochondrial biogenesis was observed after exercise training in some studies (34), changes in SIRT3 protein were confirmed using a specific mitochondrial housekeeping (voltage-dependent anion channel; VDAC) to exclude possible SIRT3 changes related to differences in the mitochondrial fraction of the sample. The data were then normalized to the values of the control group of the same bath (100%) to counteract any between-batch variability. Finally, the results were expressed as mean  $\pm$  SEM.

### Statistical Analysis

All data were obtained from at least three independent experiments. Data were then normalized to the values of the control group of the same batch and expressed as a mean  $\pm$  standard error of the means (SEM). Two-group comparisons were carried out with the Student's *t* test. The normality of samples (Kolmogorov–Smirnov test) and homogeneity of variances (Levene median test) were tested before each analysis. Differences were considered significant at  $p < .05$ . Statistical analyses were carried out with the SigmaPlot 11.0 (Systat Software, Inc., CA).

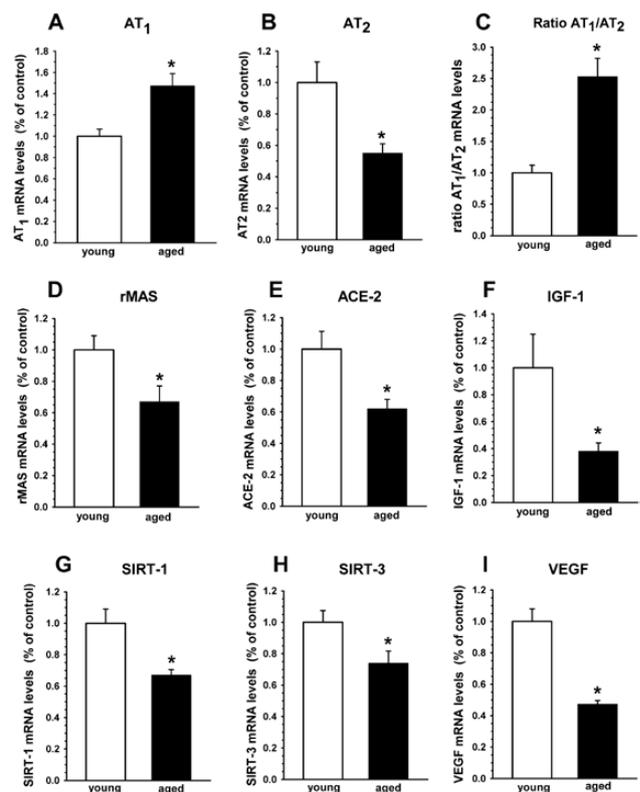
## Results

### Effects of Aging on Expression RAS Components, IGF-1, SIRT1, SIRT3, and VEGF Levels in the Nigral Region

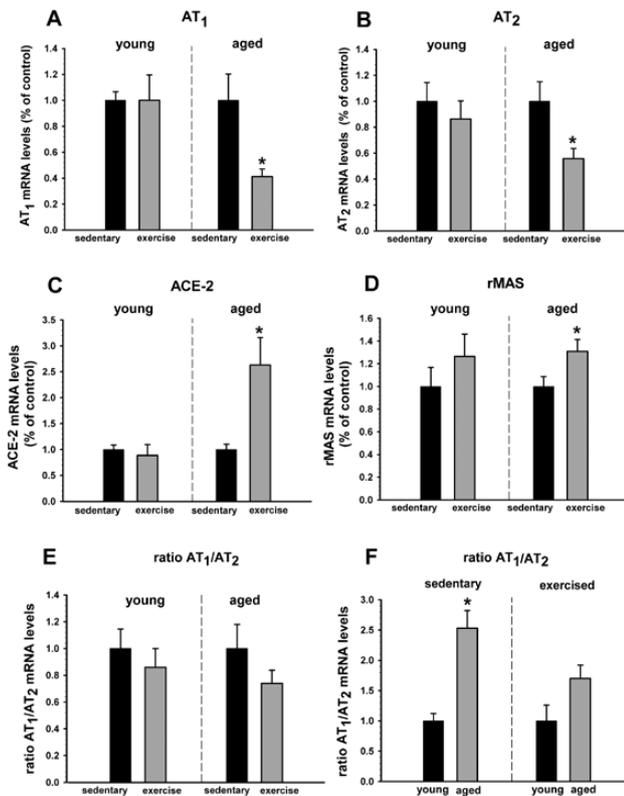
Aged sedentary rats showed a marked increase in the expression of the pro-oxidative pro-inflammatory angiotensin receptor AT1 and a marked decrease in the expression of the protective arm receptors AT2 and Mas, as well as a decrease in the expression of ACE2 which generates the protective peptide Ang 1–7. The nigral region of aged sedentary rats also showed a significant decrease in the expression of IGF-1, SIRT1, and SIRT3, and VEGF mRNA in comparison with young controls (Figure 1).

### Effects of Physical Exercise on the Expression of Different RAS Components in the Nigral Region of Aged and Young Rats

Exercise induced a marked decrease in the expression of mRNA for AT1 receptors in aged rats (Figure 2A). We also observed an exercise-induced decrease in AT2 receptor expression, so that the decrease in the ratio AT1/AT2 was not significantly different after exercise (Figure 2B and E). However, the aged/young AT1/AT2 ratio



**Figure 1.** Effects of aging on the expression of different RAS components (AT1, AT2, and Mas receptors, and ACE2), and IGF-1, SIRT1, SIRT3, and VEGF in the nigral region. Real-time quantitative RT-PCR analysis of changes in mRNA levels in sedentary aged rats relative to control rats (young sedentary rats). The expression of each gene was determined relative to the housekeeping transcripts ( $\beta$ -actin). The data were then expressed relative to control group values. Data are means  $\pm$  SEM. \* $p < .05$  relative to the young controls (Student's *t* test). ACE-2 = angiotensin converting enzyme-2; AT1 = angiotensin type 1 receptor; AT2 = angiotensin type 2 receptor; rMas = Mas receptor; IGF-1 = insulin-like growth factor-1; SIRT = sirtuin; VEGF = vascular endothelial growth factor.

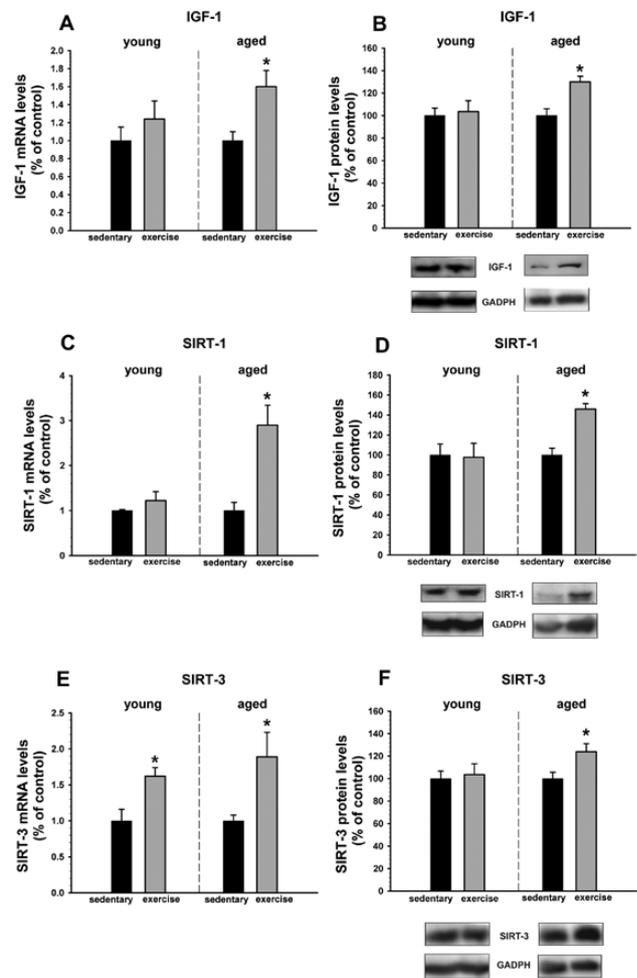


**Figure 2.** Effects of exercise on the expression of different RAS components in the nigral region of young and aged rats. Real-time quantitative RT-PCR analysis of changes in mRNA levels of AT<sub>1</sub>, AT<sub>2</sub>, Mas receptors, and ACE2 in exercised rats relative to sedentary rats of the same age (ie, young or aged rats). The expression of each gene was determined relative to the housekeeping transcripts ( $\beta$ -Actin). The data were then expressed relative to sedentary group values. Data are means  $\pm$  SEM. \* $p < .05$  relative to the sedentary controls (Student's  $t$  test). ACE-2 = angiotensin converting enzyme-2; AT<sub>1</sub> = angiotensin type 1 receptor; AT<sub>2</sub> = angiotensin type 2 receptor; rMas = Mas receptor.

was lower in exercised rats (Figure 2F). Physical exercise induced a marked increase in the mRNA expression for ACE2 (ie, Ang 1–7 converting enzyme) and a significant increase in the mRNA expression for Mas (ie, Ang 1–7 receptor) in aged rats (Figure 2C and D). In young rats, no significant changes were induced by exercise in the expression of the above-mentioned RAS components (Figure 2). WB analysis of protein expression of different RAS components in sedentary and exercised young and aged rats showed results similar to those observed by real-time quantitative PCR analysis (see Supplementary Figure 1).

### Effects of Physical Exercise on the Expression of IGF-1, SIRT1, SIRT3, and VEGF Levels in the Nigral Region of Aged and Young Rats

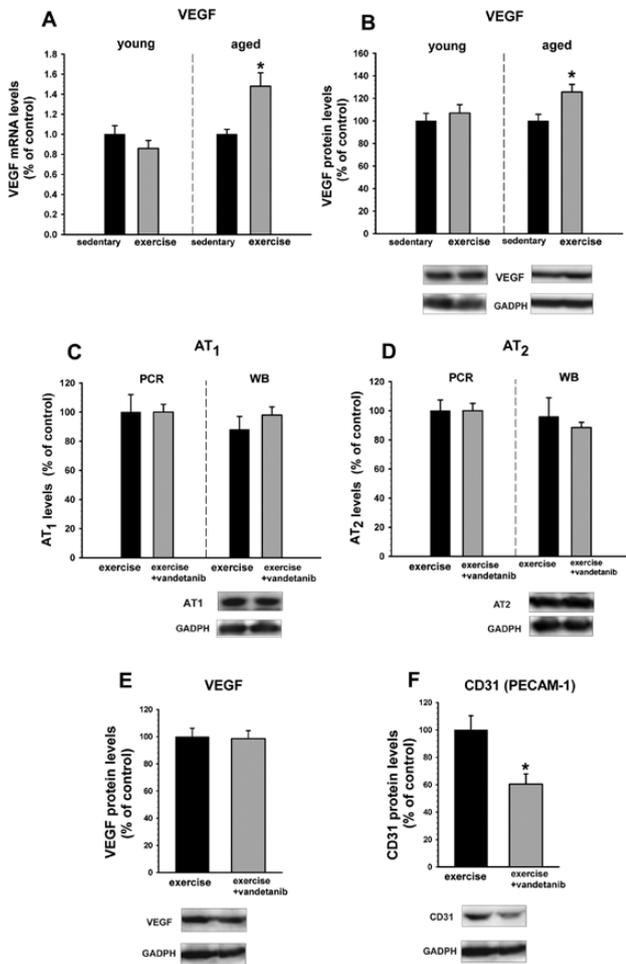
Exercise induced a significant increase in levels of IGF-1 mRNA and protein in aged rats but not in young rats (Figure 3A and B). We also observed that physical exercise induced a marked increase in levels of SIRT1 and SIRT3 mRNA and protein in aged rats. The increase in SIRT3 protein was confirmed using a specific mitochondrial housekeeping (VDAC) to exclude possible changes related to differences in the mitochondrial fraction of the sample (Supplementary Figure 2). Differences in the expression of IGF-1, SIRT1, SIRT3 mRNA, and protein between sedentary young rats and exercised young rats



**Figure 3.** Effects of exercise on the expression of IGF-1 (A, B), SIRT1 (C, D), and SIRT3 (E, F) in the nigral region of young and aged rats. Real-time quantitative RT-PCR (A, C, E) and Western blot (B, D, F) analysis of mRNA and protein levels in exercised rats relative to sedentary rats of the same age (ie, young or aged rats). Protein expression was determined relative to that of the GAPDH band value, and the expression of each gene was determined relative to the housekeeping transcripts ( $\beta$ -Actin). Data are means  $\pm$  SEM. \* $p < .05$  relative to the sedentary controls (Student's  $t$  test). IGF-1 = insulin-like growth factor-1; SIRT = sirtuin.

did not reach statistical significance excepting for SIRT3 mRNA (Figure 3C–F). In the nigral dopaminergic system, we have recently shown mutual regulation between IGF-1 and RAS, and between RAS and sirtuins (see refs. 28,29 for details).

Exercise also induced a significant increase in VEGF mRNA and protein expression in aged rats. Again, no differences in VEGF levels were observed between young sedentary rats and young exercised rats, at least using the present protocol of exercise (Figure 4A and B). As possible mutual regulation between VEGF and RAS was not previously investigated in the substantia nigra, we firstly investigated the effect of the VEGF receptor blocker vandetanib on the effects of exercise on AT<sub>1</sub>, AT<sub>2</sub>, VEGF levels, and angiogenesis. We observed that the VEGF receptor blocker did not induce any significant change in the effect of exercise on RAS components. However, vandetanib induced a significant decrease in the marker of angiogenesis CD31 (PECAM-1; Figure 4C–F). Secondly, possible effects of RAS changes on VEGF levels were investigated using AT<sub>1</sub> and AT<sub>2</sub> ko mice. No significant changes in VEGF mRNA were observed in young AT<sub>1</sub>

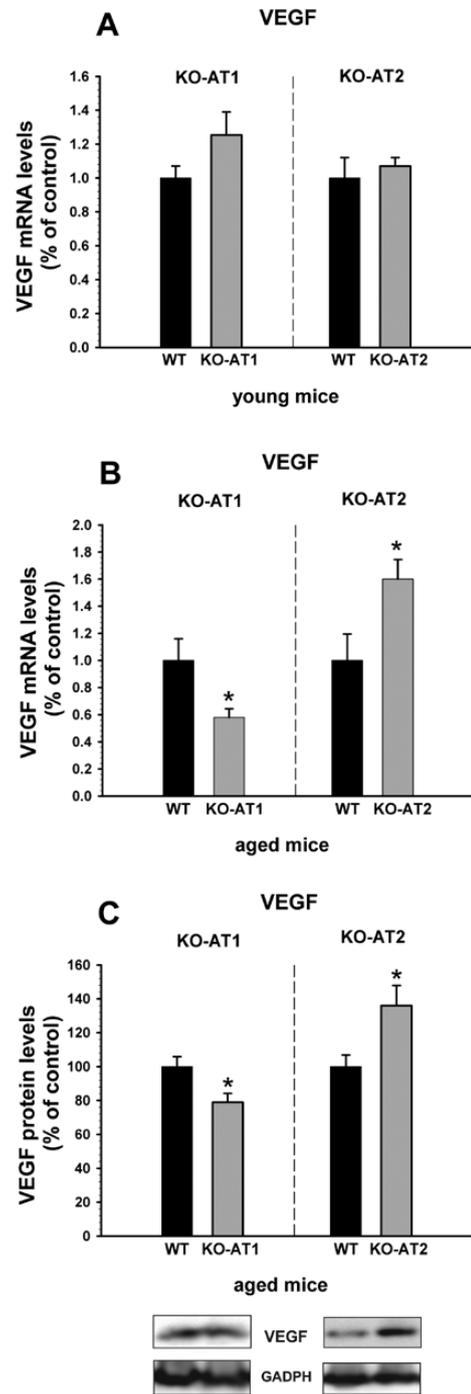


**Figure 4.** Effects of exercise on the expression of VEGF in the nigral region of young and aged rats (A, B), and effects of the VEGF receptor blocker vandetanib on the effects induced by exercise on AT1 and AT2 receptors, VEGF levels, and markers of angiogenesis (CD31, PECAM-1) in the substantia nigra of aged rats (C–F). Protein expression was determined relative to that of the GAPDH band value and the expression of each gene was determined relative to the housekeeping transcripts ( $\beta$ -Actin). Data are means  $\pm$  SEM. \* $p < .05$  relative to the sedentary controls (Student's *t* test). AT1 = angiotensin type 1 receptor; AT2 = angiotensin type 2 receptor; VEGF = vascular endothelial growth factor.

and AT2 ko mice in comparison with the corresponding wild type young mice (Figure 5A). However, we observed a significant decrease in VEGF mRNA expression in aged AT1 ko mice relative to aged wild-type mice (Figure 5B). Aged AT2 ko mice showed a significant increase in VEGF mRNA expression in comparison with the corresponding aged wild type mice (Figure 5B). Changes observed in AT1 and AT2 mRNA expression in aged mice were confirmed by WB analysis (Figure 5C).

### Discussion

The present study indicates that sedentary aged rats have an increase in activity of the pro-oxidative proinflammatory arm of the nigral RAS (ie, Ang II/AT1 axis), and a decrease in the activity of the protective arm (ie, Ang II/AT2 and Ang 1–7/Mas) in comparison with young rats. This is consistent with our previous studies (35,36), and



**Figure 5.** Effects of AT1 and AT2 receptor deletion (ie, AT1 and AT2 ko mice) on the expression of VEGF in the nigral region. Using real-time quantitative RT-PCR analysis, no significant changes were observed in VEGF mRNA levels of young AT1 or AT2 ko mice relative to young WT controls (A). However, VEGF mRNA expression was decreased in aged AT1 ko mice and increased in aged AT2 ko mice relative to aged WT controls (B). Changes in expression of VEGF in the nigra of aged ko mice were confirmed by western blot analysis (C). Protein expression was determined relative to that of the GAPDH band value and the expression of each gene was determined relative to the housekeeping transcripts ( $\beta$ -Actin). Data are means  $\pm$  SEM. \* $p < .05$  relative to the sedentary controls (Student's *t* test). AT1 = angiotensin type 1 receptor; AT2 = angiotensin type 2 receptor; VEGF = vascular endothelial growth factor; WT = wild type.

may increase vulnerability of nigral dopaminergic neurons to neuroinflammation and neurodegeneration. In addition, sedentary aged rats showed a decrease in levels of IGF-1, SIRT1, SIRT3, and VEGF. The effects of brain IGF-1 on aging and neurodegeneration is still controversial (reviewed in ref. 37). IGF-1 is actively transported to the central nervous system (CNS) from plasma, and it is also locally produced in the brain by neurons and glial cells. IGF-1 is involved in the neuroinflammatory response and possibly in other brain functions, and fine tuning of IGF-1 levels may be critical, which may explain controversial results obtained with different experimental approaches. SIRT1 and pharmacological SIRT1 activators such as resveratrol counteract the effects of aging (38) and inhibit the development of neurodegenerative diseases (39). SIRT3 is also involved in aging and neurodegeneration (40). A number of studies have revealed that aging induces a reduction in blood vessel density and VEGF levels, and that VEGF is a potent regulator of angiogenesis (18,30). In addition, it has been observed that VEGF is also involved in neurodegeneration (5) and, particularly, that VEGF has neuroprotective and neurorescue effects on dopaminergic neurons (41).

In this study, we observed that physical exercise leads to a significant increase in levels of IGF-1, SIRT1, SIRT3, and VEGF in the nigral region of aged rats, as well as a significant increase in the expression of ACE2/Mas receptor protective arm of the RAS. Physical exercise also induced an inhibition of the pro-oxidative proinflammatory arm as revealed by a decrease in AT1 receptor expression. AT2 receptor expression was also decreased by exercise. This may be related with the marked decrease in AT1 expression induced by exercise and the exercise-induced increase in other components of the protective arm (Ang 1–7/Mas). AT2 receptor expression is markedly decreased in aged sedentary animals, which is consistent with that observed in our previous studies (33,35). However, the present observation (ie, a decrease in AT2 expression with exercise) suggests that there is some degree of compensatory response of AT2 expression against the marked up-regulation of AT1 in aged sedentary brains, which is decreased after exercise-induced reduction in AT1 expression. The ratio AT1/AT2 does not change significantly with or without exercise, which support this view. The discrete upregulation of AT2 receptors in aged sedentary rats is, however, insufficient as the ratio AT1/AT2 is much higher aged sedentary rats than in young controls. However, an increase in AT1 expression in young animals led to a marked compensatory increase in AT2 receptor expression to maintain the AT1/AT2 ratio (42). Therefore, the beneficial effects of exercise appear mostly related to the decrease in the activity Ang II/AT1 axis and the increase in the activity of the Ang 1–7/Mas axis. Several previous studies have related AT1 depletion and AT1 blocking treatments with longevity (9,11) and neuroprotection (43,44), which is consistent with the beneficial effects of exercise against aging-related diseases, and PD in particular (19,20). It is also interesting to note that we did not observe significant changes in levels of IGF-1, SIRT, VEGF, and RAS components in young rats subjected to the same exercise protocol. This suggests that exercise may improve dysregulated factors induced by aging, and reduce differences with young controls. However, exercise does modify physiological levels of the same factors, controlled by effective regulatory systems in young animals. We cannot exclude that a different protocol of exercise can induce changes in young animals.

The mechanisms involved in the regulation of nigral RAS by exercise remain to be totally clarified. However, our recent studies have revealed a mutual regulation between RAS and IGF-1 (28,37) and RAS and SIRT1 (29). IGF-1 inhibited Ang II/AT1 activity in dopaminergic neurons and glial cells; IGF-1 also decreased markers of

the M1 neuroinflammatory microglial phenotype and decreased the dopaminergic neuron death induced by neurotoxins (28). Therefore, an initial decrease in levels of IGF-1 in aged animals may lead to the observed increase in the activity of the RAS pro-inflammatory arm, which may be down-regulated by the exercise-induced increase in IGF-1 levels. Previous studies in different tissues have suggested that an exercise-induced increase in IGF-1 levels may be a major factor responsible for the beneficial effects of exercise (25).

In aged transgenic mice over-expressing SIRT1, we have recently observed that levels of AT1 and NADPH-oxidase activity (ie, the pro-inflammatory RAS arm activity) were lower than in aged wild-type controls, and that the pharmacological SIRT1 activator resveratrol induces inhibition of the Ang II/AT1/NADPH-oxidase axis (29). Therefore, the decrease in levels of SIRT1 in aged animals may also contribute to the observed increase in the activity of the RAS pro-inflammatory arm, which may be down-regulated by the exercise-induced increase in SIRT1 levels. Interactions between SIRT3 and RAS have been observed in other tissues (45). In the kidney, disruption of the AT1 has been shown to induce SIRT3 upregulation and promote longevity in mice (9). In the dopaminergic system, we also observed a RAS/SIRT3 interaction, and that inhibition of SIRT3 leads to Ang II/AT1 axis over activity (Diaz-Ruiz and Labandeira-Garcia, unpublished observations).

We had not previously investigated the possible interaction between RAS and VEGF in the nigrostriatal system. However, we have observed a significant decrease in the density of microvessels in the substantia nigra of aged animals that may be related to an aging-related decrease in VEGF levels (18). Furthermore, we have shown that chronic brain hypoperfusion induces an increase in the activity of the Ang II/AT1 axis together with an increase in vulnerability of dopaminergic neurons to neurotoxins, which was inhibited by AT1 receptor antagonists (46). Altogether suggests a possible interaction between brain hypoperfusion-angiogenesis-levels of VEGF and RAS activity. The present results show that aged sedentary rats have down-regulation of VEGF levels, up-regulation of the proinflammatory Ang II/AT1/NADPH-oxidase axis and down-regulation of the anti-inflammatory anti-oxidant axis (AT2, Mas receptors). Both VEGF depletion and RAS changes are partially reverted by physical exercise. However, the results do not suggest a direct inhibitory effect of the exercise-induced increase in VEGF levels on RAS activity, since treatment of rats with the VEGF receptor blocker vandetanib inhibited exercise induced angiogenesis but not exercise-induced changes in RAS components. Although the lack of responses in RAS components may be attributed to other factors, the present results suggest that exercise may regulate nigral VEGF and nigral RAS by separate pathways. The exercise-induced increase in IGF-1 may be a common trigger for RAS down-regulation (28), VEGF up-regulation (47,48), and SIRT1 up-regulation (49). Although we did not observe a direct effect of VEGF on RAS, we observed that changes in RAS activity may affect VEGF levels. A decrease in AT1 or AT2 activity (AT1 and AT2 ko mice) led to decreased (AT1 ko mice) or increased (AT2 ko mice) levels of VEGF in aged mice, which indicates that the RAS, directly or indirectly, regulates VEGF expression. The high level of AT1 expression and low level of AT2 expression observed in aged animals may be related to compensatory changes to increase the low levels of VEGF observed in aged rats, and to counteract aging-related decrease in microvessel density and hypoperfusion.

In conclusion, physical exercise down-regulates the pro-inflammatory-pro-oxidative and up-regulates the protective arm of the local RAS in the substantia nigra, which are dysregulated in aged rats. This may decrease the pro-oxidative proinflammatory state

that increase dopaminergic neuron vulnerability and risk of PD with aging. In aged rats, physical exercise increased the levels of IGF-1 and SIRT1 and SIRT3 in the nigral area, which are down-regulated in aged rats in comparison with young controls. The exercise-induced increase in IGF-1 and sirtuins may mediate the effects of exercise on the nigral RAS. VEGF levels are also decreased in the nigra of aged animals, which may lead to reduced angiogenesis, hypoperfusion and higher dopaminergic vulnerability with aging. VEGF levels were also increased with exercise; however, the results suggest that exercise induces the increase in VEGF and the decrease in RAS activity by different pathways, as AT1 deletion induced a decrease in VEGF levels in aged mice. This suggests that high levels of AT1 activity in aged animals may also be related to a compensatory response against low levels of VEGF, angiogenesis, and brain hypoperfusion.

## Supplementary Material

Supplementary data is available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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## Conflict of interest statement

None declared.

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