Exercise decreases oxidative stress and inflammation and restores renal dopamine D1 receptor function in old rats

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Recent studies demonstrate that exercise extends average life span and improves survival in rats (14, 18, 19). Available reports also show that aerobic exercise reduces age-related increase in oxidative stress and inflammation and increases anti-oxidant defenses in mice and rats (23, 24, 28). Although exercise has been shown to attenuate age-associated increases in oxidative stress and inflammation, its effect in restoring/preventing age-related decline in renal D1 receptor function has not been studied. We designed experiments to investigate the effect of treadmill exercise on dopamine D1 receptor numbers and G protein activation and D1 receptor agonist (SKF 38393)-mediated natriuresis in old (23 mo) rats. Furthermore, markers of inflammation [C-reactive protein (CRP)], anti-inflammation (IL-10), oxidative stress [malondialdehyde (MDA)], and anti-oxidant defense [superoxide dismutase (SOD)] were also studied. We hypothesized that exercise in old rats, while decreasing inflammation and oxidative stress, restores age-related decline in D1 receptor numbers and G protein activation and D1 receptor agonist-mediated natriuresis.

MATERIALS AND METHODS

Treadmill exercise. Male Fischer (F344/NiaHsd) rats of 23 mo old were purchased from National Institute on Aging (Bethesda, MD) raised by Harlan Sprague-Dawley (Indianapolis, IN). The animals were kept in the University’s Animal Care Facility and used in the study with the approval of Institution’s Animal Care and Use Committee. The rats with similar body weights were divided into two groups. One group of rats was placed on treadmill exercise protocol for 6 wk (15 min · min⁻¹ · 60 min⁻¹, 15 degree grade, 5 days/wk) according to a published protocol (13). The other group of rats was not exercised and considered as sedentary control. Both of the groups of rats had free access to standard rodent chow and water. The average life span of the animals included in the study is 25–29 mo (42, 43).

Animal surgery. The rats were anesthetized with inactin (100 mg/kg body wt) 48 h after completion of the exercise protocol together with sedentary control group. The left carotid artery was catheterized with a PE-50 tubing for blood collection. RPT were prepared by the method routinely used in our laboratory (35). Plasma was isolated by centrifuging blood at 3,000 rpm for 15 min at 4°C.

Plasma membrane. RPT were homogenized in sucrose buffer (in mM: sucrose 250, Tris 10, PMSF 1, pH 7.4), and membranes were prepared using differential centrifugation method (35).

CRP. CRP was measured by ELISA using the protocol and reagents from Immunology Consultants Laboratory (Newberg, OR).

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**IL-10.** IL-10 was determined using rat IL-10 Quantikine ELISA Kit from R&D Systems (Minneapolis, MN).

**MDA.** MDA was measured as thiobarbituric acid-reactive substances by the method described in our publication (35). MDA was quantitated using molar extinction coefficient, $1.56 \times 10^5$ M/cm.

**Total SOD.** Total SOD activity was measured using Superoxide Dismutase Assay Kit from Cayman Chemical (Ann Arbor, MI). Cu/ZnSOD protein in the homogenate (25 μg) was determined by Western blotting using specific Cu/ZnSOD (Upstate, Chicago, IL) and horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibodies.

**Radioligand bindings.** Basal D1 receptor numbers and G protein activation in the plasma membranes were determined using $^{[3]}$H SCH 23390 (20 nM) and $^{35}$S-GTPyS (0.6 nM, ~100,000 cpm), respectively, by our published methods (35).

**D1 receptors and G protein α-subunits.** Plasma membranes (20 and 10 μg proteins for D1 receptors and Gα, respectively) were resolved by SDS-PAGE, electroblotted on PVDF membranes, and immunoblotted for D1 receptors and Gα using respective antibodies from Chemicon (Temecula, CA) and Calbiochem (San Diego, CA), respectively, by Western blotting. HRP-conjugated goat anti-rabbit was used as secondary antibody.

**Transcription factor Nrf2.** Nuclear fraction from RPT was isolated using a kit following the manufacturer’s protocol (Pierce, Rockford, IL). Nuclear proteins (15 μg) were resolved by SDS-PAGE, transferred, and immunoblotted using specific Nrf2 (Santa Cruz Biotechnology, Santa Cruz, CA) and HRP-conjugated goat anti-rabbit antibodies.

**Protein measurement.** Proteins were measured using BCA protein assay kit (Pierce) and BSA as standards.

**Renal function studies.** Another set of sedentary and exercised rats was used to measure SKF 38393 (D1 receptor agonist)-mediated sodium and water excretion according to the protocol routinely used in our laboratory (5, 35). Briefly, under inactin anesthesia (100 mg/kg) tracheotomy was performed to facilitate breathing. Left carotid artery (to measure blood pressure and heart rate) and left jugular vein (to infuse drug) were catheterized with PE-50 tubing. After midline abdominal incision, left ureter was isolated and catheterized with PE-10 tubing for urine collection. At this point, bladder urine was obtained with syringe for protein measurement. At the completion of surgery, normal saline (1% body wt/h) was infused continuously (for 45 min) throughout the experimental period to maintain a stable urinary output. Blood

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**Fig. 1.** Exercise decreases malondialdehyde (MDA) levels and increases total superoxide dismutase (SOD) activity and Cu/ZnSOD protein and nuclear Nrf2 levels in renal proximal tubules (RPT) of old rats. A: MDA was measured in the RPT homogenate as a marker of oxidative stress as described in MATERIALS AND METHODS. B: total SOD activity in the RPT homogenate was determined using a SOD assay kit. RPT homogenates (25 μg proteins; C) and nuclear proteins (15 μg; D) were resolved by SDS-PAGE and immunoblotted for Cu/ZnSOD (top blot in C) and Nrf2 (top blot in D) using specific Cu/ZnSOD and Nrf2 antibodies. The same blots in C and D were stripped off and reblotted for actin (bottom blot in C) and histone deacetylase 1 (bottom blot in D) using monoclonal actin and histone deacetylase 1 antibodies, respectively, as protein loading controls. Lanes 1–4 in C and lanes 1–3 in D: sedentary control old rats, lanes 5–8 in C and lanes 4–7 in D: exercised old rats. Bars in C and D are the ratios between the densities of Cu/ZnSOD and actin in C, and Nrf2 and histone deacetylase 1 in D. Results are means ± SE; n = 4 animals in A and B. *Significantly different from sedentary old rats (P < 0.05, t-test).
pressure and heart rate were continuously recorded on a Grass polygraph (model 7D, Grass Instrument, Quincy, MA). Thereafter, five consecutive 30-min collection periods comprising control period (CP; 2 CP, 30 min each, saline alone was infused), drug period (SKF 38393, 1 mg/kg/min in saline was infused), and recovery period (RP; 2 RP, 30 min each, saline alone was infused) were performed. Urine samples were collected throughout the 30-min period and sodium and potassium were measured using a flame photometer 480 (Ciba Corning Diagnostics, Norwood, MA). The values of two CP and RP were averaged and are presented in RESULTS.

RESULTS

The body weight decreased significantly in exercised old rats (before vs. 6 wk after: 419 ± 4 vs. 393 ± 8 g) compared with sedentary control old rats (before vs. 6 wk after: 415 ± 5 vs. 412 ± 3 g).

The levels of MDA (an oxidative stress marker) decreased (Fig. 1A) and SOD (an anti-oxidant defense marker) activity (Fig. 1B) and protein (Fig. 1C) increased in RPT of exercised compared with sedentary old rats. Furthermore, exercise increased the nuclear levels of transcription factor Nrf2 in RPT (Fig. 1D).

The levels of CRP (marker of inflammation) decreased (Fig. 2, A and B), whereas IL-10 (marker of anti-inflammation) increased (Fig. 2, C and D) in plasma and RPT of exercised rats.

The membranous D1 receptor radio-antagonist [3H]SCH 23390 basal binding and D1 receptor proteins increased in RPT of exercised old rats (Fig. 3, A and B). Also, basal [35S-GTPγS binding and Gq protein were increased in RPT membranes of exercised rats (Fig. 4, A and B).

The levels of proteins in the urine decreased in exercised rats (Fig. 5A). D1 receptor agonist SKF 38393 produced natriuresis
DISCUSSION

The present studies demonstrate that exercise in old rats decreased oxidative stress, reduced inflammation, increased anti-oxidant defense, increased D1 receptor numbers and G protein activation in RPT. Furthermore, D1 receptor agonist SKF 38393 produced natriuresis and diuresis in exercised old rats. Moreover, body weight and proteinuria decreased with exercise in old rats, which are in agreement with previous studies (6, 14). Previously, we reported defect in D1 receptor and G protein coupling in RPT and diminished natriuretic response to SKF 38393 in sedentary old (24 mo) rats but not in adult (6 mo) rats (5). Therefore, old but adult rats were included to determine the effect of exercise in restoring renal D1 receptor function in aging.

Both inflammation and oxidative stress increase with aging, which have been implicated in many diseases including arthritis, cancer, diabetes, Alzheimer’s and renal diseases (2, 10, 30). CRP is an inflammatory biomarker, which is now being recognized as a reliable clinical indicator in predicting cardiovascular diseases associated with inflammation (12). CRP also increases during aging suggesting an age-related increase in inflammation (4). On the other hand, decline in the activities of anti-oxidant enzymes (such as SOD, catalase, glutathione peroxidase, etc) has been linked with aging (2, 4, 25, 34, 40), which may contribute to age-associated increase in oxidative stress (2, 35). However, there also are studies demonstrating no change or even increased activity of some of the anti-oxidant enzymes in aging (9, 36).

Regular physical exercise is reported to slow down physiological dysfunction, which is characteristic of the aging process (1, 41, 45). Furthermore, regular exercise offers protection and improves symptoms in diseases associated with inflammation (16, 26) and oxidative stress (28). In the elderly, it improves the capacity of drug metabolism in the liver (27), whereas in aged mice it decreases oxidative stress and increases anti-oxidant defenses and survival (28). It delays the onset of heart failure in aging spontaneously hypertensive heart failure rats (14) and reduces inflammation by decreasing plasma CRP.

Fig. 4. Exercise increases $^{35}$S-GTPyS binding and protein levels of Gq in RPT of old rats. A: $^{35}$S-GTPyS binding (an index of G protein activation) was measured in the membranes of RPT. B: RPT membranes (10 µg) were resolved by SDS-PAGE. Gq proteins were determined by Western blotting using specific antibody. Lanes 1–3: sedentary control old rats, lanes 4–6: exercised old rats. Bars in C represent densitometric analysis of the protein bands. Results are means ± SE of 3 animals in each group. *Significantly different from sedentary old rats ($P < 0.05$, t-test).

Fig. 5. Exercise decreases proteinuria and enhances the D1 receptor agonist SKF 38393-mediated natriuresis and diuresis in old rats. A: total urinary proteins were measured using a protein assay kit as described in MATERIALS AND METHODS. Urinary sodium excretion (B) and urine flow (C) before, during, and after 1 µg·kg$^{-1}$·min$^{-1}$ SKF 38393. Control (control period), basal values before drug administration; drug (drug period, SKF 38393, 1 µg·kg$^{-1}$·min$^{-1}$), values during drug administration; recovery (recovery period), values after drug infusion was terminated. All the time intervals (control, drug, and recovery in B and C) were 30 min. Two control and two recovery collections were averaged and shown in the figure. Bars represent means ± SE ($n = 5–6$ animals). *Significantly different from sedentary old rats in A ($t$-test) and control values within the group in B and C (ANOVA and Newman-Keuls test, $P < 0.05$). #P < 0.05 from sedentary rats in B and C ($t$-test).
levels both in elderly and in aged F344 rats (11, 23). We also found that similar exercise protocol (14, 28) in F344 rats decreased the plasma CRP in old rats. In addition to plasma, CRP levels decreased in RPT of exercised rats. This suggests that in addition to systemic inflammation, there is also inflammation in RPT in old rats, which decreased with exercise. Previously, mRNA for CRP has been reported in the kidneys (21). In addition to decline in CRP levels, we also found that exercise decreased oxidative stress in RPT of old rats. This is consistent with previous studies showing exercise-induced reduction in oxidative stress and inflammation (23, 26, 28).

Recent studies attempted to identify the mechanism by which regular exercise reduces inflammation. It is believed that regular exercise reduces inflammation by providing an anti-inflammatory environment. The levels of anti-inflammatory cytokines such as IL-1ra and IL-10 increase in the circulation after exercise (29, 31). These mediators are produced and released by exercising skeletal muscle and exert their effects through cell surface receptors in several organs (44). IL-10 inhibits the production of inflammatory cytokines (TNF-α, IL-1β, IL-10) by transcription and posttranscription mechanisms (7, 44). In our studies, we found that exercise increased the levels of anti-inflammatory cytokine, IL-10, and decreased inflammatory marker, CRP, in rats. Although we did not measure inflammatory cytokines such as TNF-α in our studies, TNF-α is reported to increase the systemic levels of CRP (31). Therefore, it is likely that IL-10 produced during exercise inhibits the production of TNF-α resulting in the reduction of CRP levels in old rats.

One intriguing question is how regular exercise reduces oxidative stress. Does it involve anti-inflammatory cytokines such as IL-10 produced during exercise? It may involve cellular anti-oxidant defense system in combating oxidative stress as evidenced by an upregulation of Cu/ZnSOD (an anti-oxidant enzyme; Fig. 1, B and C) and Nrf2 transcription factor (Fig. 1D), required for transcription of anti-oxidant defense genes. Furthermore, our preliminary studies in primary cultures of RPT suggest Nrf2 stimulation by IL-10 (data not shown). Moreover, IL-10 receptors are present in the kidney (33) and nonsteroidal anti-inflammatory drugs increase cellular anti-oxidant defenses (39). Therefore, it is likely that anti-inflammatory cytokine (IL-10)-mediated upregulation of anti-oxidant defenses (such as Cu/ZnSOD) via Nrf2 may represent the underlying mechanism for exercise-induced reduction of oxidative stress in RPT of old rats.

Earlier, we reported that reduced D1 receptor number and decreased G protein activation contribute to the diminished natriuretic response to dopamine in old rats (5). The most interesting findings in this study are the ability of D1 receptor agonist to increase sodium excretion accompanied by an increase in basal D1 receptor numbers, G protein activation, and Gaq proteins in exercised old rats. To our knowledge, this is the first report showing beneficial effects of exercise in reversing age-related decline in D1 receptor numbers and restoring natriuretic effect of dopamine D1 receptor activation in F344/NNiaHsd rats. The study presented here in old rats may also prove beneficial in the elderly where reduced ability of dopamine to excrete sodium load is linked to age-related hypertension (46). In rodents, aging per se, generally, does not lead to hypertension. However, it should be noted that laboratory rodents including F344/NNiaHsd rats are aged in a controlled environment contrary to humans, who age in constantly changing environment (e.g., food intake, energy expenditure, salt consumption, etc). However, when F344 rats were aged with food intake with imbalanced compositions (high sucrose/low protein), they developed salt sensitivity and hypertension (32, 37).

Nevertheless, our present study demonstrates an improved kidney function in terms of increased natriuresis and reduced proteinuria (Fig. 5) in exercised old rats. It would be interesting to study the effect of exercise on blood pressure in F344/NNiaHsd rats aged with high-sucrose/low-protein diet. We believe the present study in aged F344/NNiaHsd rats has relevance to human aging in particular to kidney function, as it relates to restoration of the natriuretic response to D1 receptor activation. However, it can be argued whether a 16% increase in D1 receptor numbers in exercised rats will be meaningful in terms of restoring functional responsiveness. It should be noted that perhaps more important than number of receptors, it is the ability of these receptors to couple to G proteins, which leads to changes in second messengers. As evidenced in our findings, exercise also caused an increase in activation of Goq to which D1 receptor coupling occurs in RPT. Therefore, we believe that a combination of these two factors is beneficial in restoring age-related decline in renal D1 receptor function.

We conclude that exercise increases anti-inflammatory environment, increases anti-oxidant defense possibly via Nrf2, decreases oxidative stress, reverses age-related decline in D1 receptor numbers and G protein activation, and restores D1 receptor function in old rats. It remains to be determined whether anti-inflammatory cytokine (IL-10) produced during exercise plays a role in upregulating anti-oxidant defense and reducing oxidative stress in RPT in aging.

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GRANTS
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