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Influence of sedentary versus physically active conditions on regulation of plasma renin activity and vasopressin

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Physical inactivity is an independent risk factor for cardiovascular disease. Sedentary animals compared to physically active controls exhibit enhanced sympathoexcitatory responses, including arterial baroreflex-mediated sympathoexcitation. Hypotension-induced sympathoexcitation is also associated with the release of vasoactive hormones. We hypothesized that sedentary conditions may enhance release of the vasoactive hormones AVP and ANG II. To test this hypothesis, the humoral response to hypotension was examined in conscious rats after 9–12 wk of sedentary conditions or “normally active” conditions. Normally active conditions were produced by allowing rats access to running wheels in their home cages. Running distance peaked after 4 wk (4.5 ± 0.7 km/day), and the total distance run after 9 wk was 174 ± 23 km (n = 25). Similar levels of hypotension were induced in conscious sedentary or physically active animals with the arterial vasodilator, diazoxide (25 mg/kg iv). Control experiments used a saline injection of equivalent volume. Plasma samples were collected and assayed for plasma AVP concentration and plasma renin activity (PRA). Sedentary conditions significantly enhanced resting and hypotension-induced PRA relative to normal physical activity. In contrast, resting and hypotension-induced AVP levels were not statistically different between groups. These data suggest that baroreflex-mediated activation of the renin-angiotensin system, but not AVP secretion, is enhanced by sedentary conditions. We speculate that augmented activation of the renin-angiotensin system may be related to enhanced sympathoexcitatory outflow observed in sedentary animals and may contribute to increased risk of cardiovascular disease in the sedentary population.

**METHODS**

All procedures were approved by the Animal Care and Use Committee of the University of Missouri-Columbia and conducted in accordance with the American Physiological Society’s *Guiding Principles in the Care and Use of Animals*. All animals received food (Formulab Diet, 0.28% sodium; Purina, St. Louis, MO) and tap water ad libitum.

*Daily spontaneous running.* Fifty-four male Sprague-Dawley rats weighing 75–100 g at a time of arrival in the vivarium were used for these studies. Animals were housed individually in standard cages outfitted with custom-made running wheels (physically active group, n = 25) or without running wheels (sedentary group, n = 29) for 9–12 wk. Daily running distances, cumulative running distances, average

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Running time and average running speed were recorded daily with the use of bicycle computers (Sigma Sport, Olney, IL) calibrated to the diameter of the running wheel.

Surgical instrumentation. Two days prior to experimentation, animals were instrumented under halothane anesthesia and aseptic conditions with femoral arterial and venous catheters [polyethylene (PE)-50 fused to PE-10] to record mean arterial pressure (MAP) and heart rate (HR) and for intravenous injection, respectively, similar to our previous study (35). Catheters were tunneled under the skin and exteriorized between the scapulae. Following surgery, catheters were flushed with heparinized saline (10 U/ml) and capped with sterile plugs. Each animal was given subcutaneous fluids (10 ml, 0.9% saline) and returned to its home cage for recovery. Wheels were removed from the cages of physically active animals just prior to surgery. This was done to reduce the effects of an acute bout of exercise on AVP and ANG II levels (51, 53). Animals remained in the experiment room during the 2-day recovery period to acclimate to the experimental surroundings.

Despite the reduction in exercise, no effects on body weight, hematocrit, plasma protein, and liver weight were observed in rats maintained sedentary for 9–12 wk. Similar results were observed when rats were weighed and examined at the end of the previous sedentary condition (35). The Student’s t-test was used to determine group differences in body weight, hematocrit, plasma protein, and liver weight. A probability of P < 0.05 was considered statistically significant. Data are expressed as means ± SE.

RESULTS

Running wheel distances. Figure 1 demonstrates weekly distances run by rats provided with running wheels over the first 9 wk. Animals progressively increased their running distance over the first 5 wk and then exhibited a small decline from this peak until the day of experimentation. On a daily basis, running distance peaked between the 4th and 5th wk (day 32, 4.6 ± 0.7 km/day) and was associated with peak average running time during this same week (day 31, 1:46:04 ± 13:26). Average running speed varied over time from 29 ± 1 m/min (day 1) to 41 ± 1 m/min (five different days).

Effect of sedentary conditions vs. physical activity. Table 1 contains data regarding the effects of 9–12 wk of sedentary conditions compared with physical activity. As expected, physically active rats weighed significantly less than sedentary rats. Prior to injection of saline or diazoxide, resting mean arterial pressure appeared lower in physically active animals, but this effect did not reach significance (P = 0.212). In addition, resting heart rates were similar between physically active and sedentary rats immediately prior to injection of saline or diazoxide. Plasma samples for hematocrit and plasma protein were not influenced by injections of diazoxide relative to injections of saline whether analyzed by t-test (combining sedentary and physically active groups) or by 2-way ANOVA (separating sedentary and physically active groups; P > 0.05 for both tests). As shown in Table 1, hematocrits did not differ between physically active and sedentary rats, but there was no difference in plasma protein concentrations.

Fig. 1. Daily distances for rats provided with running wheels over 9 wk. On average, running distance peaked between the 4th and 5th wk (day 32, 4.6 ± 0.7 km/day).
Table 1. Baseline effects of 8 wk of physical activity vs. sedentary conditions

<table>
<thead>
<tr>
<th></th>
<th>Active Rats (n = 25)</th>
<th>Sedentary (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>359±8</td>
<td>404±6*</td>
</tr>
<tr>
<td>Resting MAP, mmHg</td>
<td>115±2</td>
<td>119±2</td>
</tr>
<tr>
<td>Resting HR, beats/min</td>
<td>357±7</td>
<td>358±6</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>39.5±0.4</td>
<td>39.6±0.3</td>
</tr>
<tr>
<td>Plasma protein, g/dl</td>
<td>5.8±0.1</td>
<td>6.0±0.1†</td>
</tr>
</tbody>
</table>

*P < 0.05 compared to active rats. †P value = 0.06.

a trend for a small, relative decrease in plasma protein in physically active rats (P = 0.06, t-test).

Hemodynamic responses. Figure 2 demonstrates AP and HR responses to intravenous diazoxide (25 mg/kg) or equivalent volumes of saline (0.9%) in sedentary or physically active rats. Injection of saline produced no significant effect on MAP in either group. Saline injection did produce a small, but significant, increase in HR that was similar between groups. In contrast, injection of diazoxide produced a rapid and sustained decrease in MAP that was significantly different from saline. Importantly, the hypotension produced by diazoxide was sustained similarly in both groups for the 20-min time period with some slight recovery toward control levels. The hypotensive responses were associated with increases in HR that were equally sustained over the time course and were not significantly different between groups.

Regulation of PRA. Figure 3A demonstrates plasma renin activity in physically active and sedentary animals in response to diazoxide (25 mg/kg) or equivalent volumes of saline (0.9%). In saline-treated animals, PRA was very similar to resting PRA reported in previous studies using conscious rats (35, 46–48). In both groups of animals, injection of diazoxide produced a significant increase in PRA compared with saline injection. A significant overall effect of sedentary conditions on PRA indicated that sedentary conditions enhanced PRA under both control conditions and during hypotension with diazoxide (overall effect P < 0.05; interaction P = 0.402).

Regulation of plasma vasopressin. Figure 3B depicts the effects of diazoxide or saline on vasopressin concentration in sedentary vs. physically active rats. In saline-treated animals, plasma vasopressin was very similar to resting plasma vasopressin values reported in previous studies using conscious rats (35, 46). In both groups, injection of diazoxide produced a significant increase in plasma vasopressin compared with saline injection. Neither resting nor hypotension-
induced vasopressin release appeared to be altered by sedentary conditions (overall effect \( P = 0.468 \); interaction \( P = 0.507 \)).

**DISCUSSION**

The purpose of the present study was to test the hypothesis that imposition of sedentary conditions enhances hypotension-induced activation of the renin-angiotensin system and AVP secretion relative to "normally active" conditions produced by spontaneous wheel running. This hypothesis was based on previous studies that demonstrated that baroreflex-mediated sympathoexcitation is greater in sedentary vs. physically active animals (6, 13, 36). The results of our experiments are consistent with our hypothesis that sedentary conditions produce a relative enhancement of resting and hypotension-induced increases in PRA. However, the data argue against an enhancement of resting and hypotension-induced increases in plasma vasopressin levels. These results suggest that there is an uncoupling between the effects of sedentary vs. physically active conditions on regulation of PRA and vasopressin secretion. Collectively, the results of this study and others (6, 13, 36) suggest that baroreflex-mediated increases in PRA and sympathetic nervous system outflow are enhanced in sedentary animals relative to physically active animals. We speculate that these enhancements contribute importantly to the overall detrimental effects of a sedentary lifestyle on arterial blood pressure regulation and cardiovascular health.

Consistent with our original hypothesis, sedentary conditions produced a relative enhancement of resting and hypotension-induced PRA. Higher PRA could be a result of higher resting and baroreflex-mediated sympathoexcitation observed in sedentary vs. physically active animals (11, 13, 24, 36). Specifically, the activity of renal sympathetic nerves, as assessed by renal norepinephrine turnover in humans (28) or by direct recordings in animals (24, 36), is enhanced in sedentary subjects relative to physically active controls. Although it is well known that renal nerves are responsible, at least in part, for stimulation of renin release from the kidney (12), additional experiments are needed to confirm whether other factors are involved, and whether enhanced renal nerve activity observed in previous studies (24, 28, 36) translates into the functionally related increases in PRA observed in the current study.

The underlying mechanisms by which sedentary conditions enhance, or physically active conditions reduce activation of the renin-angiotensin system are not fully known. In the present study, sedentary rats exhibited a trend for a slightly higher plasma protein content \( (P = 0.06) \), which could suggest a slightly lower relative plasma volume. A lower plasma volume alone could result in a higher sympathetic tone and increases in PRA via a relative unloading of volume-sensitive cardiopulmonary receptors (2, 3). However, studies by DiCarlo and colleagues (7, 43) suggest that sedentary conditions vs. physically active conditions produced by spontaneous wheel running have no effect on arterial baroreceptor and cardiopulmonary receptor afferent input. Therefore, alterations in the central nervous system processing of arterial and cardiopulmonary receptor input have been proposed to mediate activity-related changes in arterial baroreflex function (7, 43). In support of this hypothesis, we and others have reported physical activity-dependent changes within brain regions involved in cardiovascular control (33). These changes include alterations in neuronal firing properties (21), differences in neuronal structure and innervation (19, 37), and sensitivity to glutamate microinjections (26, 34). Interestingly, exercise training in rabbits with heart failure reduces components of the central renin-angiotensin system and is associated with reductions in elevation plasma ANG II levels (32). Thus, there is substantial evidence to suggest that physical activity-related synaptic plasticity occurs in central nervous system structures that impact regulation of the sympathetic nervous system and the renin-angiotensin system.

The physiological significance of higher PRA in sedentary animals relative to physically active animals is unknown. Higher PRA could result in a number of cardiovascular alterations associated with a sedentary lifestyle, including hypertension (50). A recent report suggests that elevations in sympathetic nervous system activity and the renin-angiotensin system occur prior to the development of heart failure in a mouse model, despite normal resting arterial pressures during this time (15). Similarly, previous reports suggest that disease states, such as heart failure and certain forms of hypertension, are associated with overactivity of both the sympathetic nervous system and the renin-angiotensin system (38, 52, 54). Physical activity (vs. inactivity) appears to be beneficial in reducing overactivity of the sympathetic nervous system and the renin-angiotensin system in congestive heart failure patients and animals (5, 18, 40). Although the current study was performed on otherwise “healthy” animals, it is intriguing to speculate that a sedentary lifestyle alone, or in combination with other cardiovascular risk factors, predisposes individuals to cardiovascular disease via effects on the sympathetic nervous system and the renin-angiotensin system.

Neither resting nor hypotension-induced vasopressin release was affected by sedentary vs. physically active conditions. This lack of effect was surprising to us and contrary to our hypothesis that vasopressin levels would be enhanced in sedentary animals. Since in our previous study performed in hindlimb-unloaded rats (35), we were able to observe significant differences in vasopressin levels using similar methods, we believe that the hypotensive stimulus used was sufficient enough to observe potential differences in vasopressin secretion. The mechanisms by which vasopressin release is stimulated are multifactorial and are regulated by such factors as decreases in blood pressure or blood volume, increased ANG II, and increases in plasma osmolality (44, 45, 49). Enhanced levels of PRA in sedentary animals would have been predicted to result in a greater level of plasma ANG II, since PRA is strongly correlated with plasma ANG II levels in conscious rats (48). Furthermore, Stern and colleagues (21) reported that magnocellular neurons in the PVN that are associated with vasopressin secretion have greater intrinsic excitability in sedentary vs. treadmill-exercised rats. Nonetheless, the data from the present study argue that these differences do not result in an enhancement of vasopressin secretion.

**Limitations.** It is possible that obtaining our samples at a single time point and following a brief period of anesthesia reduced our chances of seeing a significant difference in vasopressin between groups. We believe these to be unlikely given the half-life of vasopressin (~7 min in rat) and the extended period of hypotension (20 min) that preceded sample collection. In our previous study, we were able to identify significant differences in vasopressin levels in hindlimb-unloaded rats using similar methods (35). Therefore, although we
cannot discount the possibility that there may be physical activity-dependent alterations in the time course of plasma vasopressin levels, we can state that vasopressin levels are not statistically different in sedentary animals compared with physically active animals after 20 min of sustained hypotension.

We did not directly measure plasma volume or plasma osmolality in this study and so cannot completely eliminate alterations in these variables between sedentary and physically active rats as contributing factors. Previous studies have reported that plasma osmolality is unaffected when comparing sedentary and physically active subjects (10, 16). Even if higher plasma osmolalities were associated with the trend for lower plasma volumes in sedentary animals, we would have expected this to enhance resting and hypotension-induced AVP levels. Future studies will be necessary to determine directly whether changes in plasma volume, osmolality or other factors contribute to a lack of effect on vasopressin release.

As mentioned, the significant effects on PRA, but not vasopressin, suggest that there is a disassociation between activity-induced changes in baroreflex-mediated increases in PRA and vasopressin release. Previously, we have demonstrated differential alterations in baroreflex regulation of the renin-angiotensin system and vasopressin release in hindlimb-unloaded rats (35), a model of cardiovascular deconditioning (31). However, in contrast to our current study, we observed that hindlimb unloading enhanced both resting and hypotension-induced levels of vasopressin but had no effect on resting or hypotension-induced increases in PRA. These data suggest collectively that the spectrum of physical activity encompassing cardiovascular deconditioning, sedentary conditions, physically active conditions, and endurance training differentially influences baroreflex control of sympathetic nervous system activity, PRA, and vasopressin.

Summary/conclusions. In summary, sedentary conditions vs. physical activity produced by daily spontaneous running alters neurohumoral regulation of the circulation by enhancing activation of the renin-angiotensin system. In contrast, neither resting nor hypotension-induced vasopressin levels appear to be altered in sedentary compared with physically active rats. Interestingly, these data suggest that resting and baroreceptor reflex control of the renin-angiotensin system, vasopressin secretion, and the sympathetic nervous system are altered differentially by various levels of physical activity and inactivity.

Perspectives and Significance

Although it has been well documented that regular physical activity can lower blood pressure and sympathetic activity in normal and hypertensive humans (4, 20, 39) and animals (1, 9, 22–24, 36), the conclusions of the majority, if not all, of these studies have focused on the role of exercise training rather than the effects of remaining sedentary. This is a subtle, yet important difference in perspective, as a sedentary lifestyle is not only a risk factor for cardiovascular disease but is now considered a chronic disease process that is modifiable by regular exercise (25). It has been suggested recently that physically active or trained subjects should be identified as the control or “normal” group, and the sedentary group should be considered the disease group (25). This is logical from an evolutionary standpoint since human beings have relied on being physically active to maintain their survival for the majority of our existence (25). Thus, the identified “healthy” effects of physical activity may actually be related to the detrimental effects of remaining sedentary. As mentioned, the current study was performed on otherwise “healthy” animals. We speculate that a sedentary lifestyle alone, or in combination with other cardiovascular risk factors, predisposes individuals to cardiovascular disease via effects on the sympathetic nervous system and the renin-angiotensin system.

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