Effects of Norepinephrine Infusion and Exercise on Blood Pressure in Rats (44125)

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Abstract. **Two experiments were conducted to test the hypotheses that norepinephrine (NE) infusion would increase blood pressure and wheel running activity level, and, secondly, that voluntary exercise would lower NE-induced increases in blood pressure. NE-bitartrate was infused into male Sprague-Dawley rats using an implanted osmotic pump (3.75 μg/kg/min in 0.9% saline). Control rats received the vehicle solution. Systolic blood pressure was measured by the tail-cuff method. Voluntary wheel running activity expressed as wheel revolutions per 24 hr was measured on the 5th, 9th, and 13th day. Blood pressure on the 13th day and wheel running activity on the 9th day were significantly higher in NE-infused rats. The NE content of heart tissue was not altered, but urinary excretion of NE and epinephrine was increased in the NE-infused animals. Food intake, body weight gain, and kidney weight per 100 g body wt were not changed but heart weight per 100 g body wt was increased by NE infusion. Urinary total calcium excretion was higher in the NE-infused rats. Spontaneous voluntary exercise in running wheels attenuated increases of blood pressure in NE-infused rats. The results of the present study suggest that rats receiving exogenous NE exhibit increased blood pressure and voluntary wheel running activity. Voluntary wheel running exercise also reduces blood pressure in NE-infused rats.**
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Sympathetic nervous system activity which controls the renin-angiotensin system (1) is of major importance in the control of blood pressure. Therefore, numerous attempts have been made to determine a possible role of altered sympathetic nervous system activity in development and maintenance of hypertension, focusing on the production and metabolism of norepinephrine (NE). Despite these efforts, no clear-cut evidence of an increased sympathetic nerve drive could be obtained either in hypertensive patients (2) or in hypertensive animals (3). This is partially due to the complex regulation of the metabolism of catecholamines.

Several large studies, which allowed for age, anthropometric characteristics, and other confounders, have reported an inverse relationship between blood pressure and either habitual physical activity assessed by interview or measured physical fitness (4, 5). In a study analyzing 48 controlled intervention studies (6), the weighted net change in conventional blood pressure caused by endurance aerobic training averaged −5.3 mm Hg for systolic and −4.8 mm Hg for diastolic blood pressure, depending on their initial blood pressure. In addition to the long-term effects of regular exercise, a single bout of physical exercise induces a significant post exercise reduction in blood pressure (7).

Plasma catecholamines have been reported to increase during dynamic exercise (8, 9) and various forms of stress (9, 10). Epinephrine (E), released by the adrenal glands, is more responsive than NE to psychological stimuli (9, 10), whereas NE, released predominantly from sympathetic nerve endings, increases more than E in response to physical stresses such as cold pressor testing and bicycle exercise (9–11). However, the plasma NE concentration post exercise training has been reported to be reduced (12, 13). It is therefore important to determine the effects of exogenous NE on blood pressure and exercise activity to study the relationship among NE, blood pressure, and physical activity level.

Incremental infusions of NE increased mean arterial pressure, decreased urinary sodium excretion and effective

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renal plasma flow, and increased proximal tubular sodium reabsorption (14). Several large studies have reported a highly significant positive correlation between serum total calcium and blood pressure (15, 16). Lower serum ionized calcium and elevated urinary calcium excretion were also observed in essential hypertension (17) and spontaneously hypertensive rats (18).

The present study, therefore, first investigated whether NE infusion would increase blood pressure and voluntary activity level, and if increased blood pressure is associated with abnormal renal handling of electrolytes; and, second, tested the hypothesis that exercise lowers NE-induced increases in blood pressure.

Materials and Methods

Animals. Two experiments were conducted to study the effects of NE infusion and exercise on blood pressure. In the first experiment, 20 male Sprague-Dawley rats weighing 150–200 g were housed in individual cages and randomly assigned into two groups of 10 rats each. Animals of one group were implanted subcutaneously with Alzet Mini-Osmotic Pumps (Alza, Palo Alto, CA) filled with NE-bitartrate (Sigma Chemical Co., St. Louis, MO) to deliver 3.75 μg/kg/min (2.86 mg NE/kg/day) in 0.9% saline for 14 days. The other group (control) was implanted with the same pump filled with the saline solution used as a vehicle. The American Institute of Nutrition-76 diet and tap water were provided *ad libitum* for 2 weeks. Spontaneous voluntary exercise in the running wheel was measured on the 5th, 9th, and 13th day after implantation of pump using standard Wahmann activity wheels (1.125 m in circumference) as described previously (19). The systolic blood pressure of prewarmed (for tail vein dilation), conscious rats was measured at the beginning and on 13th day of the experiment by a tail-cuff method after rats were rested for 2 hr. Body weight and food consumption were determined weekly. At the end of the experiment, animals were placed in individual metabolism cages and two successive 24-hr urine samples were collected in flasks containing 2 *N* HCl. Urine samples were weighed, filtered, mixed, and kept frozen at −80°C until analyzed for electrolytes and catecholamines. Blood samples were collected by heart puncture using heparinized syringes under light ether anesthesia. Plasma samples were separated by centrifugation and kept at −80°C. The heart and kidney were quickly removed, rinsed with chilled saline solution, and weighed. Hearts were kept frozen at −80°C for analysis of NE.

In the second experiment, a group of five male Sprague-Dawley rats weighing 300–350 g received NE infusion as in Experiment 1, and were housed in individual cages with access to a standard Wahmann activity wheel for 14 days. These rats had access to the voluntary activity wheels for the whole experimental period. Another group of five rats (control) received NE infusion and were housed in normal rat cages. Systolic blood pressure was determined at the beginning and end of the experiment as in the first experiment. Body weight and food intake were determined weekly. At the end of experiment, animals were sacrificed, and heart and kidney weights were determined.

Chemistry. Catecholamine (CA) concentrations in urine samples were determined by HPLC and electrochemical detection (20). For analysis of CA concentration in the heart, tissues were homogenized in ice-cold 0.4 *N* perchloric acid using a Polytron homogenizer, centrifuged, and the supernatant was analyzed in the same way as urine samples. The sodium and potassium concentrations in the urine and plasma samples were determined by flame photometry, and the total and ionized calcium concentrations were analyzed by calcium-specific electrodes using a Nova-7 analyzer (Nova Biomedical, Waltham, MA).

Data Analysis. Data were analyzed using the analysis of variance and Student's *t* test was used for comparisons between the control and NE groups (21).

Results

Experiment 1. Systolic blood pressure in the NE-infused rats was increased to the hypertensive level, whereas control rats remained normotensive (Table I). Body weight gain, food intake, and kidney weight per 100 g body wt were not affected by NE infusion. However, the heart weight per 100 g body wt in NE-infused rats was greater than that in control rats (Table I). Plasma sodium and potassium concentrations and urinary excretion of these electrolytes were similar in NE-infused and control rats (Tables II and III). Plasma total and ionized calcium concentrations of NE-infused rats were similar to those of control rats (Table II), but urinary calcium excretion of NE-infused rats was greater than that of controls (Table III).

Urinary excretion of NE and E of NE-infused rats was higher than that of control rats, whereas the dopamine (DA)

Table I. Body Weight, Food Intake, Organ Weight, and Blood Pressure of Saline- (Control) and NE-Infused Rats That Performed Spontaneous Voluntary Exercise in a Running Wheel: Experiment 1

	Saline	NE
Body weight gain (g)	138.2 ± 10.4	132.6 ± 12.9
Food intake (g/day)	16.0 ± 1.0	15.1 ± 1.4
Heart weight (g/100 g body wt)	0.33 ± 0.02	0.38 ± 0.03[a]
Kidney weight (g/100 g body wt)	0.82 ± 0.05	0.86 ± 0.08
Initial systolic blood pressure (mm Hg)	108 ± 6	110 ± 5
Final systolic blood pressure (mm Hg)	117 ± 7	170 ± 12[a]

Note. Values are mean ± SD of 10 rats.
[a] Significantly ($P < 0.01$) different from the saline group.

Table II. Plasma Electrolyte Concentrations of Saline- (Control) and NE-Infused Rats That Performed Spontaneous Voluntary Exercise in a Running Wheel: Experiment 1

	Saline	NE
Total Ca (m*M*)	2.51 ± 0.05	2.50 ± 0.05
Ca^{++} (m*M*)	1.08 ± 0.09	1.10 ± 0.12
Na (m*M*)	133 ± 1.4	135 ± 2.8
K (m*M*)	5.5 ± 0.3	5.4 ± 0.4

Note. Values are mean ± SD of 10 rats.

excretion was similar in the two groups. The NE concentration in the heart of NE-infused rats was 16% higher than that of control rats, but this difference was not statistically significant because of a wide variation among rats (Fig. 1).

The voluntary wheel running activity, expressed as wheel revolutions/24 hr in either a clockwise or a counterclockwise direction, is given in Figure 2. The NE-infused rats tended to exercise more than control rats, but the significant difference was observed only on the 9th day after implantation.

Experiment 2. The average number of wheel revolutions per rat during 24 hr was 971 ± 72 (mean ± SD) in the running wheel exercise group. Body weight gain, food intake, and heart and kidney weights were similar between control (sedentary) and experimental (running wheel exercise) groups (Table IV). However, blood pressure was significantly ($P < 0.05$) reduced in the rats housed in cages with access to running wheels compared with those housed in normal rat cages.

Discussion

The sympathetic nervous system is an important mediator of the body's physiological adjustment to a variety of physical, environmental and behavioral stressors. The principal neurotransmitter released by the sympathetic nervous system is NE. The present study confirmed previous reports that exogenous NE increases blood pressure and produces moderate cardiac hypertrophy (22, 23). The most common detrimental consequence of chronic hypertension is left ventricular hypertrophy (24) and the increased heart weight is confirmation of increased blood pressure in NE infused rats in the present study.

Table III. Urinary Electrolyte and Catecholamine Excretion in Saline- (Control) and NE-Infused Rats That Performed Spontaneous Voluntary Exercise in a Running Wheel: Experiment 1

	Saline	NE
Total Ca (μmole/24 hr)	45.7 ± 17.1	66.4 ± 18.9[a]
Na (mmole/24 hr)	1.20 ± 0.19	1.08 ± 0.26
K (mmole/24 hr)	1.42 ± 0.27	1.47 ± 0.34
NE (μg/24 hr)	1.6 ± 0.3	36.8 ± 8.5[b]
E (μg/24 hr)	0.4 ± 0.1	1.5 ± 0.5[b]
DA (μg/24 hr)	4.5 ± 0.3	3.9 ± 1.1

Note. Values are mean ± SD of 10 rats.
[a] Significantly ($P < 0.05$) different from the saline group.
[b] Significantly ($P < 0.01$) different from the saline group.

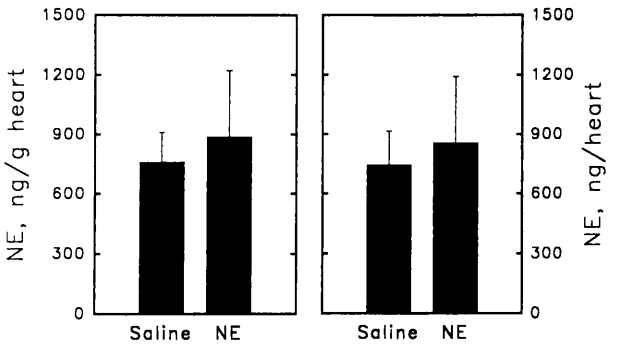

Figure 1. The NE concentration in the heart of saline- (control) and NE-infused rats that performed spontaneous voluntary exercise in running wheels (mean ± SD of 10 rats). Experiment 1.

The urinary excretion rates of free NE and DA in spontaneously hypertensive and stroke-prone spontaneously hypertensive rats were reported to be higher than those of Wistar-Kyoto rats throughout the developing and established stages of hypertension (25), indicating that alterations in the catecholaminergic system are involved in the development of hypertension in those animals. The significant blood pressure response to infused NE was not due to changes in body weight, since body weight was not changed by NE infusion. The urinary sodium concentration between control and NE-infused hypertensive rats was similar. There is an obvious limitation in the present study since the sodium retention during the developmental stage of hypertension in NE-infused rats was not determined. The possibility that NE-infused rats may increase sodium retention during the developmental stage of hypertension can therefore not be ruled out. Normally, an increase in blood pressure would elevate sodium excretion, a phenomenon often referred to as pressure natriuresis (26). The increased blood pressure in NE infused rats might counteract the direct sodium retaining action of NE (14), by which sodium balance was maintained

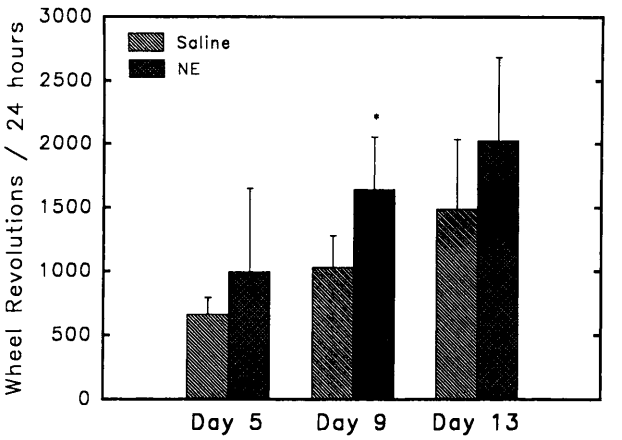

Figure 2. The spontaneous voluntary exercise in running wheels of saline- (control) and NE-infused rats (mean ± SD of 10 rats). *Significantly ($P < 0.05$) different from the saline group. Experiment 1.

Table IV. Effects of Spontaneous Voluntary Exercise in a Running Wheel on Body Weight Gain, Food Intake, Organ Weights and Blood Pressure in NE-Infused Rats: Experiment 2

	Control (sedentary)	Running wheel exercise[a]
Body weight gain (g)	112.6 ± 14.8	126.0 ± 18.8
Food intake (g/day)	16.7 ± 2.4	17.3 ± 0.9
Heart weight (g/100 g body wt)	0.34 ± 0.03	0.36 ± 0.04
Kidney weight (g/100 g body wt)	0.83 ± 0.04	0.84 ± 0.03
Initial systolic blood pressure (mm Hg)	115 ± 3	118 ± 5
Final systolic blood pressure (mm Hg)	180 ± 7	161 ± 10[b]

Note. Values are mean ± SD of five rats.
[a] The average wheel revolution of running wheel exercise group was 971 ± 72/rat/24 hr.
[b] Significantly ($P < 0.05$) different from the control group.

during the later stage of hypertension in the present study. The sodium balance, despite increased blood pressure in the NE-infused group, is therefore evidence of altered renal function. This is because the elevated pressure would cause a normal kidney to increase sodium excretion.

Imaoka *et al.* (27) reported concurrent changes in the renin angiotensin system and calcium metabolism in elderly hypertensives. Hypertensives had lowered plasma renin and aldosterone, and decreased levels of serum calcitonin and 1,25-$(OH)_2$cholecalciferol, which were associated with calciurea. Therefore, the increased calcium excretion in the present study might be due to secondary effects of hypertension in the NE-infused rats.

One possibility for NE-induced hypertension is due to increased stimulation of sympathetic nerves surrounding the renal artery, since previous studies showed that renal denervation inhibited the development of hypertension in spontaneously hypertensive (28, 29) and DOCA salt rats (30). Other investigators reported that the blood pressure-lowering effect of renal denervation was not associated with changes in urinary sodium excretion or plasma renin activity (31, 32). Elevated plasma NE in sham-operated control rats but normal NE values in rats subjected to renal denervation were found in those studies (31, 32). Therefore, it is possible that sympathetic vasomotor tone is influenced by signals carried along with renal afferent nerves to the hypothalamus, which has been shown to control arterial blood pressure and vascular resistance (33).

It is not presently clear how the infused NE enabled animals to increase voluntary exercise in the running wheels. A possible mechanism could be through its circulatory effects by adjusting blood flow to metabolic demands (34).

The NE-infused rats that were allowed to exercise voluntarily in running wheels for 14 days attenuated increases of blood pressure compared with sedentary rats. This result confirmed the previous report with genetically hypertensive rats performing voluntary exercise in running wheels (35, 36). The reduction of blood pressure after exercise in hypertensive animals was primarily associated with the reduction of cardiac output in a study with spontaneously hypertensive rats (36). Others found reduced peripheral resistance after endurance training (37, 38). One area of progressive vasodilation during and after exercise might be the skin circulation, which needs to fulfill its thermoregulatory role (39). NE has been known to cause α-mediated systemic vasoconstriction to all vascular beds, whereas E causes a decrease in total systemic vascular resistance (40). Concentrations of plasma E are known to increase with sustained exercise (8, 11) and might contribute to systemic vasodilation, which would counteract the action of NE. However, the actual mechanism responsible for lowering blood pressure is still unknown, and further studies are needed to elucidate it.

Tipton *et al.* (41) reported that when spontaneously hypertensive rats, under forced exercise conditions, were trained at a high intensity level (70%–95% maximal oxygen uptake), resting blood pressure increased, whereas a significantly reduced blood pressure was observed with lower intensities (40%–60% maximal oxygen uptake). These findings were analogous to those stemming from the observation in humans (7) that moderate aerobic exercise carried out regularly lowered resting blood pressure. The previous findings and the results of the present studies suggest that lower-level voluntary exercise may have potential in the control of hypertension associated with stress or central nervous system activity.

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