Norepinephrine Reuptake Is Impaired in Skeletal Muscle of Hypertensive Rats In Vivo

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Abstract—Certain forms of experimental hypertension are characterized by organ-specific alterations of catecholaminergic pathways. The purpose of this study was to evaluate, in the same awake and freely moving normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) before and after the development of arterial hypertension, the norepinephrine (NE) turnover and, in particular, the neuronal NE reuptake activity that ends its effects once released from nerve terminals, in subcutaneous adipose tissue and in skeletal muscle, whose sympathetic efferents are respectively independent or dependent from baroreflexes. Plasma and tissue interstitial NE and 3,4-dihydroxyphenylethylene glycol (DHPG), its major deaminated metabolite, were measured before and after blockade of NE reuptake by tissue perfusion of desipramine through microdialysis probes. Arterial pressure and plasma NE in SHR were similar to those in WKY at 5 weeks of age but increased at 16 weeks of age. In contrast, plasma DHPG was already higher in young SHR. Basal interstitial NE and DHPG were increased in both tissues of young and old SHR compared with age-matched WKY. Desipramine induced a higher rise of interstitial NE in SHR of both ages, with a lesser increase in the skeletal muscle of old compared with young SHR. These results indicate an increased NE turnover in prehypertensive and hypertensive SHR in both baroreflex-dependent and -independent tissues, not shown by plasma NE levels in young SHR. In the skeletal muscle, where sympathetic efferents are baroreflex dependent, the reduced interstitial NE reuptake contributes to the higher availability of interstitial NE for postsynaptic effects in old SHR. (Hypertension. 2001;37[part 2]:698-702.)

Key Words: muscle, skeletal □ adipose tissue □ nervous system, sympathetic □ norepinephrine □ rats, inbred SHR

Anormalities of the central neural mechanisms regulating peripheral sympathetic outflow and catecholamine metabolism after the release of the neurotransmitter from the nerve endings have been described in the development of hypertension in humans and in spontaneously hypertensive rats (SHR), the animal model of essential hypertension most used. In SHR tissues, age-related differences have been reported in the enzymes implicated in the synthesis (such as tyrosine hydroxylase and dopamine β-hydroxylase) and metabolism (such as monoamine oxidase and catechol-o-methyltransferase) of norepinephrine (NE), which has been found to be higher in resistance arteries but normal in the heart in the early phases of hypertension development, with an inverse relationship in adult rats. Neuronal uptake of NE, the active mechanism responsible for removing NE from the synaptic cleft once released from the nerve terminal, has been found to be normal in cardiac and vascular tissue, increased in the kidney and skeletal muscle of young SHR, but reduced in the heart and kidney of adult SHR. Most of these data on NE turnover are obtained from in vitro or ex vivo tissue studies that may not exactly reflect the in vivo situation. The purpose of the present study was to investigate, in vivo, the turnover of NE and neuronal NE reuptake by using microdialysis techniques in 2 peripheral tissues, skeletal muscle and subcutaneous adipose tissue, which are controlled differently by baroreflexes, in the same awake and freely moving Wistar-Kyoto rats (WKY) and SHR before and after the development of arterial hypertension. In vivo catecholamine turnover was evaluated by measuring in the tissue interstitium the levels of basal NE and its major deaminated metabolite, 3,4-dihydroxyphenylethylene glycol (DHPG), before and after blockade of the neuronal presynaptic uptake mechanism by local tissue desipramine perfusion.

Methods

The rats were cared for according to the Institutional Animal Care guidelines and the protocols approved by the Institutional Animal Ethics Committee of the University of Parma. Male SHR (n = 17) and WKY (n = 19) were obtained at 4 weeks of age from Charles River Italia (Calco-Como, Italy) and were housed 1 per cage in a room maintained at a temperature controlled between 23°C and 25°C and a 12-hour light/dark cycle. Standard rat chow and water were supplied ad libitum.
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minutes (basal period), the perfusion was changed to the Ringer’s

8 per each group). Five days

DHPG levels during the microdialysis procedure in cannulated

young rats were housed again for a longitudinal study. The same

on NE and DHPG plasma levels were observed until 240 minutes of

chosen because in our experience, no systemic effects of desipramine

pmol/min in the tissue interstitium), which was perfused for 120

position of the tip of the probes in white adipose tissue or skeletal

controlled by cryostatic sections for histological confirmation of the

probes with membranes (10-mm length, 0.5-mm outside diameter,

and molecular weight cutoff 20 kDa; CMA/20, CMA/Microdialysis

as we have previously described.18 Two small biopptic samples taken

after the insertion of the guide were quickly frozen and controlled by cryostatic sections for histological confirmation of the position of the tip of the probes in white adipose tissue or skeletal muscle. The probes were then connected to a microperfusion pump (CMA100, CMA/Microdialysis AB) and perfused at a flow rate of 2.0 μL/min with a Ringer’s solution of the following millimolar composition: NaCl 140, KCl 3.0, MgCl 2 1.0, and CaCl 2 1.2. The rats took a few minutes to recover from experimental handling, and after a 30-minute equilibration period, the dialysates from microdialysis probes were collected at 30-minute intervals over a period of 210 minutes and immediately analyzed for NE and DHPG. After 90 minutes (basal period), the perfusion was changed to the Ringer’s solution containing desipramine hydrochloride (concentration of 5 μmol/L at a flow rate of 2 μL/min, giving a presumed quantity of 10 pmol/min in the tissue interstitium), which was perfused for 120 minutes. This concentration and perfusion rate of desipramine were chosen because in our experience, no systemic effects of desipramine on NE and DHPG plasma levels were observed until 240 minutes of perfusion at this concentration. At the end of the experiment, the young rats were housed again for a longitudinal study. The same groups of animals were reexamined at the age of 16 weeks.

To exclude the possibility that the insertion of the small microdialysis probes in the dorsal region may have altered sympathetic activity in these strains, we simultaneously measured plasma NE and DHPG levels during the microdialysis procedure in cannulated conscious and unrestrained rats (n = 8 per each group). Five days before the microdialysis procedure, young and old WKY and SHR were instrumented, after anesthesia with sodium pentobarbital (60 mg/kg body wt IP) on a temperature-regulated table to maintain rectal temperature between 36°C and 38°C, by placing catheters (polyethylene tubing [PE-10] welded to a PE-50 catheter) in the femoral artery for blood sampling and arterial pressure measurements and in the femoral vein for volume replacement (same amount of iso-oncotic artificial rat plasma: 2.5 g/dL of each BSA and immunoglobulin in Ringer’s solution; volume replacement rate: 30 mL/kg per hour for young and old rats for 10 minutes and then 1 mL/kg per hour). The catheters, filled with heparinized saline, were positioned in the abdominal aorta and in the vena cava, tunnelled subcutaneously, extruded at the back in the low interscapular region, and protected from the rat by insertion into a stainless-steel tether. After that procedure, the rats were again placed in individual cages. The arterial catheter was coupled to a pressure transducer (Statham P231D, Gould Statham Inc), and the signal was amplified and recorded by a data-acquisition system (MP100WS, Harvard Apparatus). Before and during the microdialysis procedure, blood samples (500 μL) were repeatedly taken (every 30 minutes). NE and DHPG concentrations in plasma and dialysates from tissue interstitia were immediately analyzed after collection by high-performance liquid chromatography with electrochemical detection as previously described.18 To avoid possible analytical interferences of NE with 3,4-dihydroxyphenylalanine, the pH of the mobile phase was rigorously maintained at <2.65. Peak identification was performed on the basis of retention time of external standards and on cyclic voltametry. All drugs and chemicals were purchased from Sigma Chemical Co.

**Microdialysis Probe Calibration and In Vivo Recovery Experiments**

Microdialysis probes were calibrated in vivo for the relative recovery rate of NE and DHPG in all animals undergoing experimental procedures by perfusing 3,4-dihydroxybenzylamine (DHBA), an internal standard that is thought to behave like catecholamines and have similar diffusion characteristics. DHBA in the Ringer’s perfusion solution at a concentration of 0.4 nmol/L has not been shown to possess any pharmacological activity on NE or its metabolite levels.18 The recovery rate is needed to estimate the absolute NE interstitial concentration from the concentration in the dialysate; NE and DHPG concentrations measured in the dialysate obtained from the skeletal muscle and adipose tissue were corrected for an in vivo recovery of DHBA.

**Statistical Analysis**

Values are presented as mean±SEM. Statistical analysis was based on a 2-way ANOVA model for repeated measures, in which the dependent variable (NE or DHPG) represents the same measurement taken at various times. The Student t test either for independent or paired samples was used to compare group means when ANOVA showed a significant effect of the factor. A value of P<0.05 was considered statistically significant.

**Results**

**Body Weight, Arterial Pressure, and Heart Rate**

As shown in the Table, compared with age-matched WKY, 5-week-old SHR showed no differences in mean arterial pressure and heart rate. Body weight was also at this time comparable in both strains. Mean arterial pressure was higher in 16-week-old SHR than in younger SHR and age-matched WKY (P<0.01, t test). Heart rate was reduced in old rats compared with young rats (P<0.01, t test) without differences between the 2 strains. Body weight was higher in old WKY compared with age-matched SHR (P<0.01, t test).
Plasma Catecholamine Levels
As depicted in Figure 1 and indicated in the Table, young SHR and WKY showed similar levels of plasma NE, whereas DHPG was higher in still normotensive SHR compared with age-matched WKY. After the development of hypertension, compared with young SHR and age-matched WKY, SHR showed increased plasma NE (Table and Figure 1A). DHPG levels were also confirmed to be higher in old SHR compared with old WKY (Table and Figure 1B). Tissue perfusion of desipramine through the microdialysis probes did not alter the plasma concentrations of either NE or DHPG (Figure 1A and 1B).

Interstitial Catecholamines in Skeletal Muscle
Basal NE levels in dialysate from skeletal muscle (adjusted for the in vivo recovery of DHBA) were much higher in SHR compared with WKY (P<0.01, t test) (Table and Figure 2A). In old animals (16 weeks), a further increase in NE levels was observed in SHR compared with younger SHR, whereas similar NE concentrations were found in young and old WKY. As shown in Figure 2A, both young and old WKY as well as old SHR exhibited a 2- to 3-fold increase in interstitial NE concentrations over the basal levels after desipramine perfusion; in contrast, a 5-fold increase of interstitial NE over the basal value was observed in young SHR. ANOVA revealed a significant effect of desipramine over time in all groups (ANOVA, P<0.001) and a significant difference between young and old SHR (F=4.9, P=0.023, 1 df) and between age-matched SHR and WKY (ANOVA, P<0.001). Basal interstitial DHPG was significantly higher in young and old SHR compared with age-matched WKY (P<0.01, t test; Figure 2B). The inhibition of presynaptic reuptake by desipramine perfusion halved DHPG levels in the interstitium of young and old WKY and old SHR, whereas a much higher reduction was reached in young SHR (Figure 2B). ANOVA showed a significant effect of desipramine over time in all groups (ANOVA, P<0.001) and a significant difference between young and old SHR (F=4.9, P=0.023, 1 df) and between age-matched SHR and WKY (ANOVA, P<0.001).

Interstitial Catecholamines in Adipose Tissue
Marked increases in the basal levels of NE and DHPG were observed in age-matched SHR compared with WKY (P<0.01, t test; Table and Figure 3A and 3B). Whereas young and old WKY exhibited a 2- to 3-fold increase in NE levels after desipramine perfusion, a 5- to 6-fold increase was observed in age-matched SHR (Figure 3A). ANOVA revealed a significant effect of desipramine over time in all studied groups (ANOVA, P<0.001) as well as a significant difference between age-matched SHR and WKY (ANOVA, P<0.001). Desipramine perfusion halved interstitial DHPG levels in all groups (ANOVA, P<0.001, over time for all groups and between groups of age-matched SHR and WKY; Figure 3B).

Discussion
In certain forms of experimental hypertension as well as in a subgroup of young hypertensive patients, sympathetic traffic to the cardiovascular system is increased, especially in the early phases.1 In the present study, we have applied the microdialysis technique to investigate, in vivo and in a longitudinal study performed on the same group of awake and freely moving normotensive WKY and in SHR, the regional turnover of NE in 2 peripheral tissues before and after the development of hypertension. Sympathetic neural activity and NE turnover, as expressed by basal interstitial NE and DHPG concentrations, were higher in both skeletal muscle and subcutaneous adipose tissue in young still normotensive SHR than in age-matched WKY. The increased basal interstitial NE levels in both tissues from young SHR suggest an
increased release from nerve endings of the neurotransmitter associated with a higher local neuronal uptake and metabolism by intraneuronal monoamine oxidase, as expressed by elevated interstitial DHPG levels. These data obtained in vivo confirm the ex vivo results obtained by others in skeletal muscle tissue of 5-week-old SHR and provide new findings on an increased sympathetic activity discharge and turnover of NE in subcutaneous adipose tissue, where sympathetic outflow is independent from baroreflex function. Hence, because it is thought that skeletal muscle sympathetic activity, but not subcutaneous adipose tissue sympathetic outflow, is related to baroreflex function, it appears that in the SHR model of hypertension the increased sympathetic activity is not simply the result of decreased baroreflex inhibition but in all likelihood a more diffused peripheral sympathetic activation. Other groups have previously shown an early activation of sympathetic nervous activity in SHR by measuring plasma NE levels19,20 and provide new findings in young SHR (5-fold for NE) than in age-matched WKY (2- to 3-fold), thereby indicating a more active presynaptic reuptake output, as found in the early phases of hypertension in young SHR, after local perfusion of desipramine, is much higher in young SHR than in age-matched WKY. Basal NE interstitial levels in skeletal muscle and in subcutaneous adipose tissue, measured as the increase in NE levels in dialysates from the striated muscle of young SHR, a normal level of blood pressure was measured. Obviously, the elevated activity of the sympathetic nervous system at this time was not sufficient to cause a rise in vascular tone and consequently in blood pressure. At this stage of hypertension, the increased sympathetic activity seems to be better with increased sympathetic activity than is NE.

Therefore, in the present study, conclusions about sympathetic activity based solely on NE plasma levels in young SHR seem to be limited and not representative of the higher levels found in interstitial tissues. This discrepancy is probably due to the various processes to which NE is submitted after release into the synaptic gap and before spilling into the blood stream. Hoeldtke et al24 have observed that the quantity of NE that spills into the plasma from the synaptic cleft appears to be ~12% to 20% of the quantity released from nerve endings in the whole body. This difference between the high interstitial concentration in the synaptic cleft (and thereby at the receptor site) and the concentration in the plasma appears to be greatly influenced by local NE metabolism. Another factor to consider is that plasma NE concentrations depend on NE clearance. A variety of circumstances can change the rate at which NE is removed from the plasma in the 2 strains. An increased cardiac output, as found in the early phases of hypertension in young SHR, can determine a parallel rise in NE removal from plasma, contributing to the discrepancy between interstitial and plasma NE levels in this strain.1,3,25

However, in the presence of increased sympathetic discharge from the striated muscle of young SHR, a normal level of blood pressure was measured. Obviously, the elevated activity of the sympathetic nervous system at this time was not sufficient to cause a rise in vascular tone and consequently in blood pressure. In young SHR, this may depend on the efficiency of other counterbalancing mechanisms (such as endothelium-derived relaxing factors) that modulate the increased sympathetic activity.

After the development of hypertension in SHR, plasma NE and DHPG levels were higher than the levels found in young SHR and age-matched WKY. Basal NE interstitial levels in skeletal muscle were greater in old SHR than in young SHR, whereas a blunted increase in NE after desipramine perfusion (2- to 3-fold), indicating
a reduction in presynaptic NE reuptake at this age compared with a younger age (5-fold increase), was found. Such a behavior in older SHR allows for a higher availability of NE for postsynaptic vascular effects leading to the sustained increase of vascular resistance observed at this age. This higher NE interstitial level may also affect muscle metabolism in SHR of this age by inhibiting at a concentration in the same range the oxygen consumption and glucose uptake.\(^{26}\) The explanation for reduced in vivo NE reuptake in skeletal muscle of SHR after the development of hypertension is not clear even if some hypotheses may be put forward propounding a potential effect of angiotensin II, which at very low concentrations can markedly inhibit NE uptake.\(^{27}\) In a recent study,\(^{2}\) the authors showed an impairment in total and cardiac neuronal NE reuptake in patients with essential hypertension compared with normotensive patients, suggesting a functional reduction in NE presynaptic transport activity linked to possible NE transporter gene mutations.\(^{28}\) Moreover, in subcutaneous adipose tissue, basal NE levels in dialysates were higher in old SHR than in age-matched WKY but were similar to levels in young SHR. In contrast to the observation in skeletal muscle, the responses to desipramine perfusion on NE interstitial levels in subcutaneous adipose tissue were higher in old SHR compared with age-matched WKY but not different from those in respectively younger rats. In white adipose tissue, adrenergic nerve fibers are present around vessels, but they are also abundantly distributed directly on fat cells.\(^{29}\) Their activation, which is independent of baroreflex control, is mainly involved in the regulation of lipolysis.\(^{29}\) Compared with age-matched WKY, SHR show a lower increase in body weight during their life spans, and this could be related to the continuously increased activity of the sympathetic nervous system in their white adipose tissue throughout their lives.\(^{17}\)

In conclusion, sympathetic nervous system activation is increased and appears to be independent of baroreflex function, being present in skeletal muscle and subcutaneous adipose tissue in awake and freely moving hypertensive rats. In addition, the present findings obtained in vivo support the notion of increased release, reuptake, turnover, and storage of NE in SHR of both ages. Finally, after the development of hypertension in SHR, neuronal NE reuptake is reduced in skeletal muscle and may be responsible for the enhanced availability of NE for postsynaptic vascular effects, contributing to the increased vascular resistance in this tissue and hence to the elevation of blood pressure.

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**References**

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