In Vivo Hypothalamic Release and Synthesis of Catecholamines in Spontaneously Hypertensive Rats

Karel Pacák, Gal Yadid, Gabor Jakab, Jacques W.M. Lenders, Irwin J. Kopin, David S. Goldstein

Juvenile spontaneously hypertensive rats (SHR) have higher plasma levels of catechols and markedly larger catechol responses to yohimbine than do normotensive Wistar-Kyoto rats, indicating increased sympathoadrenal outflow and increased α2-adrenergic receptor-mediated restraint of peripheral catecholamine release during hypertension development in SHR. Yohimbine-induced catecholamine release and metabolism in the posterolateral hypothalamus of the brain were assessed in juvenile (6 to 7 weeks) and adult (15 to 16 weeks) SHR and Wistar-Kyoto rats. In vivo microdialysis was used to obtain samples for measurements of norepinephrine, dihydroxyphenylglycol, methoxyhydroxyphenylglycol, and dihydroxyphenylacetic acid in conscious animals before and after yohimbine injection (1 mg/kg IV) beginning 24 hours after probe implantation. Catecholamine synthesis was examined from elevations of 3,4-dihydroxyphenylalanine levels after probe perfusion with NSD-1015, an inhibitor of L-aromatic acid decarboxylase. In adults, SHR had higher dialysate norepinephrine (277±38 versus 181±35 pg/mL), dihydroxyphenylglycol (3260±509 versus 2231±201 pg/mL), methoxyhydroxyphenylglycol (2659±369 versus 1890±144 pg/mL), and dihydroxyphenylacetic acid (46 312±5512 versus 13 187±1963 pg/mL) levels and markedly larger increases in 3,4-dihydroxyphenylalanine levels after NSD-1015 than Wistar-Kyoto rats. In juveniles, SHR had larger proportionate increments in microdialysate norepinephrine levels after yohimbine than Wistar-Kyoto rats (85% versus 25%). Although juvenile SHR and Wistar-Kyoto rats had similar NSD-1015-elicited increments in 3,4-dihydroxyphenylalanine levels, systemic yohimbine enhanced the NSD-1015-elicited 3,4-dihydroxyphenylalanine elevations in juvenile SHR but not in Wistar-Kyoto rats. These findings suggest augmented norepinephrine release and catecholamine synthesis in the posterolateral hypothalamus of adult SHR and augmented α2-adrenergic receptor restraint of both norepinephrine release and catecholamine synthesis in juvenile SHR. (Hypertension. 1993;22:467-478.)

KEY WORDS • DOPA • methoxyhydroxyphenylglycol • 3,4-dihydroxyphenylacetic acid • hypertension • sympathetic nervous system • receptors, adrenergic

Spontaneously hypertensive rats (SHR) provide an animal model for studying pathophysiological mechanisms that may participate in the development of hypertension.1 Many studies of mechanisms of hypertension in SHR have focused on peripheral catecholaminergic systems. SHR have increased directly recorded sympathetic nerve activity2-5 and vascular hyperinnervation6-9 as well as augmented release of the sympathetic transmitter norepinephrine during sympathetic stimulation10-18. These abnormalities seem most prominent in juvenile animals, suggesting abnormal peripheral catecholaminergic function during the development of hypertension in SHR. Juvenile SHR also have augmented α2-adrenergic receptor-mediated restraint of norepinephrine release from sympathetic nerves10-17; in particular, these animals have markedly exaggerated increases in plasma levels of catechols after systemic administration of the α2-adrenergic receptor antagonist yohimbine.19

Whether SHR have abnormal catecholaminergic function in the brain is much less clear. Many studies have examined brain tissue levels of norepinephrine or its metabolites, catecholamine-synthesizing enzymes, adrenergic receptors, or release of [3H]norepinephrine from brain slices in SHR. The implications of the results in terms of brain catecholaminergic function remain uncertain because of tenuous inferences from in vitro data about in vivo neurochemical mechanisms and because of the interplay of catecholamine synthesis and exocytotic norepinephrine release with modulation by receptors on noradrenergic terminals.

The present study explored these issues by using microdialysis, which enables sampling extracellular fluid from particular brain regions in conscious, freely moving animals. By measuring dialysate concentrations of norepinephrine and its metabolites at baseline and after systemic yohimbine administration, we assessed whether SHR have increased release of norepinephrine in the brain and whether juvenile SHR have augmented...
\(\alpha_1\)-adrenergic receptor-mediated restraint of norepinephrine release. By measuring dialysate concentrations of the catecholamine precursor dihydroxyphenylalanine (DOPA) after local perfusion with 3-hydroxybenzylhydrazine dihydrochloride (NSD-1015), an irreversible inhibitor of L-aromatic amino acid decarboxylase, we also assessed whether SHR have augmented tyrosine hydroxylation and therefore catecholamine biosynthesis\(^{20,21}\) and whether juvenile SHR have augmented \(\alpha_1\)-adrenergic receptor-mediated restraint of tyrosine hydroxylation.

The microdialysis probe was implanted in the posterolateral hypothalamus (PLH) for four reasons. First, SHR have excessive sympathoadrenergic responses to emotional stressors,\(^{22-32}\) and the PLH participates in autonomic responses to emotional distress\(^{23,34}\); second, posterior hypothalamic stimulation produces marked, autonomously mediated pressor responses in SHR\(^{35}\); third, grafting hypothalamic neurons from embryonic SHR into the hypothalamus of adult normotensive Wistar-Kyoto (WKY) rats produces hypertension\(^{36}\); and fourth, systemic yohimbine administration increases hypothalamic microdialysate concentrations of norepinephrine and its metabolites.\(^{37}\)

**Methods**

The procedures were approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke. Male young SHR and WKY rats (6 to 7 weeks old) and adult SHR and WKY rats (15 to 16 weeks old) from Taconic Farms (Germantown, NY) were housed two to three per cage at room temperature (22°C) with food and water ad libitum and with a 12-hour light/dark cycle at least 4 days before the acute experiment.

**Animal Preparation**

Each animal was anesthetized with pentobarbital (juvenile rats: 30 mg/kg; adult rats: 50 mg/kg IP) 20 to 24 hours before the acute experiment. Cannulas (PE-10 connected to PE-50 tubing for juvenile rats, PE-50 for adult rats) were inserted into the femoral artery and vein, sutured in place, and led subcutaneously to the nape, where they were exteriorized and attached to a metal spring, which also contained the inlet to the microdialysis probe. The dead space of the cannulas was approximately 150 \(\mu L\).

Each animal was then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, Calif.), with the incisor bar 3.2 mm below the interaural line. The skull was exposed, and a small hole was drilled. The microdialysis probe (1 mm; molecular weight cutoff, 20 000 D; BAS/Carnegie Medicine, West Lafayette, Ind) was placed with the following coordinates with respect to the bregma: juvenile rats: posterior, 3.6; lateral, 1.5; vertical: -9.8 mm, according to the atlas of Konig and Klippel\(^{38}\) and Palkovits (personal communication); adult rats: posterior, 3.7; lateral, 1.5; vertical: -9.3 or -9.9 mm for SHR or WKY rats, respectively, according to the atlas of Paxinos and Watson\(^{39}\) (Fig 1). The implanted microdialysis probe was anchored to the skull with three stainless-steel screws and acrylic dental cement. After surgery, the animals recovered in cylindrical Plexiglas cages for 20 to 24 hours before the acute experiment. Cannula patency was maintained by flushes of 0.30 mL of 0.9% saline solution containing 50 IU/mL heparin sodium for juvenile rats and 200 IU/mL heparin sodium for adult rats.

**Microdialysis Procedure**

The microdialysis probe was connected to a microinfusion pump (CMA 100, BAS/Carnegie Medicine) immediately after surgery and continuously perfused at 1.0 \(\mu\)L/min with artificial cerebrospinal fluid (189 mmol/L NaCl, 3.9 mmol/L KCl, and 3.37 mmol/L CaCl\(_2\); pH 6.3). Each collection vial contained 20 \(\mu\)L of 0.2N acetic acid. After each collection period, the dialysate was frozen.

**Experiment 1**

Acute experiments began between 9 and 10 AM on the day after operation. The dialysis experiment began with the collection of three baseline samples during 40-minute intervals, with blood samples obtained at the middle of each interval. Heparinized normal saline (30 IU/mL, 1 mL/kg) was injected after the first interval. Juvenile SHR (n=7; initial body weight, 153±6.1 g) and WKY rats (n=6; initial body weight, 165±3.6 g) and adult SHR (n=7; initial body weight, 326±3.5 g) and WKY rats (n=6; initial body weight, 421.5±18.7 g) received yohimbine (1 mg/kg) intravenously after the third baseline microdialysate collection. This was followed by four consecutive 40-minute collection periods. Blood samples (0.4 to 0.5 mL) were collected into tubes containing heparin (5 \(\mu\)L, 1000 IU/mL) and immediately centrifuged for 90 seconds to separate the plasma. The plasma was stored at -70°C until analyzed. Heparinized saline (30 IU/mL) was added to the red blood cells, and the same volume as had been drawn was injected into the animal immediately after each blood sample was collected.

**Experiment 2**

Juvenile SHR (n=6; initial body weight, 151±6.6 g) and WKY rats (n=6; initial body weight, 204±11.0 g) and adult SHR (n=6; initial body weight, 304±4.5 g) and WKY rats (n=6; initial body weight, 444±7.6 g) received 100 \(\mu\)mol/L NSD-1015 according to the method of Nisenbaum et al.\(^{40}\) The decarboxylase inhibitor was administered via the implanted microdialysis probe after the second baseline microdialysate collection. This was followed by seven consecutive 40-minute collection periods.

For the evaluation of possible \(\alpha_2\)-autoreceptor-mediated modulation of tyrosine hydroxylation activity, groups of juvenile SHR (n=6; initial body weight, 161±4.2 g) and WKY rats (n=6; initial body weight, 193±6.1 g) received yohimbine (1 mg/kg) intravenously followed by NSD-1015 (100 \(\mu\)mol/L, 40 minutes after yohimbine administration) via the microdialysis probe. Acute experiments began between 9 and 10 AM on the day after operation. The dialysis experiment began with collection of two baseline samples during 40-minute intervals. After the second interval, either NSD-1015 or yohimbine and NSD-1015 were administered, and seven consecutive collection periods followed. Dissolved NSD-1015 is unstable, so the solution of NSD-1015 in artificial cerebrospinal fluid was prepared immediately before each experiment.
FIG 1. Diagram of coronal section shows position of microdialysis probe in posterolateral hypothalamus (PLH) 3.6 mm caudal to the bregma. AH, Ammon's horn; CPu, caudate putamen; IC, internal capsule; Md, mediodorsal thalamic nucleus; PmCo, posteromedial cortical amygdaloid nucleus; Po, posterior thalamic nuclear group; Vp, ventral posterior thalamic nucleus; 3V, third ventricle; ZI, zona incerta. Arrows indicate direction of flow of artificial cerebrospinal fluid.

Probe Location

At the end of the experiment, the brain was removed and stored in 10% Formalin solution for later histological verification of probe position. Serial coronal sections (30 to 40 μm thickness) were cut through the hypothalamus, and the position of the probes was validated by microscopy.

Drugs

Yohimbine hydrochloride was obtained from Sigma Chemical Co, St Louis, Mo, and NSD-1015 from Research Biochemicals Inc, Natick, Mass.

Assays

Concentrations of norepinephrine, dihydroxyphenylglycol (DHPG), methoxyhydroxyphenylglycol (MHPG), dihydroxyphenylacetic acid (DOPAC), and DOPA in the dialysate and plasma were assayed using reversed-phase high-performance liquid chromatography (HPLC) with electrochemical detection after partial purification by adsorption on alumina. Recovery through the alumina extraction step averaged 70% to 75% for norepinephrine and DHPG, 65% to 70% for DOPA, and 40% to 50% for DOPAC. Catechol concentrations in each sample were corrected for recovery of an internal standard, dihydroxybenzylamine. Levels of DOPA and DOPAC were corrected further for differences in recovery of the internal standard and recoveries of these compounds in a mixture of external standards. The limits of detection for norepinephrine and DHPG were approximately 65 to 100 pg/mL; for MHPG and DOPA, approximately 160 to 220 pg/mL; and for DOPAC, approximately 260 to 330 pg/mL.

Procedures for plasma MHPG determinations were adapted from previous methods for measurement of urinary MHPG. Microdialysate concentration of MHPG was assayed in the supernatant after adsorption of the catechols onto alumina. Briefly, 10 mL HPLC-grade ethyl acetate was added to the supernatant, shaken for 10 minutes, and centrifuged; the supernatant was then taken to dryness in a vacuum centrifuge. The residue was dissolved by adding 100 μL of a solution of 0.2N acetic acid (80%) and 0.2 mol/L phosphoric acid (20%), and the mixture was vortexed for 5 seconds, centrifuged, and the supernatant injected into the HPLC system. MHPG recovery through this procedure averaged 50% to 60%.

For conversion of picograms per milliliter to picomoles per liter, multiply by 5.92 for norepinephrine, 5.44 for epinephrine, 5.88 for DHPG, 5.49 for MHPG, 5.95 for DOPAC, 6.49 for dopamine, and 5.08 for DOPA.
Statistical Analysis
Results are presented as mean±SEM. Strain effects for baseline levels of catechols as well as effects of either yohimbine or NSD-1015 were analyzed by two-way analyses of variance for repeated measures, the between-groups factor being strain (SHR versus WKY rats) and the within-groups factor being time (0 to 400 minutes), followed by the Newman-Keuls post hoc test.

Baseline values were calculated as the mean of the last three baseline samples. To express results as percent changes from baseline, the average of the baseline values was defined as 0%, and all subsequent measures were related to this value, with differences between SHR and WKY groups compared by analyses of variance for repeated measures and the Newman-Keuls post hoc test. A value of P<.05 was defined as statistically significant.

Results
Baseline Concentrations of Catechols
In juveniles, SHR had significantly decreased dialysate DHPG levels and nonsignificantly increased dialysate DOPAC levels at baseline compared with values in WKY rats (484±110 versus 907±83 pg/mL, P<.05; 4624±885 versus 3323±904 pg/mL; Fig 2). Juvenile SHR had nonsignificantly lower microdialysate levels of norepinephrine (74±14 versus 91±12 pg/mL) and slightly higher levels of MHPG (963±173 versus 926±97 pg/mL).

In adults, SHR had higher dialysate levels of norepinephrine (277±38 versus 181±35 pg/mL), DHPG (3260±509 versus 2231±201 pg/mL), and MHPG (2659±369 versus 1890±144 pg/mL) and markedly higher levels of DOPAC (4632±512 versus 13187±1963 pg/mL, F=44, P<.001) than WKY rats (Fig 3).

In juveniles, SHR had nonsignificantly higher baseline arterial plasma levels of norepinephrine (247±20 versus 215±26 pg/mL) and DOPAC (686±43 versus 614±88 pg/mL) but significantly higher levels of DHPG (865±76 versus 561±11 pg/mL, P<.01), MHPG (3602±481 versus 2269±272 pg/mL, P<.05), DOPA (746±38 versus 506±47 pg/mL, P<.01), and epinephrine (177±23 versus 85±29 pg/mL) than WKY rats (Fig 4).

In adults, SHR tended to have lower baseline plasma concentrations of several catechols than WKY rats (norepinephrine, 183±33 versus 240±41; DHPG, 809±107 versus 1014±141; MHPG, 1841±174 versus 2043±383; DOPA, 687±101 versus 749±24; DOPAC, 662±38 versus 851±56; and epinephrine, 113±38 versus 188±23 pg/mL; Fig 5), but none of these strain differences were statistically significant.

Effects of Yohimbine
Systemic injection of yohimbine increased dialysate levels of norepinephrine, DHPG, MHPG, and DOPAC in juvenile and adult SHR and WKY rats (Figs 2 and 3). In juveniles, absolute responses of dialysate levels of these catechols in SHR were slightly and statistically nonsignificantly larger than in WKY rats, as judged from the strain×time interaction effect in the analysis of variance; however, proportionate responses of microdialysate norepinephrine levels in juvenile SHR after yohimbine administration were significantly larger than in WKY rats (F=6.2, P<.01, Fig 2).

In contrast, in adults, SHR had smaller absolute and percent responses of microdialysate norepinephrine after yohimbine injection than WKY rats, and there were no strain differences in responses of microdialysate DHPG, MHPG, and DOPAC levels after yohimbine administration (Fig 3).

Yohimbine administration produced markedly larger responses of arterial plasma levels of norepinephrine (F=8.2, P<.001), DHPG (F=8.8, P<.001), MHPG (F=2.8, P<.05), DOPA (F=5.1, P<.001), and epinephrine (F=2.6, P<.05) in juvenile SHR than in juvenile WKY rats (Fig 4). In contrast, in adults, none of the strain differences were statistically significant (Fig 5).

Effects of Centrally Administered NSD-1015
DOPA was detected only occasionally at baseline in microdialysate samples. Administration of NSD-1015 via the microdialysis probe increased microdialysate DOPA concentrations in all animals. Steady-state DOPA levels (defined by DOPA concentrations that did not differ significantly in two consecutive samples) were attained within 160 minutes in juvenile and 240 minutes in adult rats.

In adult SHR, DOPA accumulation after NSD-1015 administration was more rapid than in WKY rats (F=5.0, P<.001, Fig 6), whereas in juveniles, DOPA accumulated similarly in the two strains (Fig 6).

Marked decreases in dialysate DOPAC levels attended the increases of microdialysate DOPA concentrations after NSD-1015 administration in all animals (Fig 6). DHPG levels also decreased (data not shown), whereas norepinephrine levels increased, with peak levels during the second or third collection period, followed by slow declines thereafter to levels above baseline values by the end of the experiment (data not shown).

In juveniles, after yohimbine followed by NSD-1015, SHR had much larger increases in microdialysate DOPA levels (F=5.9, P<.001) than WKY rats (Fig 7).

Discussion
In the present study, age-dependent differences were observed between SHR and WKY rats in hypothalamic norepinephrine release and catecholamine biosynthesis and in modulation of both processes by α2-adrenergic receptors. These differences can be explained in terms of two underlying pathophysiological mechanisms: increased catecholamine turnover and increased α2-adrenergic receptor restraint of norepinephrine release, as explained below.

Microdialysate basal levels of norepinephrine, DHPG, and MHPG were all higher in adult SHR than in age-matched WKY rats. Because DHPG and MHPG are the main neuronal and extraneuronal metabolites of norepinephrine, this pattern indicates increased release, reuptake, and metabolism of norepinephrine in the PLH of adult SHR, ie, increased norepinephrine turnover.

Under steady-state conditions, catecholamine synthesis equals catecholamine turnover. Adult SHR had strikingly higher dialysate concentrations of DOPAC than WKY rats. DOPAC is the product of oxidative metabolism of dopamine, which is considered a marker of dopaminergic nerve terminals. This further supports the hypothesis that catecholamines play a role in the pathophysiology of hypertension, as suggested by previous studies. Furthermore, systemic administration of yohimbine increased microdialysate levels of catecholamines, further supporting the role of these neurotransmitters in the regulation of blood pressure.
Fig 2. Graphs show effects of intravenous administration of yohimbine (YOH, 1 mg/kg) on microdialysate concentrations of norepinephrine (NE), dihydroxyphenylglycol (DHPG), methoxyhydroxyphenylglycol (MHPG), and dihydroxyphenylacetic acid (DOPAC) in the posterolateral hypothalamus of conscious juvenile spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) normotensive rats. %Δ, Percent of baseline. Asterisks show significant absolute or relative difference between strains based on strain×time interaction effect of analysis of variance or Newman-Keuls test (*P<.05, **P<.01). Results are mean±SEM.
FIG 3. Graphs show effects of intravenous administration of yohimbine (YOH, 1 mg/kg) on microdialysate concentrations of norepinephrine (NE), dihydroxynphylglycol (DHPG), methoxyhydroxyphenylglycol (MHPG), and dihydroxynphenylacetic acid (DOPAC) in the posterolateral hypothalamus of conscious adult spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) normotensive rats. %Δ, Percent of baseline. Asterisks show significant absolute or relative differences between strains based on strain x time interaction effect of analysis of variance for repeated measures or Newman-Keuls test (*P<.05, **P<.01). Results are mean ± SEM.
deamination of dopamine in the neuronal cytoplasm. In steady states, the rates of synthesis of dopamine from DOPA and of metabolism of dopamine to DOPAC are limited by the rate of synthesis of DOPA from tyrosine. Because DOPAC rapidly enters extracellular fluid, high extracellular fluid levels of DOPAC probably reflect increased tyrosine hydroxylase activity. Consistent with this explanation for elevated microdialysate DOPAC levels in adult SHR, after local perfusion of NSD-1015, an irreversible inhibitor of L-aromatic amino acid decarboxylase, the rates of decline in microdialysate DOPAC levels were faster in the adult SHR than in age-matched WKY rats. This was not the case in juveniles (see below).

Acute inhibition of L-aromatic amino acid decarboxylase increases DOPA levels; the rate of increase in DOPA levels would be expected to be proportional to the rate of DOPA production by tyrosine hydroxylation. The markedly faster and larger increases in microdialysate DOPA concentrations after decarboxylase inhibition in adult SHR than in WKY rats provide further support for the view that, at least in the PLH, tyrosine hydroxylation and therefore catecholamine biosynthesis is greater in adult SHR than in WKY rats.
Fig 5. Graphs show effects of intravenous administration of yohimbine (YOH, 1 mg/kg) on arterial plasma concentrations of norepinephrine (NE), dihydroxyphenylglycol (DHPG), methoxyhydroxyphenylglycol (MHPG), dihydroxyphenylalanine (DOPA), dihydroxyphenylacetic acid (DOPAC), and epinephrine (EPI) in conscious adult spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) normotensive rats. Results are mean±SEM.

The magnitude of the proportionate difference in baseline microdialysate DOPAC levels between the adult SHR and adult WKY rats exceeded by far the differences in baseline norepinephrine, DHPG, and MHPG levels. Because the PLH possesses both dopaminergic and noradrenergic terminals, the results suggest that turnover of both dopamine and norepinephrine may be increased in adult SHR; however, in the present study, levels of homovanillic acid, the main product of dopamine metabolism, were not measured.

As previously reported for responses of levels of catechols in arterial plasma, after systemic yohimbine administration, increments in dialysate levels of catechols generally did not differ between adult SHR and age-matched WKY rats.

In contrast to the strain difference in microdialysate catechol levels in adults, juvenile SHR did not appear to have higher rates of catecholamine biosynthesis or norepinephrine turnover at baseline than juvenile WKY rats. Dialysate DHPG levels were actually significantly lower in juvenile SHR than in juvenile WKY rats, whereas DOPAC and MHPG levels tended to be higher, suggesting decreased conversion of dopamine to norepinephrine or increased conversion of DHPG to MHPG in juvenile SHR. After administration of NSD-1015 via the microdialysis probe in juvenile animals, the strains did not differ in either the rate of accumulation of DOPA or the rate of decline of DOPAC levels.

In juvenile animals, SHR had proportionately larger increments of dialysate norepinephrine levels after sys-
FIG 6. Line graphs show effects of central administration of 3-hydroxybenzylhydrazine dihydrochloride (NSD, 100 μmol/L) on microdialysate concentrations of dihydroxyphenylalanine (DOPA) and dihydroxyphenylacetic acid (DOPAC) in the posterolateral hypothalamus of conscious juvenile or adult spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) normotensive rats. Asterisks show significant absolute differences between strains in strain x time interaction effect of analysis of variance for repeated measures or by Newman-Keuls test (*P<.05, **P<.01). Results are mean±SEM.

temically administration of yohimbine than WKY rats, analogous to previously reported differences in responses of plasma catechol levels in the two strains.19 The strain differences were highly significant, not only for the proportionate increases in microdialysate norepinephrine levels after yohimbine administration but also for the absolute rate of DOPA accumulation and the rate of decline of DOPAC levels after administration of NSD-1015. The NSD-1015 results indicate that in juvenile SHR, α2-adrenergic receptors tonically restrain not only hypothalamic norepinephrine release but also tyrosine hydroxylation.

Fig 8 summarizes how interactions between α2-adrenergic receptors and dopamine and norepinephrine synthesis can explain the present neurochemical findings in SHR. The main catecholaminergic abnormality in the PLH of SHR appears to increase tyrosine hydroxylation, leading to high microdialysate levels of DOPAC and accelerated increases in dialysate DOPA levels after NSD-1015 administration. Juvenile SHR also have (perhaps compensatorily) increased α2-adrenergic receptor-mediated restraint of catecholamine synthesis and turnover, masking the underlying abnormality of tyrosine hydroxylation. In adult SHR, an age-related decline in α2-adrenergic receptor restraint of catecholamine synthesis and turnover results in an unopposed increase in catecholamine synthesis and norepinephrine release at baseline, and smaller responses to α2-adrenergic receptor blockade reflect the decline in α2-adrenergic receptor modulation.

Research literature to date about brain tissue levels of catecholamines, catecholamine-synthesizing enzymes, and α2-adrenergic receptor numbers has not revealed any consistent abnormality in SHR. This inconsistency can be explained by increased α2-adrenergic receptor–mediated suppression in juvenile SHR of what becomes apparent in adult SHR when α2-adrenergic receptor modulation decreases and by the indirectness of tissue assays to indicate in vivo synthesis and release of catecholamines.

Regarding tissue levels of catecholamines or their metabolites, Dawson et al44 reported slightly higher hypothalamic norepinephrine and DOPAC concentrations in 8-week-old SHR than in age-matched WKY rats. McKeon and Hendley45 reported that hypothalamic norepinephrine concentrations did not differ between SHR and WKY rats at either 6 or more than 26 weeks of age. Hypothalamic DOPAC levels were about twice as high in adult SHR than in WKY rats, whereas in 6-week-old rats, the strain difference was much less apparent. Howes et al46 reported that hypothalamic tissue DHPG, norepinephrine, and DOPAC levels did
FIG 7. Line graph shows effects of intravenous administration of yohimbine (YOH, 1 mg/kg) followed by central administration of 3-hydroxybenzylhydrazine dihydrochloride (NSD, 100 μmol/L) on microdialysate concentration of dihydroxyphenylalanine (DOPA) in the posterolateral hypothalamus of conscious juvenile spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) normotensive rats. Asterisks show significant absolute differences between strains in strain x time interaction effect of analysis of variance for repeated measures or by Newman-Keuls test (*P<.05). Results are mean±SEM.

not differ between SHR and WKY rats of various ages, whereas striatal DOPAC levels increased with increasing age, and the rate of this increase was greater in SHR.

Regarding tissue tyrosine hydroxylase activity and norepinephrine turnover, Koulu et al reported more rapid accumulation of DOPA after decarboxylase inhibition in the locus ceruleus of juvenile SHR than in juvenile WKY rats. The strain difference was not apparent in 14-week-old animals. Patel et al summarized discrepant previous studies about norepinephrine turnover in brain and peripheral organs of SHR and measured tissue norepinephrine turnover by the rate of decline in norepinephrine content after tyrosine hydroxylase blockade. Norepinephrine turnover in hypothalamic tissue did not differ between SHR and WKY rats at 5 or 9 weeks of age but was significantly higher in SHR at 18 weeks of age. Fujino reported lower norepinephrine concentrations and decreased rates of catecholamine synthesis in the anterior hypothalamus of juvenile SHR compared with findings in juvenile WKY rats.

With regard to α2-adrenergic receptors, Morris et al reported that juvenile SHR had greater numbers of hypothalamic α2-adrenergic receptors by radioligand binding than WKY rats. Increased α2-adrenergic receptor responsiveness in SHR has been suggested from mydriatic and growth hormone responses to exogenously administered clonidine. In 8-week-old animals, Dawson et al reported a more marked acute decrease in hypothalamic norepinephrine levels and a larger increase in tissue DOPAC after yohimbine administration in SHR than in WKY rats.

Dopamine has been implicated in the development of hypertension in SHR. Lesions of the substantia nigra attenuate both the development of hypertension and the behavioral hyperactivity in SHR, and attenuation of hypertension development after intracerebroventricular administration of 6-hydroxydopamine in SHR at 5 weeks of age appears to depend on destruction of dopaminergic rather than of noradrenergic terminals. These findings suggest a rather global involvement of dopaminergic pathways in the development of hypertension in SHR. Yohimbine can act as a dopamine receptor antagonist, resulting in increased dopaminergic activity. Another mechanism by which yohimbine may influence dopaminergic activity is via noradrenergic neurons that terminate on dopaminergic cells mainly in the substantia nigra. Only very low densities of α2-adrenergic receptors have been found in brain dopaminergic areas. Systemically administered yohimbine (1 mg/kg IV) does not change extracellular striatal DOPAC levels, whereas higher doses of yohimbine (5 to 10 mg/kg IP) increase extracellular dopamine levels in the striatum. Thus, yohimbine may affect both noradrenergic and dopaminergic neuronal activities; however, the mechanisms of these actions remain poorly understood.

In conclusion, the present findings and model help to explain previously obtained discrepant results about brain tissue catecholamine levels and adrenergic receptor numbers in SHR. Our results suggest that during the development of hypertension in SHR, augmented restraint by α2-adrenergic receptors limits what would otherwise produce increased catecholamine biosynthe-
sis and norepinephrine release in the PLH of SHR and that as SHR mature, the extent of α-2-adrenergic receptor-mediated restraint declines, unmasking genetically determined increases in catecholamine synthesis and norepinephrine release.

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