Original Article

Treatment with Hypotensive Agents Affects the Impaired Relaxation of the Penile Corpus Cavernosum in Hypertensive Rats

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Treatment of erectile dysfunction (ED) in hypertensive subjects remains to be formally established. There is currently no standardized treatment for ED in hypertensive subjects. In this study, we tested our hypothesis that hypotensive drugs would improve impaired relaxation in the corpus cavernosum of spontaneously hypertensive rats (SHR). Ten-week-old SHR was treated with amlodipine, imidapril or hydralazine for 4 weeks. Although all three drugs achieved an equivalent decrease in systolic blood pressure (SBP), only amlodipine and imidapril induced an increase in relaxation in response to electrical field stimulation (EFS) of the corpus cavernosum. In the case of amlodipine, this effect was dose- and SBP-dependent. Nitric oxide (NO)–dependent relaxation was increased by amlodipine over a wide range of EFS frequencies, was increased by imidapril at low EFS frequencies, and was decreased by hydralazine. Carbon monoxide (CO)–dependent relaxation was only increased by hydralazine, and this increase occurred over a wide range of frequencies. The NOx and cGMP levels in the EFS-stimulated corpus cavernosum were increased by amlodipine. Amlodipine did not affect the thiobarbituric acid–reacting substance levels in the serum and the corpus cavernosum, but did decrease superoxide dismutase activity in the tissue. Imidapril and hydralazine inhibited the acetylcholine-induced relaxation in the corpus cavernosum. Sodium nitroprusside–induced relaxation in the tissue was increased by amlodipine. All three agents similarly inhibited the phenylephrine-induced contraction. These results suggest that impaired neurogenic relaxation in the corpus cavernosum of SHR is improved by amlodipine and imidapril through an increase in the synthesis and/or release of neuronal NO, but not CO, and presumably the inhibited detumescence of erection, which is induced by norepinephrine being released from sympathetic neuron. These findings indicate that amlodipine and imidapril may ameliorate the decreased relaxation of cavernous smooth muscle in the setting of hypertension. (*Hypertens Res* 2006; 29: 523–532)

***Key Words*: hypertension, spontaneously hypertensive rat, Ca^{2+} antagonist, nitric oxide, carbon monoxide**

Introduction

Penile erection is evoked by elevated pressure in the corpus cavernosum, resulting from the relaxation of the cavernous smooth muscles and its arterioles. The main relaxant substance has been determined to be nitric oxide (NO), which is liberated from neurons and endothelium in the corpus cavernosum (*1*). Erectile dysfunction (ED) often occurs in the elderly and subjects with diabetes mellitus (*2*). Hypertension has also been thought to be a risk factor for ED (*2*). The pathogenesis of ED in the setting of hypertension has been reported to involve atherosclerotic vascular damage (*3*, *4*). Using spontaneously hypertensive rats (SHR), we recently demonstrated

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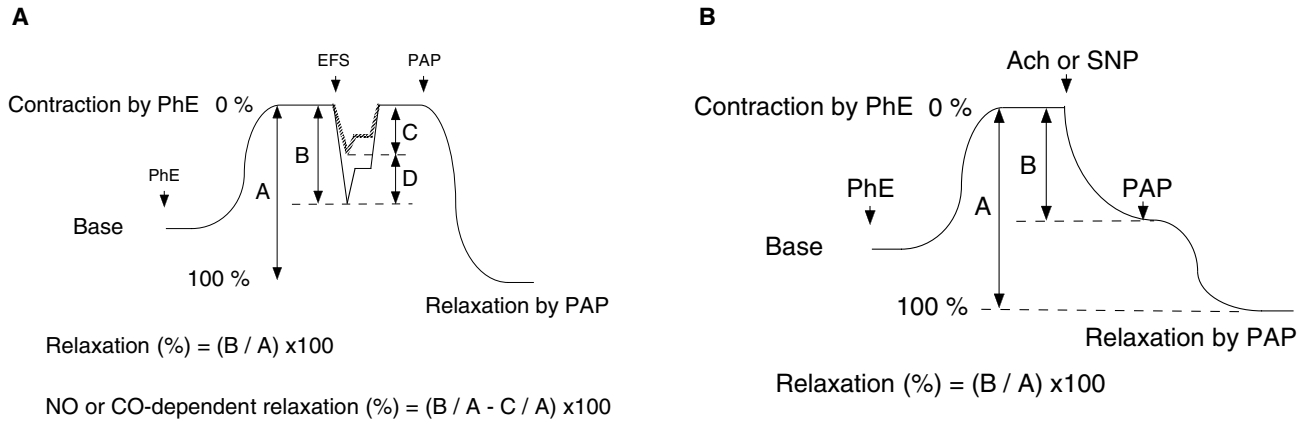

Fig. 1. *Calculation of relaxation in response to EFS, ACh and SNP. A: Calculation of relaxation in response to EFS in the corpus cavernosum. "C" is relaxation in response to EFS after treatment with* N$^{\omega}$*-nitro-L-arginine or zinc protoporphyrin-IX. B: Calculation of relaxation in response to ACh or SNP in the corpus cavernosum. At the end of the examinations, the corpus cavernosum was treated with PAP. EFS, electrical field stimulation; PhE, phenylephrine hydrochloride; ACh, acetylcholine; SNP, sodium nitroprusside; PAP, papaverine.*

that neuronal NO- and carbon monoxide (CO)–dependent relaxation in the corpus cavernosum is impaired in ED in the setting of hypertension (*5*). However, it remained unclear whether hypotensive drugs improve ED in the setting of hypertension. Some hypotensive drug classes, such as β-blockers, α1-blockers, α-methyldopa, angiotensin-converting enzyme (ACE) inhibitors and calcium antagonists, have been reported to induce ED in humans (*6*). However, it has not been determined whether the impaired erectile function is induced as a side effect of the hypotensive drug or by hypertension *per se*. In particular, it remains unclear whether treatment with a hypotensive drug affects the neuronal system of the penile corpus cavernosum in the setting of hypertension. Calcium antagonists (*7*) and ACE inhibitors (*8*) have been shown to augment NO release from the endothelium of blood vessels. Some dihydropyridine calcium antagonists (*9*) and ACE inhibitors (*10*) have been shown to reduce oxidative stress in hypertensive patients. Therefore, it is possible that treatment with a hypotensive agent will improve ED in the setting of hypertension, as a result of improving endothelial function and/or augmenting NO in response to diminished oxidative stress.

In view of the above, we used SHR as a model of hypertension (*5*, *11*) to elucidate whether hypotensive drugs improve the impaired relaxation of the corpus cavernosum in hypertension state, and if so, whether the neuronal or endothelial systems of the corpus cavernosum are involved in this improvement.

Methods

These experiments were approved by the Animal Research Committee of Saitama Medical School.

Animals and Hypotensive-Drug Treatments

We used 10-week-old male SHR/Izm as a model of hypertension (Funabashi Farms, Funabashi, Japan). The animals were housed in a temperature-controlled (24°C) facility under a 12-h on/12-h off light cycle. The animals had free access to laboratory rat chow and tap water. The rats were treated with tap water containing amlodipine besilate (5, 10 or 20 mg/kg body weight), imidapril hydrochloride (1, 5 or 10 mg/kg body weight) or hydralazine hydrochloride (2.5 mg/day) for 4 weeks from the age of 10 weeks.

Reagents

Amlodipine besilate (amlodipine) and imidapril hydrochloride (imidapril) were obtained from Sumitomo Pharmaceuticals Co., Ltd. (Tokyo, Japan) and Tanabe Seiyaku Co., Ltd. (Osaka, Japan), respectively. Hydralazine hydrochloride (hydralazine), guanethidine sulphate (guanethidine), atropine sulphate (atropine), phenylephrine hydrochloride (PhE), papaverine (PAP), tetrodotoxin (TTX), acetylcholine chloride (ACh), sodium nitroprusside (SNP), *N*$^{\omega}$-nitro-L-arginine (L-NNA) and zinc protoporphyrin-IX (ZnPP) were obtained from Sigma Chemical Co. (St. Louis, USA). Amlodipine, imidapril and hydralazine were dissolved in tap water for drinking. All other reagents were dissolved in distilled water.

Measurement of Blood Pressure

Systolic blood pressure (SBP) and heart rate in control rats and rats treated with each drug were measured without anesthesia by the tail-cuff method using a plethysmograph (PS-600; Riken Kaihatsu, Tokyo, Japan) before and weekly after the initiation of the treatment with each drug.

Table 1. Systolic Blood Pressure Changes in SHR

	Blood pressure changes (mmHg)				
	0 week	1 week	2 weeks	3 weeks	4 weeks
Control SHR	159.3±8.3	160.0±7.0	164.0±8.1	168.0±5.0	169.8±9.8
Imidapril 5 mg/kg	159.7±9.6	143.0±10.5	145.5±9.9	138.2±8.7*	138.6±10.6*
Amlodipine 10 mg/kg	158.8±8.6	153.7±11.3	146.9±8.8	150.4±7.7*	141.9±5.7*
Hydralazine 2.5 mg/day	159.6±6.4	143.4±7.9	143.9±7.1	145.4±9.0*	139.1±8.1*

Values are mean±SEM. $n=20$ each. *$p<0.05$ *vs*. control SHR. SHR, spontaneously hypertensive rats.

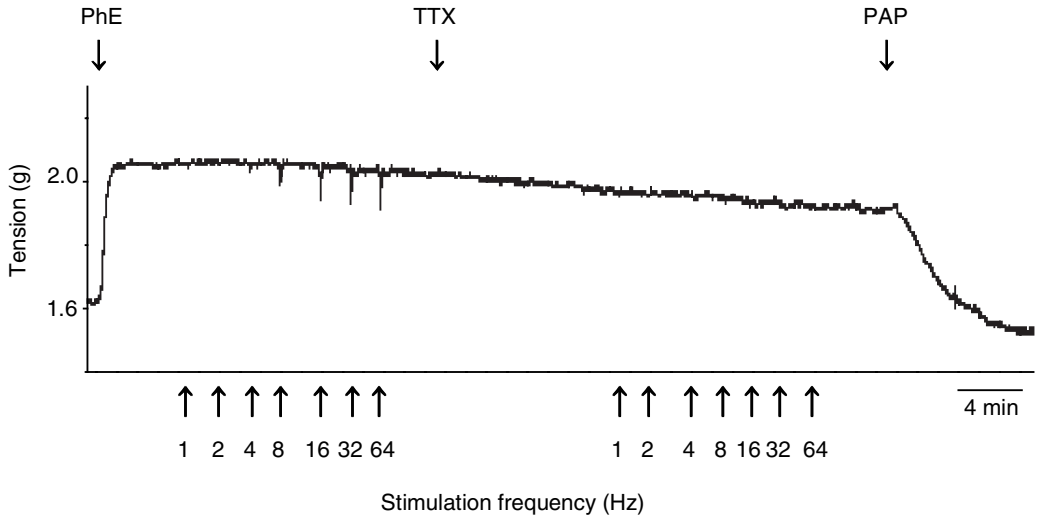

Fig. 2. *Effect of TTX on relaxation in response to EFS in the corpus cavernosum of SHR. After strips of corpus cavernosum were incubated with tetrodotoxin (TTX: 10^{-7} mol/l, final concentration) for 10 min, the relaxation in response to the electrical field stimulation (EFS) was examined. An arrow indicates EFS. Results shown are a typical chart tracing. PhE, phenylephrine hydrochloride; PAP, papaverine.*

Preparation

Rats were anesthetized with intraperitoneal injection of pentobarbital sodium (0.4 mg/kg) and sacrificed by bleeding from the carotid arteries. The penis was rapidly removed, the corpus cavernosum (approximately 3 × 12 mm) was set in a Magnus chamber filled with 10 ml Krebs solution, and isometric tension was measured as described previously (*5*). The initial resting tension applied to each strip was adjusted to 2 g (*12*, *13*). At the end of the experiments, PAP (10^{-4} mol/l, final concentration) was added to the corpus cavernosum to attain the maximal relaxation, and the weight of each strip was measured after being removed from the organ bath.

Experimental Design

Measurements of Relaxation in Response to Electrical Field Stimuration

The corpus cavernosum obtained from rats with or without hypotensive treatment was equilibrated with Krebs solution containing guanethidine (5×10^{-6} mol/l, final concentration) and atropine (10^{-6} mol/l, final concentration) for 90 min to deplete endogenous norepinephrine and block cholinergic endothelial relaxation. After stimulating contraction through the addition of PhE (10^{-5} mol/l, final concentration) for 10 min, the strip was subjected to relaxation in response to electrical field stimulation (EFS) using sequential frequencies of 1, 2, 4, 8, 16, 32 and 64 Hz delivered as 10-s trains (50 V, 0.8 ms) at 2-min intervals. After the strip was washed with fresh medium containing guanethidine and atropine for 30 min, L-NNA (*14*) (nitric oxide synthase [NOS] inhibitor; 10^{-5} mol/l, final concentration) and/or ZnPP (*11*) (heme oxygenase [HO] inhibitor; 3×10^{-5} mol/l, final concentration) was added. The strips were equilibrated for 20 min after treatment with L-NNA and/or for 40 min in the dark after treatment with ZnPP. After PhE-stimulated contraction, the strip was relaxed by EFS as described above. Any strips that failed to show less than 4% relaxation in response to EFS at 16 Hz were discarded to remove unsuccessful preparations. Relaxation and NO- or CO-dependent relaxation were calculated as shown in Fig. 1A.

Measurements of the Contraction Induced by Phenylephrine or Endothelium-Dependent or -Independent Relaxation

SHR were treated with amlodipine (10 mg/kg body weight, $n=10$), imidapril (5 mg/kg body weight, $n=10$) or hydralazine (2.5 mg/day, $n=10$). After the corpus cavernosum strips were equilibrated with Krebs solution containing guanethidine and atropine for 90 min as described above, the contraction in response to PhE (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} or 10^{-4} mol/l, final concentration) was determined. The relaxation in response to ACh (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} or 10^{-1} mol/l, final concentration) or SNP (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} or 10^{-1} mol/l, final concentration) was determined after the equilibration in Krebs solution containing guanethidine (5 μmol/l, final concentration) for 90 min and the contraction induced by PhE (10 μmol/l, final concentration) for 10 min. The contraction induced by PhE was measured as the difference between the baseline tension before the application of PhE and the tension after 10 min exposure to PhE. The measured contraction was normalized to the weight of the strip (unit: g/g wet tissue). The relaxation induced by ACh or SNP was calculated as shown in Fig. 1B.

Nitrate/Nitrite Levels in the Corpus Cavernosum Stimulated by EFS

Levels of nitrate/nitrite (NOx) in the corpus cavernosum tissues obtained from amlodipine-treated SHR (10 mg/kg body weight, $n=8$) and control SHR ($n=8$) were measured after stimulation by EFS of 16 Hz using a Nitrate/Nitrite assay kit (Cat No. 780001; Cayman Chemical Co., Ann Arbor, USA). The concentration of NOx was normalized to the weight of a strip of the corpus cavernosum and expressed as nmol/mg weight.

Measurements of Guanosine 3´,5´-Monophosphate Levels

After stimulation by EFS of 16 Hz, the levels of guanosine 3′,5′-monophosphate (cGMP) in tissues obtained from amlodipine-treated (10 mg/kg body weight, $n=8$) and control SHR ($n=8$) were measured using a cGMP enzyme immunoassay system (Amersham Pharmacia Biotech Inc., Piscataway, USA).

Thiobarbituric Acid Reactive Substance Levels and Superoxide Dismutase Activity in the Serum or Corpus Cavernosum

Thiobarbituric acid reactive substance (TBARS) levels in the serum or corpus cavernosum obtained from amlodipine-treated (10 mg/kg body weight; $n=8$) and control SHR ($n=8$) were measured by the thiobarbituric acid assay method of Yagi as described previously (*15*). Superoxide dismutase (SOD) activity in the serum or corpus cavernosum was measured by the modified assay method of Oyanagui as described previously (*16*). The TBARS levels and SOD activity in the corpus cavernosum were normalized to the weight of the strip (unit: nmol/g wet tissue or U/g wet tissue).

Statistical Analysis

Results were expressed as the mean±SEM. Statistical analysis was performed using paired or unpaired two tail *t*-test or a one-way analysis of variance (ANOVA). When ANOVA was used and when this analysis indicated significance (at the 0.05 level), post-hoc test analysis (Fisher's protected least significant difference) was used to determine which conditions were significantly different from each other. Values of $p<0.05$ were considered to indicate statistical significance.

Results

Effect of Hypotensive Drugs on SBP

Amlodipine (10 mg/kg body weight), imidapril (5 mg/kg body weight) and hydralazine (2.5 mg/day) achieved similar reductions in SBP after 4 week-drug treatment (Table 1).

Effect of TTX on EFS-Induced Relaxation in the Corpus Cavernosum of SHR

To clarify the mechanisms of the relaxation in response to EFS, TTX was added to organ bath, and the tissues were left to stand for 10 min. The relaxation in response to EFS was abolished by TTX (10^{-7} mol/l, final concentration; Fig. 2), indicating that EFS-induced relaxation is a neuronal response in the corpus cavernosum.

Effect of Hypotensive Drugs on the Response to EFS in the Corpus Cavernosum

The EFS-induced relaxation response was examined in strips of the corpus cavernosum treated with each hypotensive drug (amlodipine, imidapril or hydralazine). Treatment with amlodipine and imidapril improved the impaired relaxation of SHR in response to EFS from 2 Hz to 64 Hz and from 4 Hz to 16 Hz (Fig. 3A, $p<0.05$ or $p<0.01$, $n=20$), respectively, while hydralazine did not affect the relaxation in response to EFS (Fig. 3A, $p>0.5$, $n=20$). The relaxation in response to EFS increased in a dose-dependent manner (Fig. 3B-a; $n=10$ each), and in an SBP-dependent manner (Fig. 3C; $n=10$ each, correlation coefficient$=-0.41$, $p<0.01$) in the amlodipine-treated SHR, but not in imidapril-treated SHR (Fig. 3B-b and C; $n=10$ each, correlation coefficient$=-0.25$).

Effect of Hypotensive Drugs on the Relaxation Caused by NO or CO Derived from the Nerve in the Corpus Cavernosum

The corpus cavernosum in SHR treated with hypotensive drugs was preincubated with L-NNA to block NO synthesis or with L-NNA and ZnPP to block NO and CO synthesis. The relaxation in response to EFS was then examined. NO-dependent relaxation in response to EFS from 2 Hz to 64 Hz or from

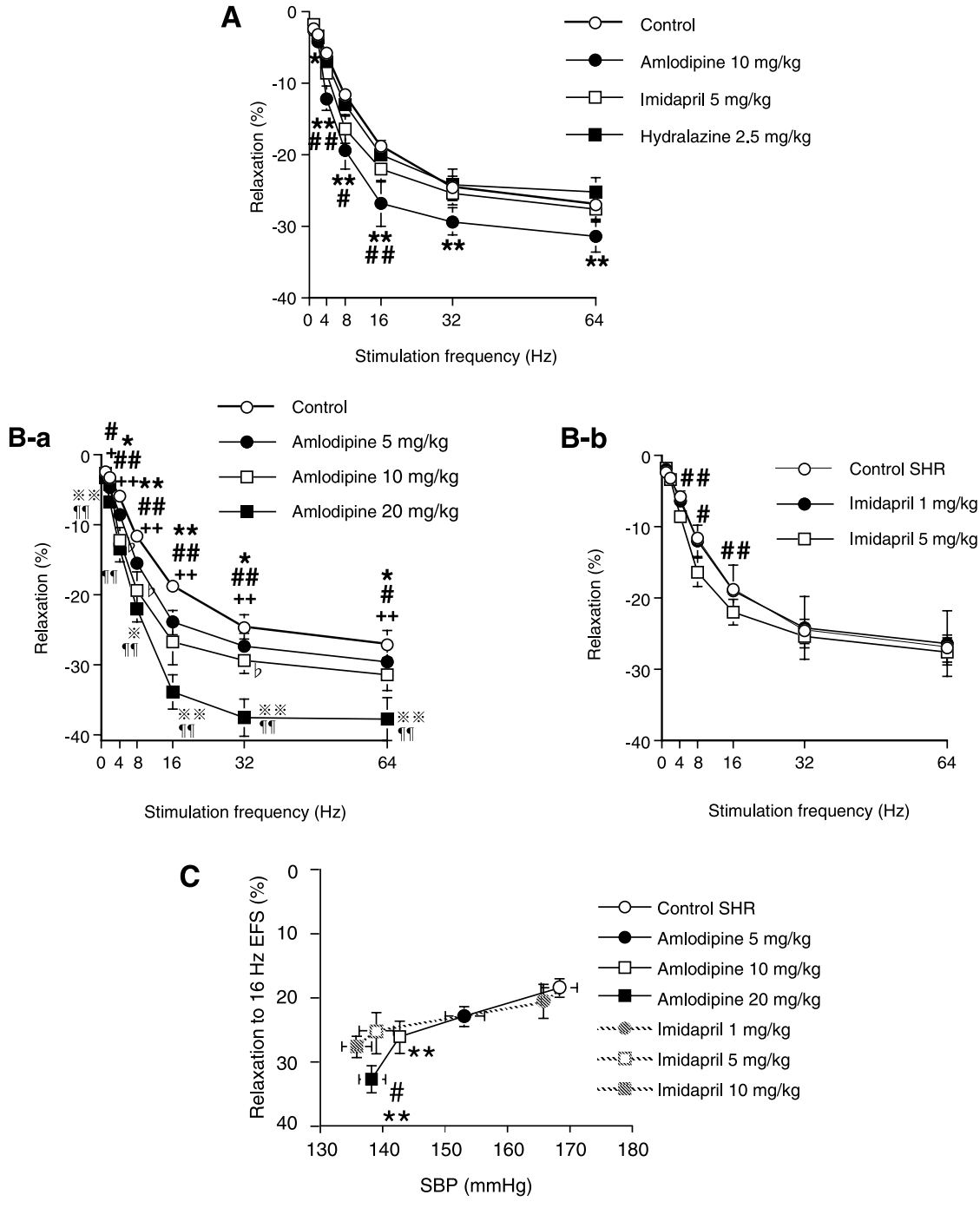

Fig. 3. *Effect of hypotensive drugs on relaxation in response to EFS in the corpus cavernosum of SHR. A: Comparison of the relaxation in response to EFS among controls, amlodipine (10 mg/kg)-, imidapril (5 mg/kg)-, and hydralazine (2.5 mg/day)-treated SHR (*n = *20 each).* *p < *0.05,* **p < *0.01 for amlodipine-treated SHR* vs. *controls.* #p < *0.05,* ##p < *0.01 for imidapril-treated SHR* vs. *controls. B: Comparison among different doses of amlodipine (0, 5, 10 and 20 mg/kg,* n = *10 each) and imidapril (0, 1, 5 and 10 mg/kg,* n = *10 each).* *p < *0.05,* **p < *0.01 for amlodipine (5 mg/kg)-treated SHR* vs. *controls.* #p < *0.05,* ##p < *0.01 for amlodipine (10 mg/kg)- or imidapril (5 mg/kg)-treated SHR* vs. *controls.* +p < *0.05,* ++p < *0.01 for amlodipine (20 mg/kg)-treated SHR* vs. *controls.* ♭p < *0.05 for amlodipine (5 mg/kg)-treated SHR* vs. *amlodipine (10 mg/kg)-treated SHR.* ※p < *0.05,* ※※p < *0.01 for amlodipine (5 mg/kg)-treated SHR* vs. *amlodipine (20 mg/kg)-treated SHR.* ¶¶p < *0.01 for amlodipine (10 mg/kg)-treated SHR* vs. *amlodipine (20 mg/kg)-treated SHR. C: Relationship between systolic blood pressure (SBP) and the relaxation in response to EFS with amlodipine or imidapril treatment.* **p < *0.01 for amlodipine-treated SHR (10 or 20 mg/kg)* vs. *controls.* #p < *0.05 for amlodipine (5 mg/kg)-* vs. *amlodipine-treated SHR (20 mg/kg). Values are reported as the means ± SEM.*

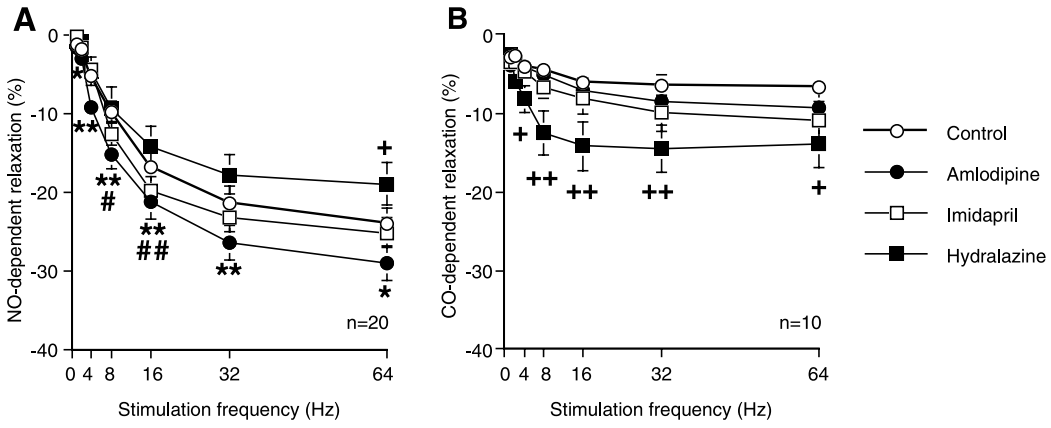

Fig. 4. *Effect of hypotensive drugs on relaxation induced by neuronal NO or CO in the corpus cavernosum of SHR. NO- and CO-dependent relaxation in response to EFS were calculated and are shown for the control, amlodipine (10 mg/kg)-, imidapril (5 mg/kg)- and hydralazine (2.5 mg/day)-treated SHR.* *p < *0.05* **p < *0.01 for amlodipine-treated SHR* vs. *controls.* #p < *0.05,* ##p < *0.01 for imidapril-treated SHR* vs. *controls.* +p < *0.05,* ++p < *0.01 for hydralazine-treated SHR* vs. *controls. Values are reported as the means±SEM.*

Table 2. Amlodipine-Induced Change of Level of Nox and cGMP in Corpus Cavernosum of SHR

	NOx in tissue (nmol/mg wet tissue)		cGMP in tissue (fmol/mg protein)	
	EFS−	EFS+	EFS−	EFS+
Control SHR	0.072±0.004	0.093±0.009#	854±98	890±42
Amlodipine 10 mg/kg	0.082±0.006	0.117±0.007*,#	902±32	1,220±112*,#

EFS+: the electrical field stimulation of 16 Hz was applied to the tissues; EFS−: no stimulation. Values are mean±SEM. $n=8$. *$p<0.05$ *vs*. control SHR. #$p<0.05$ *vs*. EFS−. Nox, nitrate/nitrite; cGMP, guanosine 3′,5′-monophosphate; SHR, spontaneously hypertensive rats.

Table 3. Amlodipine-Induced Change of Level of Thiobarbituric Acid Reactive Substance (TBARS) and Superoxide Dismutase (SOD) Activity in Corpus Cavernosum of SHR

	TBARS		SOD activity	
	Serum (nmol/ml)	Tissue (nmol/g wet tissue)	Serum (U/ml)	Tissue (U/g wet tissue)
Control SHR	2.23±0.11	52.7±3.5	20.1±1.4	261.8±2.9
Amlodipine 10 mg/kg	2.18±0.16	66.0±7.8	19.7±0.5	142.6±5.0**

Electrical field stimulation was not applied to the tissues. Values are mean±SEM. $n=8$. **$p<0.01$ *vs*. control SHR. SHR, spontaneously hypertensive rats.

8 Hz to 16 Hz was increased by amlodipine or imidapril (Fig. 4A; $p<0.05$ or $p<0.01$, $n=20$ each), respectively. NO-dependent relaxation in response to EFS of 64 Hz was decreased by hydralazine (Fig. 4A; $p<0.05$, $n=20$ each). CO-dependent relaxation in response to EFS from 4 Hz to 64 Hz was increased by hydralazine (Fig. 4B; $p<0.05$ or $p<0.01$, $n=10$ each), and was not affected by amlodipine or imidapril.

Effect of Amlodipine on NOx and cGMP Levels in the Corpus Cavernosum

To examine the mechanisms of the improvement of neuronal NO synthesis in the corpus cavernosum of SHR treated with amlodipine, NOx and cGMP levels in the corpus cavernosum were measured. Treatment with amlodipine did not affect the basal level of NOx in the corpus cavernosum, and the level of NOx in the corpus cavernosum was increased by EFS of 16 Hz, as compared with the basal level (Table 2, $p<0.05$). The NOx and cGMP levels in the corpus cavernosum of the amlo-

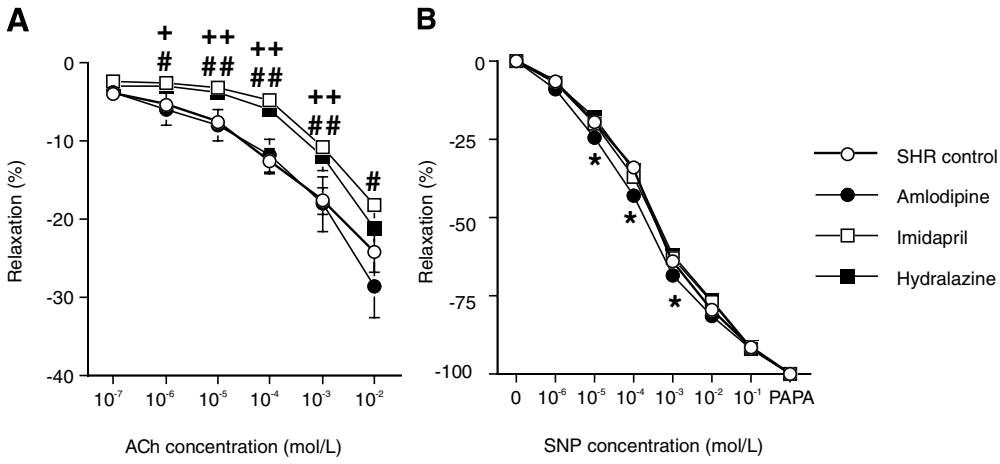

Fig. 5. *Effect of hypotensive drugs on endothelium-dependent or -independent relaxation in the corpus cavernosum of SHR. Dose-response curves of the relaxation in the corpus cavernosum in response to acetylcholine (ACh; A) or sodium nitroprusside (SNP; B) are shown in controls and SHR treated with each drug.* $^{*}p<0.05$ *for amlodipine-treated SHR* vs. *controls.* $^{\#}p<0.05$, $^{\#\#}p<0.01$ *for imidapril-treated SHR* vs. *controls.* $^{+}p<0.05$, $^{++}p<0.01$ *for hydralazine-treated SHR* vs. *controls. Values are reported as the means±SEM.*

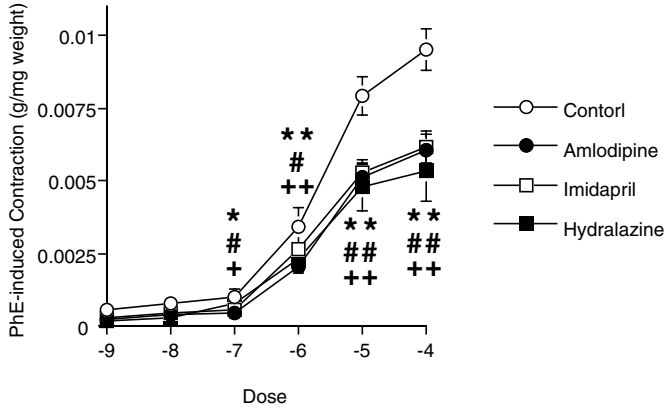

Fig. 6. *Effect of hypotensive drugs on contraction in response to phenylephrine hydrochloride (PhE) in the corpus cavernosum of SHR. Dose-response curves in response to PhE in the corpus cavernosum are shown in controls and SHR treated with each drug.* $^{*}p<0.05$, $^{**}p<0.01$ *for amlodipine-treated SHR* vs. *controls.* $^{\#}p<0.05$, $^{\#\#}p<0.01$ *for imidapril-treated SHR* vs. *controls.* $^{+}p<0.05$, $^{++}p<0.01$ *for hydralazine-treated SHR* vs. *controls. Values are reported as the means±SEM.*

dipine-treated SHR were greater than those of control SHR after EFS (Table 2, $p<0.05$).

Effect of Amlodipine on TBARS Levels and SOD Activity in the Corpus Cavernosum and Serum

To examine mechanisms of the augmentation of the neuronal NO concentration in the corpus cavernosum, we also measured levels of TBARS and SOD activity in the corpus cavernosum and serum. Levels of TBARS in the corpus cavernosum and serum were similar between control SHR and amlodipine-treated SHR (Table 3; $p>0.05$). SOD activity in the corpus cavernosum of the amlodipine-treated SHR was less than that of the control SHR (Table 2; $p<0.01$), but not less than the activity in the serum (Table 3; $p>0.05$).

Effect of Hypotensive Drugs on Relaxation in Response to ACh and SNP in the Corpus Cavernosum

The role of vascular factors in relaxation in response to each hypotensive drug (amlodipine 10 mg/kg body weight, imidapril 5 mg/kg body weight or hydralazine 2.5 mg/day) was examined in the corpus cavernosum strips of SHR. Amlo-

dipine did not affect the relaxation in response to ACh. Imidapril and hydralazine impaired the relaxation in response to ACh (Fig. 5A, $p<0.05$). Relaxation of the corpus cavernosum in SHR in response to SNP was slightly increased only by the treatment with amlodipine (Fig. 5B; $p<0.05$).

Effect of Hypotensive Drugs on PhE-Induced Contraction

Contraction in response to PhE was similarly inhibited by the treatment with each hypotensive drug, as compared with non-treated SHR (Fig. 6; $p<0.05$, $n=10$ each).

Discussion

Penile erection is impaired not only in the elderly and diabetics, but also in hypertensives (*2*). It was recently demonstrated that impaired relaxation in the corpus cavernosum of SHR might result from an impaired neuronal NO- and CO-dependent relaxation in the corpus cavernosum (*5*, *17*). In the present study, we examined the effect of treatment with hypotensive agents on impaired relaxation in the corpus cavernosum of SHR. EFS-induced relaxation and NO-dependent relaxation in the corpus cavernosum of SHR, which were measured under circumstances that excluded the possibility of ACh-induced NO release from the endothelium by atropine, was increased by amlodipine or imidapril. Further, the relaxation in response to EFS was abolished by treatment with TTX. These results indicate that neurogenic relaxation in the corpus cavernosum is improved by treatment with amlodipine or imidapril. This improvement in the relaxation results from augmented neuronal NO synthesis and/or release in the corpus cavernosum, because NO-dependent relaxation was increased by the treatment with amlodipine and imidapril, as compared with the level in control SHR. After treating with imidapril, relaxation in response to EFS was increased in a dose-dependent manner, but in an SBP-independent manner. Therefore, the improvement in neurogenic relaxation may result from a specific action of imidapril, rather than from the effect of imidapril on blood pressure. Neurogenic relaxation of the corpus cavernosum was improved in a dose-dependent manner and in an SBP-dependent manner by the treatment with amlodipine. Furthermore, the relaxation was much greater in the amlodipine-treated SHR than in the imidapril-treated SHR, although the blood pressure was lowered to the same level by both treatments. Therefore, this improvement in neurogenic relaxation may result not only from a blood pressure–lowering effect, but also from the specific action of amlodipine. On the other hand, hydralazine did not change the relaxation in response to EFS, but did inhibit NO-dependent relaxation in response to EFS of 64 Hz. Therefore, treatment with hydralazine may suppress the neuronal NO system in the corpus cavernosum. It has been reported that neuronal relaxing factors exhibit different properties at different frequencies of EFS (*5*). Thus it is possible that any of the individual components of penile erection of the rat are inhibited by hydralazine *via* an impairment of NO-induced relaxation. We also examined the effect of treatment with hypotensive agents on another neuronal relaxing system, the CO-related system, in the corpus cavernosum of SHR. CO-dependent relaxation in the corpus cavernosum of SHR was increased by hydralazine. This result may be partially due to the hydralazine-induced suppression of NO-dependent relaxation in the tissues. And this action of hydralazine may be specific, because neither amlodipine nor imidapril affected the CO-dependent relaxation in the tissues.

To clarify the specific action of amlodipine on EFS-induced relaxation in SHR, we examined the mechanisms of the improvement of neuronal NO synthesis and/or release in the corpus cavernosum by amlodipine. We found that amlodipine did not affect the base level of NOx, which was increased by EFS. The increased NOx level and cGMP level in the corpus cavernosum were greater in amlodipine-treated SHR than in control SHR. These results suggest that amlodipine improves neurogenic relaxation of the corpus cavernosum in SHR through augmented neuronal NO synthesis and/or release. In addition, increased relaxation of the corpus cavernosum muscle may have played some role in the augmentation of the NO-induced activation of soluble guanylate cyclase, because the relaxation of the corpus cavernosum in response to SNP was slightly increased by the treatment with amlodipine. It is well known that hypertensive patients have increased oxidative stress levels and that increased oxidants trap NO, resulting in an impairment of the endothelial function of blood vessels (*10*). In our previous study, we demonstrated that increased oxidative stress in SHR suppressed the bioavailability of NO, resulting in ED through a reduction of the relaxation induced by NO from neurons in the corpus cavernosum of SHR (*5*). Therefore, antioxidant therapy may improve the relaxing function in the corpus cavernosum of SHR. We examined the effect of amlodipine on oxidative stress using TBARS and SOD activity as markers, because dihydropyridine calcium channel blockers, such as amlodipine, have been reported to suppress oxidative stress in hypertensive patients (*9*). In the present study, TBARS levels in serum and tissue were not decreased by the treatment with amlodipine, while SOD activity in the tissue was suppressed, suggesting that the amlodipine-induced improvement of the relaxation in the corpus cavernosum may not be attributable to an amlodipine-induced decrease in oxidative stress. This result conflicts with our previous result. Thus the improvement of the relaxation in the corpus cavernosum by amlodipine may not be due to a specific action in SHR. Recently, it was reported that the level of asymmetrical dimethyl arginine (ADMA), as an endogenous NOS inhibitor, was increased in hypertensive subjects (*18*). It is necessary to examine whether amlodipine inhibits ADMA in the corpus cavernosum of SHR. Okamura *et al*. reported that addition of amlodipine to the medium of an organ bath impaired the relaxation in the corpus cavernosum of canines in response to EFS (*19*).

Although the exact reason for this discrepancy is not yet known, it might be due to differences in the species and treatment periods. In fact, there are also conflicting reports in humans, with one study showing that amlodipine did not affect sexual function (*20*), and another reporting that the drug increased sexual symptom distress (*21*).

In the present study, we examined endothelial function in the corpus cavernosum of hypotensive drug–treated SHR, because ACE inhibitors (*8*) and calcium antagonists have been reported to improve endothelial function through an enhanced action of NO released from the endothelium of blood vessels in dogs and hypertensive humans (*7*, *22*, *23*). We demonstrated that the relaxation of corpus cavernosum tissue in response to ACh was suppressed by treatment with imidapril or hydrazine, and was not affected by treatment with amlodipine. These results indicate that the improvement of the relaxation in response to EFS in the amlodipine- and imidapril-treated SHR does not result from a change of endothelial function in the corpus cavernosum, even though the tissues were not atropinized.

We demonstrated that the contraction in response to PhE was similarly impaired by 4-week treatment with either amlodipine, imidapril or hydralazine. The noradrenergic neuron system of the corpus cavernosum is thought to control the detumescence of penile erection (*24*). Therefore, long-term treatment with hypotensive drugs may inhibit α1-adrenoceptor function of the corpus cavernosum, thereby inhibiting the detumescence of erection. Thus, treating hypertension with the drugs used in the present study might be beneficial for ED through the inhibition of detumescence of erection by mechanisms other than enhancement of NO synthesis and/or release.

In summary, impaired relaxation in the corpus cavernosum of SHR was improved by the treatment with amlodipine or imidapril in the present study. The mechanisms of this improvement by amlodipine or imidapril were related to an augmented neuronal NO synthesis and/or release, but not to improved endothelial function. Hydralazine improved the function of the neuronal CO system, suppressed the NO system and did not affect relaxation in the corpus cavernosum of SHR. We conclude that amlodipine and imidapril may be helpful in treating the ED associated with hypertension.

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