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Effects of Calcium Channel Blockade on the Aortic Intima in Spontaneously Hypertensive Rats

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Hypertension is associated with an intimal dysfunction characterized by endothelium-dependent constriction to serotonin, decreased endothelium-dependent relaxation to acetylcholine, and a subendothelial infiltration of monocyte-macrophages. The goal of our study was to evaluate the effect of long-term calcium channel blockade with Ro 40-5967, a new long-acting calcium channel blocker, on these alterations in aortas of spontaneously hypertensive rats (SHR). Arterial blood pressure was decreased by Ro 40-5967. In aortas from Ro 40-5967–treated SHR, the serotonin ratio (maximal contraction to serotonin on rings with endothelium over maximal contraction on paired rings without endothelium) was reduced (1.14±0.10) compared with control SHR (1.72±0.12, $P<.01$) because of inhibition of maximal contraction in rings with endothelium. This effect of Ro 40-5967 was partially reversed by an inhibitor of nitric oxide (NO) synthase, N^G-nitro-L-arginine-methyl ester, and partially inhibited in the presence of the thromboxane/prostaglandin H_2 receptor antagonist AH 23848. Maximal relaxation to acetylcholine in rings with endothelium was increased by Ro 40-5967. In rings without endothelium, Ro 40-5967 treatment enhanced the sensitivity to sodium nitroprusside–induced relaxation. Cyclic GMP content, an indicator of NO release, was not increased in aortas from Ro 40-5967–treated SHR. Thus, improvement of endothelial function was probably achieved by facilitating the action of NO at the level of the smooth muscle cells and by reducing prostaglandin H_2–induced constriction. Finally, the number of monocyte-macrophages in the subendothelium was decreased by Ro 40-5967. We conclude that long-term treatment with Ro 40-5967 reverses both the functional and morphological changes of the aortic intima in hypertension. (*Hypertension*. 1993;22:569-576.)

KEY WORDS • **endothelium** • **serotonin** • **calcium channel blockers** • **nitric oxide** • **guanosine cyclic monophosphate** • **prostaglandins H** • **rats, inbred SHR**

The intima of arteries from spontaneously hypertensive rats (SHR), consisting of an endothelium and subendothelial tissue, can be distinguished from that of arteries from Wistar-Kyoto rats both morphologically and functionally. Arteries from hypertensive rats exhibit a thickened intima that is due to both increased migration of blood-borne mononuclear cells and increased deposition of extracellular material.[1-5] Functionally, whereas a normal endothelium favors relaxation and contributes to maintenance of vessel patency, the SHR endothelium has an overall proconstricting influence. This is partly due to the release of constricting factor or factors, including the unstable prostaglandin endoperoxide PGH_2,[6,7] by SHR but not Wistar-Kyoto rat aorta. However, a deficiency in either the release or action of one or more endothelium-derived relaxing factors (EDRFs) also contributes. Relaxant responses to several endothelium-dependent dilators are blunted, although the ability of the underlying smooth muscle of SHR to relax does not seem impaired relative to Wistar-Kyoto arteries.[8-10] Previous studies from this laboratory have suggested that this functional imbalance may be associated with the morphological abnormalities of the endothelium and subendothelium that characterize arteries from SHR.[11,12] Endothelial cells are generally considered to be the source of vasoconstrictor factor in SHR arteries. However, the possibility that monocyte-macrophages in the subendothelium also contribute to constrictor factor production and to intimal dysfunction cannot be excluded. Alternatively, the invasion of mononuclear cells could be a consequence of the imbalance in relaxing and constricting factors produced by the endothelium. Whatever the mechanism may be, the changes in endothelial function demonstrated in experimental models are relevant to the pathology of clinical hypertension, because abnormal endothelial responses have also been demonstrated in human essential hypertension.[13,14]

Because intimal dysfunction in hypertension may contribute to increased peripheral vascular resistance, drugs that would improve endothelium-dependent relaxation and normalize intimal morphology might have advantages over conventional antihypertensive treatment. We have recently shown that angiotensin-converting enzyme inhibitors, which can increase EDRF release[15-18] and improve endothelial dysfunction in

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SHR,[19] can also prevent and reverse subendothelial monocyte-macrophage infiltration.[11,12]

The goal of the present study was to evaluate the consequences of long-term calcium channel blockade on the function and morphology of the intima in SHR. Ro 40-5967 is a novel, potent, and long-lasting calcium antagonist[20] that has been shown to be effective in several models of hypertension.[21] An advantage of this drug for long-term treatment is that it acts for more than 24 hours after oral administration in rats, which is not the case for most of the other calcium channel blockers. Endothelial function was evaluated in SHR treated for 4 weeks with Ro 40-5967 by studying modulation of serotonin-induced contraction by the endothelium, as well as endothelium-dependent and -independent relaxation. In addition, the aortic cyclic GMP (cGMP) content was measured to assess the influence of Ro 40-5967 on the basal release of nitric oxide (NO) in SHR. These functional and biochemical studies were accompanied by morphological evaluation of the effect of Ro 40-5967 on the structure of the subendothelium of SHR aorta.

Methods

Animals and Experimental Design

Two separate studies were performed using male 10- to 12-week-old SHR (Füllinsdorf, Switzerland). In the first study, SHR (12 control rats and 12 treated with Ro 40-5967) were allocated for either functional or morphological study of their aortas. In the second study (12 control rats and 15 treated with Ro 40-5967), segments of aorta from the same SHR were prepared for further functional studies or for determination of cGMP content. In both studies, age-matched SHR were fed for 4 weeks either a standard diet or a diet mixed with Ro 40-5967 (approximately 30 mg/kg per day). All animals had free access to water. Body weight, blood pressure, and heart rate (monitored indirectly using the tail-cuff method) were measured weekly. Experiments were conducted according to the "Position of the American Heart Association on Research Animal Use" adopted November 11, 1984, by the American Heart Association.

Evaluation of Endothelial Function on Isolated Arterial Rings

At the end of the treatment regimen, the animals were killed, and the descending thoracic aortas were dissected free, cleaned, and cut into 5-mm rings. Some rings were left with intact endothelium and some were denuded of endothelium by gentle rubbing of their intimal surface. The rings were suspended under 3 g resting tension in 10-mL organ chambers containing gassed (95% O_2–5% CO_2) and warmed (37°C) Krebs-Henseleit solution of the following composition (mmol/L): NaCl, 115; KCl, 4.7; $MgSO_4$, 1.2; $KHPO_4$, 1.5; $NaHCO_3$, 25; $CaCl_2$, 2.5; and glucose, 10. The rings were connected to force transducers (Swena, Stockholm, Sweden), and isometric tension was recorded (Linearcorder mark VII, Graphtec Corp, Tokyo, Japan, or Hellig 112 037, Hellig AG, Germany).

After a 1-hour equilibration period, the rings were contracted with norepinephrine (10^{-6} mol/L). The absence or presence of endothelium was verified by the absence or presence of relaxation on further addition of acetylcholine (10^{-6} mol/L). The rings were then washed and retensioned if necessary until a stable baseline tension was obtained.

For determination of endothelium-dependent and -independent relaxation, rings were constricted with equieffective concentrations of serotonin. When a stable tension was obtained, acetylcholine (10^{-9} to 10^{-4} mol/L) or sodium nitroprusside (SNP, 10^{-10} to 10^{-5} mol/L) was added cumulatively to rings with or without endothelium, respectively. The EC_{50} was calculated as the concentration that evoked 50% of the maximum relaxation of serotonin-contracted arteries.

The rings were rewashed for 1 hour or until a stable baseline tension was again obtained. The constricting effect of serotonin was then evaluated on paired rings (one with and one without endothelium from the same aorta) by addition of cumulative doses of serotonin (10^{-8} to 10^{-4} mol/L). To investigate the role of endothelium-derived NO, we studied a second pair of rings in parallel in the presence of the inhibitor of NO synthesis N^G-nitro-L-arginine-methyl ester (L-NAME, 300 μmol/L). L-NAME was added 10 minutes before the concentration-response curve to serotonin was begun. The contribution of the endothelium-derived contracting factor PGH_2 to serotonin-induced contraction was investigated with the thromboxane/PGH_2 antagonist AH 23848.[22] AH 23848 (10 μmol/L) or its vehicle (10 μL dimethyl sulfoxide; final bath concentration, 0.1%) was added to pairs of rings 10 minutes before serotonin concentration-response curves.

The EC_{50} was calculated as the concentration of serotonin that caused half-maximal constriction. In addition, functional alteration was quantified using the "serotonin ratio" (maximal contraction to serotonin on isolated arterial rings with endothelium over maximal contraction on paired rings without endothelium), which decreases as the endothelial function normalizes and the balance is shifted in favor of relaxing factors.

Cyclic GMP Determination

Two 7-mm rings of each aorta from the second group of Ro 40-5967–treated or control rats were kept aside after preparation of the aortas for functional studies. The rings (one with intact endothelium and one denuded of endothelium by rubbing) were incubated for 90 minutes in Krebs-Henseleit solution at 37°C as described above. The purpose of the incubation period was to remove any influence of atrial natriuretic peptide (a stimulant of particulate guanylate cyclase in smooth muscle cells) circulating in vivo in the SHR on cellular cGMP content measured ex vivo. At the end of the 90-minute incubation period, the tissues were rapidly frozen with liquid nitrogen and then stored at −80°C until assayed for cGMP content.

The tissues were homogenized at 4°C in 1.0 mL of 6% trichloroacetic acid, sonicated for 10 seconds, and then centrifuged at 2500*g* for 15 minutes. Supernatants were extracted with 4 vol of water-saturated diethyl ether. The ether phase was discarded, and the samples were evaporated to dryness and then resuspended in sodium acetate buffer. Cyclic GMP was determined in the acetylated samples with a commercially available radioimmunoassay (New England Nuclear, Dreieich, Germany). The protein contents of the samples were measured according to Lowry et al[23]; cGMP content is expressed as femtomoles cGMP per milligram of protein.

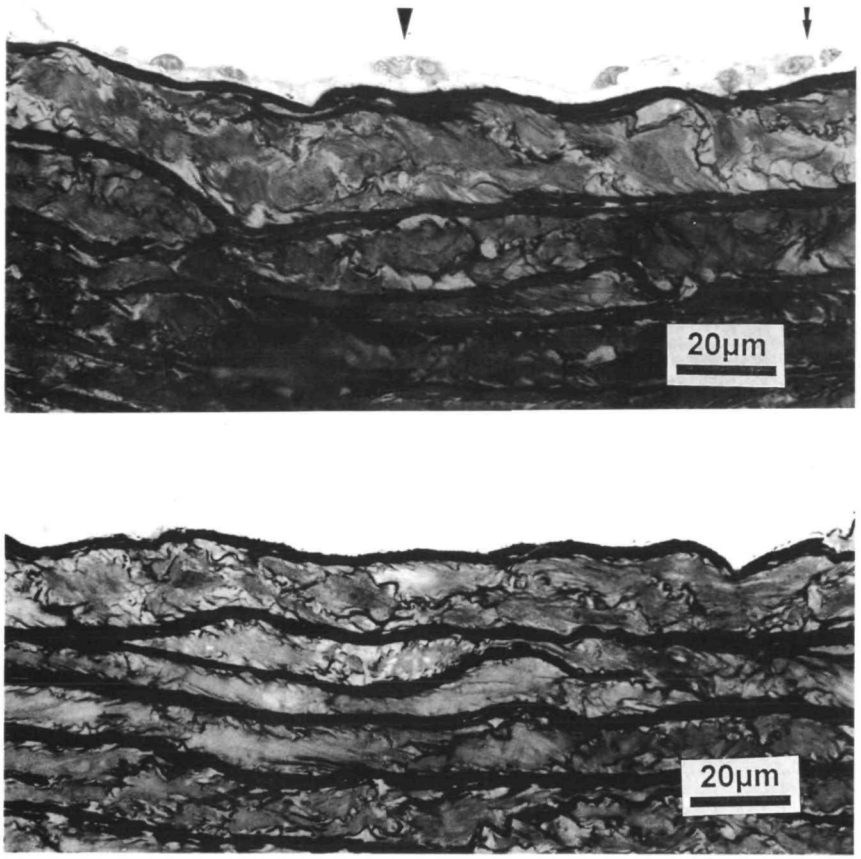

FIG 1. *Light micrographs show spontaneously hypertensive rat aorta before (top) and after (bottom) rubbing of intimal surface to remove endothelium. Note in the top panel the presence of endothelial cells (arrowhead) and monocyte-macrophages (arrow). In the bottom panel, both of these cell types are absent; rubbing resulted in complete removal of endothelium and subendothelium.*

Morphological Evaluation of Monocyte-Macrophage Infiltration on Whole Arteries

Infiltration of the subendothelium with monocyte-macrophages was evaluated using morphometry as previously described.[11,12] Briefly, anesthetized rats were perfused via a cannula implanted into the left ventricle with 5 mL Krebs-Henseleit solution containing 10^{-5} mol/L adenosine and then with 2.5% glutaraldehyde buffered with 0.1 mol/L phosphate buffer (pH 7.4, room temperature). Arterial segments were removed, fixed in 2.5% sodium cacodylate (0.1 mol/L)–buffered glutaraldehyde (pH 7.4), dehydrated with ethanol, fixed in epoxy resin, then cut into semithin sections (1 μm) and stained with toluidine blue and basic fuchsin. One whole section of good technical quality was randomly chosen for morphometric evaluation. For morphometry, the cross-sectional area of the subendothelium, the thickness of the subendothelium, the length of the internal elastic lamina, and the number of subendothelial cell nuclei (as an index of the number of monocyte-macrophages) were measured by light microscopy with the computerized morphometry system DIASYS (Datalab, Thörigen, Switzerland) connected to an IBM AT03.

Another set of experiments was performed in isolated SHR aortic rings to assess both the efficiency of the intimal rubbing on endothelium removal and the effect of rubbing on intimal monocyte-macrophage content. Briefly, the intimal surface of aortic rings from anesthetized SHR was either rubbed using a metal rod or left intact; the rings were then fixed, sectioned, and stained as described above. Analysis of the sections (representative photomicrographs in Fig 1) shows that rubbing of the intimal surface removed both the endothelium and the subendothelial monocyte-macrophages.

Drugs and Preparation

Ro 40-5967 was synthesized at F. Hoffmann–La Roche Ltd, Basel, Switzerland. Norepinephrine hydrochloride (Fluka) was stored as a stock solution (10^{-1} mol/L) in 20% HCl and diluted as required in a solution containing 0.9% NaCl and 1% ascorbate. Acetylcholine chloride (Dispersa) and SNP (Nipride, F. Hoffmann–La Roche) were stored frozen as stock solutions (10^{-2} mol/L) in water and diluted in Krebs-Henseleit solution as required. Serotonin hydrochloride and L-NAME (both from Sigma) were prepared freshly each day in Krebs-Henseleit solution. AH 23848 was synthesized de novo at F. Hoffmann–La Roche and was prepared daily (10^{-2} mol/L) in dimethyl sulfoxide.

Statistical Analysis

Comparisons of morphometric parameters, acetylcholine- and SNP-induced relaxation, and serotonin-induced constriction (individual doses, serotonin ratios, EC_{50} values, and maximum contractions of serotonin concentration-response curves) between Ro 40-5967–treated and control rats were made using the Student's unpaired *t* test. Comparisons of cGMP contents and the effects of L-NAME and AH 23848 on contractions induced by serotonin (serotonin ratios, EC_{50} values, and maximum

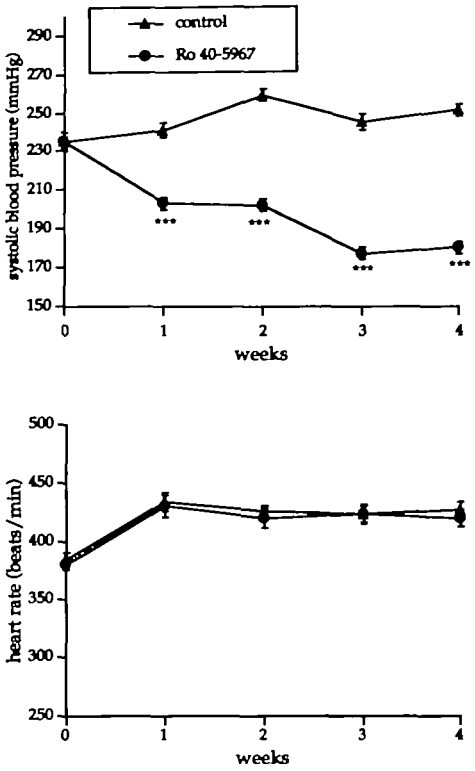

FIG 2. *Line graphs show evolution of systolic blood pressure (top) and heart rate (bottom) of spontaneously hypertensive rats fed a standard diet (control, n=24) or a diet containing Ro 40-5967 (approximately 30 mg/kg per day, n=27) over 4 weeks.* ***P<.001 *vs control group fed standard diet.*

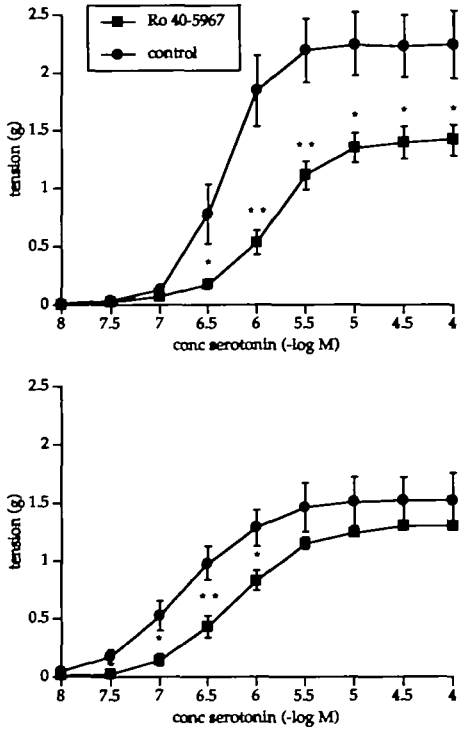

FIG 3. *Line graphs show concentration-response curves for serotonin applied to aortic rings with (top) and without (bottom) endothelium from spontaneously hypertensive rats treated (n=6) or not (n=6) for 4 weeks with Ro 40-5967.* *P<.05, **P<.01 *vs corresponding control. conc, Concentration.*

contractions of serotonin concentration-response curves) in Ro 40-5967–treated and control rats were made using two-way analysis of variance followed by a Newman-Keuls test.[24] A level of $P<.05$ was considered significant. All results are expressed as mean±SEM.

Results

In both studies, systemic blood pressures, heart rates, and body weights were comparable over the 4-week treatment period for the Ro 40-5967–treated and control SHR. Therefore, the results of the two studies were pooled. Systemic blood pressure was significantly decreased in SHR treated with Ro 40-5967 compared with controls from the first week to the end of treatment (Fig 2), and heart rate was unaffected by Ro 40-5967 over the full 4 weeks (Fig 2). The body weights of SHR receiving Ro 40-5967 (301±3 g, n=27) were not significantly different from those of control SHR (295±4 g, n=24) at the end of the treatment period.

Serotonin-Induced Contractions

In both studies, concentration-response curves of serotonin in aortic rings from Ro 40-5967–treated SHR were shifted to the right in the presence and absence of endothelium compared with controls (Fig 3). Maximum contractions to serotonin were reduced in vessels with but not without endothelium from Ro 40-5967–treated relative to control SHR. This resulted in a significant decrease in the serotonin ratio from 1.72±0.12 in the control group to 1.14±0.1 in the Ro 40-5967–treated group ($P<.01$).

Effect of L-NAME on Serotonin-Induced Contraction

L-NAME (300 μmol/L) significantly increased maximum contractions to serotonin in rings with endothelium from SHR treated with Ro 40-5967 but not from control SHR and had no effect in endothelium-denuded rings from either group. Consequently, the serotonin ratio was significantly ($P<.05$) increased by L-NAME in aortas from Ro 40-5967–treated but not from control SHR (Fig 4). In contrast, the sensitivity of aortas with endothelium from both control and Ro 40-5967–treated SHR to serotonin was increased by L-NAME (EC_{50} of serotonin reduced from 0.51±0.09 to 0.18±0.06 μmol/L, $P<.02$, and 1.2±0.2 to 0.45±0.04 μmol/L, $P<.01$, by L-NAME in control and Ro 40-5967–treated SHR, respectively).

Effect of AH 23848 on Serotonin-Induced Contraction

Serotonin-induced contractions in aortas from both Ro 40-5967–treated and control SHR were inhibited in the presence of AH 23848 (10 μmol/L) compared with its solvent dimethyl sulfoxide. The serotonin ratio was reduced by AH 23848 from 1.43±0.06 to 1.18±0.12 ($P<.01$) in rings from control SHR and from 1.11±0.09 to 0.93±0.03 ($P<.05$) in Ro 40-5967–treated SHR because of reduction of endothelium-dependent contraction. This resulted in the EC_{50} for serotonin being increased by AH 23848 from 0.26±0.06 to 0.88±0.17 μmol/L ($P<.01$) in intact rings from control SHR and

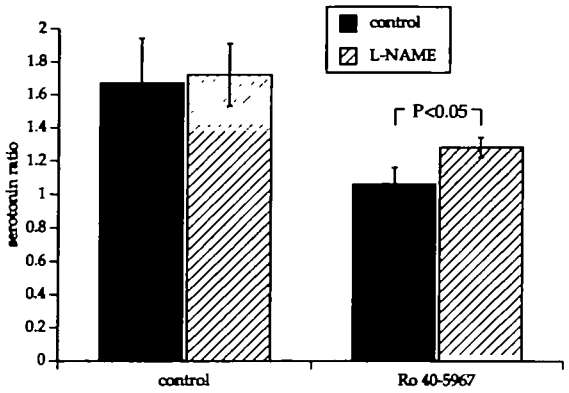

FIG 4. *Bar graph shows effect of 10 minutes of preincubation with N^G-nitro-L-arginine-methyl ester (L-NAME) (300 μmol/L) on serotonin ratio (ratio of maximal tension to serotonin in rings with endothelium to rings without endothelium) in aortic rings from spontaneously hypertensive rats fed for 4 weeks a standard diet (control, n=5) or a diet containing Ro 40-5967 (approximately 30 mg/kg per day, n=5).*

from 0.69±0.14 to 1.7±0.19 μmol/L ($P<.01$) in intact rings from Ro 40-5967–treated SHR.

Acetylcholine-Induced Relaxation

Aortic rings with endothelium were submaximally precontracted to 1.1±0.1 g for control SHR and 1.0±0.1 g for SHR treated with Ro 40-5967. The concentrations used were 1.3±1.0 and 2.8±0.3 μmol/L serotonin, respectively. In control rings, acetylcholine caused concentration-dependent relaxation that was followed by contraction at concentrations higher than 0.3 μmol/L (Fig 5). This latter response to acetylcholine was absent in rings from Ro 40-5967–treated rats, resulting in an increase in the maximal acetylcholine-induced relaxation relative to control rings.

Sodium Nitroprusside–Induced Relaxation

Aortic rings without endothelium were submaximally precontracted to 1.0±0.1 g for control SHR and 0.9±0.1 g for SHR treated with Ro 40-5967. The concentrations used were 2.6±1.5 and 2.5±0.2 μmol/L serotonin, respectively. The relaxation induced by SNP was significantly enhanced (EC_{50} reduced to 2.4±0.2 nmol/L from 6.0±0.6 nmol/L, $P<.001$) in aortas from SHR treated with Ro 40-5967 compared with control SHR (Fig 6).

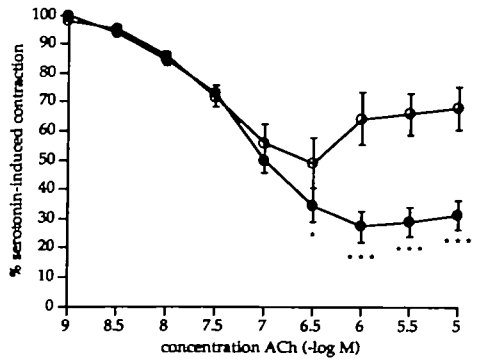

FIG 5. *Line graph shows concentration-response curves of acetylcholine (ACh) administered to endothelium-intact serotonin-precontracted aortic rings from spontaneously hypertensive rats fed for 4 weeks a standard diet (control, n=8, ○) or a diet containing Ro 40-5967 (approximately 30 mg/kg per day, n=11, ●). *P<.05, ***P<.001, compared with corresponding control.*

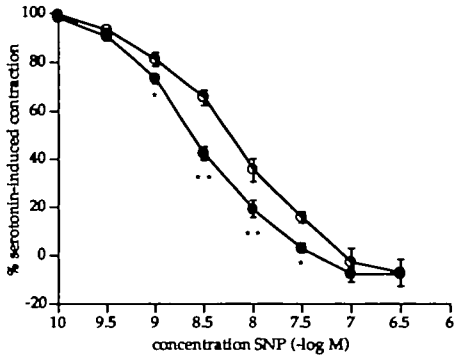

FIG 6. *Line graph shows concentration-response curves of sodium nitroprusside (SNP) administered to endothelium-denuded serotonin-contracted aortic rings from spontaneously hypertensive rats fed for 4 weeks a standard diet (control, n=8, ○) or a diet containing Ro 40-5967 (approximately 30 mg/kg per day, n=11, ●). *P<.05, **P<.01, compared with corresponding control.*

Cyclic GMP Content

The aortic cGMP content (Table 1) was significantly higher in the presence than in the absence of endothelium for both control and Ro 40-5967–treated rats. However, Ro 40-5967 had no significant effect on cGMP content, in either the presence or absence of endothelium compared with controls.

Morphological Evaluation

In agreement with earlier studies,[11] SHR aortas were characterized by a thick subendothelium clearly separating endothelial cells from the internal elastic lamina, which contained a large number of cell nuclei indicative of invading monocyte-macrophages. Both the thickness of the subendothelium and the number of cell nuclei counted in the subendothelium were significantly reduced in aortas from SHR treated with Ro 40-5967 compared with those from controls, whereas the length of the internal elastic lamina was unaffected (Table 2).

TABLE 1. Effect of Ro 40-5967 on Cyclic GMP Content of Aortas From Spontaneously Hypertensive Rats

cGMP Content, fmol/mg protein	Control	Ro 40-5967
Without endothelium	5.53±1.47	2.47±0.39*
With endothelium	11.50±2.31†	11.17±1.69*‡

Cyclic GMP (cGMP) was determined in aortas, with and without endothelium, from spontaneously hypertensive rats fed for 4 weeks a standard diet (control) or a diet supplemented with Ro 40-5967 (approximately 30 mg/kg per day, n=8 per group).
*Not significantly different from corresponding control group.
†$P<.05$, ‡$P<.01$, compared with corresponding group without endothelium.

TABLE 2. Morphological Evaluation of the Effect of Ro 40-5967 on Monocyte-Macrophage Infiltration

Variable	Control	Ro 40-5967
Length of IEL, mm	5.1±0.1	5.1±0.1
Area of SE, mm²	5.3±0.9	0.2±0.1*
Average thickness of SE, μm	2.3±0.1	0.8±0.3*
No. SE cells/mm IEL	6.1±0.8	2.7±0.6†

IEL indicates internal elastic lamina; and SE, subendothelium. Monocyte-macrophage infiltration was assessed by the number of cell nuclei in the subendothelium of spontaneously hypertensive rat aorta (n=8 per group).
*$P<.001$, †$P<.01$, compared with corresponding control.

Discussion

This study shows that reduction of blood pressure in SHR by long-term calcium channel blockade with Ro 40-5967 is accompanied by improvement of endothelial function and prevention of monocyte-macrophage infiltration into the aortic subendothelium.

The improvement of intimal dysfunction by Ro 40-5967 was reflected in both decreased endothelium-dependent contraction to serotonin and increased maximal relaxation to acetylcholine. Sensitivity to serotonin was reduced in rings both with and without endothelium from SHR treated with Ro 40-5967 relative to control SHR. Thus, the effect of Ro 40-5967 on sensitivity to serotonin is likely due to inhibition of the direct contraction of smooth muscle by serotonin. In contrast, maximal contraction to serotonin was reduced by Ro 40-5967 in rings with intact endothelium but not in rings that had been rubbed to remove the endothelium, resulting in a marked reduction of the serotonin ratio in Ro 40-5967–treated SHR. This suggests an additional interaction of the calcium antagonist, with serotonin-induced contraction dependent on the presence of an intact vascular intima. The site of this interaction could be the endothelium, the subendothelial monocyte-macrophages removed along with the endothelium, or the smooth muscle cell. It could involve an increased release of an EDRF such as NO from the endothelium, macrophages, or both; an increased smooth muscle cell sensitivity to NO; or a decrease in the release of or sensitivity to a constricting factor. To evaluate whether the effect of Ro 40-5967 was due to an interaction with the release or the action of NO, we examined the effect of the inhibitor of NO synthesis, L-NAME,[25] on the contractions induced by serotonin in control SHR and after treatment with Ro 40-5967.

L-NAME increased the sensitivity to serotonin in rings with endothelium from both control and Ro 40-5967–treated SHR. This observation is consistent with inhibition of release of NO from the SHR endothelium, resulting in decreased opposition to contraction induced by serotonin, endothelium-derived constricting factor, or both. Maximal contractions to serotonin in rings with endothelium from Ro 40-5967–treated SHR were also increased by L-NAME but were not affected in rings from control SHR. Because serotonin-induced contraction was not altered in rings without endothelium, the serotonin ratio was increased by L-NAME in Ro 40-5967–treated but not in control SHR. The fact that L-NAME partially reversed the effect of Ro 40-5967 on the serotonin ratio in SHR strongly suggests that Ro 40-5967 interferes with NO, either by increasing its formation or liberation, by increasing its transport to the media, or by increasing the responsiveness of the smooth muscle cells to NO.

Formation of NO is a calcium-dependent process, calcium entering the cells via receptor-operated, stretch-operated, and Ca^{2+}-activated calcium channels (for review, see Reference 26). Activators of voltage-operated calcium channels can elicit NO release in perfused arteries,[27] but blockers of these channels do not generally inhibit agonist-stimulated EDRF release.[28-31] In agreement, acetylcholine-induced relaxation was not inhibited in rings from Ro 40-5967–treated rats. On the other hand, endothelium-dependent relaxation was elicited by a dihydropyridine calcium antagonist, S 11568,[32] but the mechanism was thought to be distinct from the calcium channel–blocking properties of the compound. In the present study, Ro 40-5967 did not increase cGMP in aortas from SHR, suggesting that neither the basal synthesis of NO nor its transport to the medial smooth muscle from the endothelium nor activity of the guanylate cyclase enzyme responsible for cGMP formation is increased as a result of long-term treatment with Ro 40-5967. However, we cannot rule out the possibility that acetylcholine- or serotonin-induced NO release was increased by Ro 40-5967 treatment. In a recent study it was found that acute application of Ro 40-5967 to isolated canine arteries caused endothelium-dependent relaxation and enhanced relaxation induced by other stimulants of EDRF release.[33]

SNP elicits relaxation by diffusing into the smooth muscle cell and releasing NO.[34] SNP-induced relaxation was increased in rings without endothelium from Ro 40-5967–treated compared with control SHR. Thus, it seems that chronically administered Ro 40-5967 may improve endothelial function by increasing the responsiveness of the smooth muscle cells to NO. The first step in NO-induced relaxation is stimulation of soluble guanylate cyclase to produce cGMP.[35,36] It is unlikely that Ro 40-5967 acts at this level, because, as discussed above, alteration of guanylate cyclase activity would also have influenced the cGMP produced in response to basally released NO. A more likely mechanism by which Ro 40-5967 interacts with NO in smooth muscle cells is directly related to its calcium antagonistic properties. cGMP brings about relaxation by reducing the availability of calcium to the contractile proteins through various means, including inhibition of calcium influx via receptor- and voltage-operated channels.[37] Ro 40-5967, by simultaneously reducing the influx of extracellular calcium, may act by promoting the relaxant effect of NO.

Such a mechanism could also account for enhancement of maximal relaxation to the endothelium-dependent vasodilator acetylcholine in aorta from Ro 40-5967–treated SHR. However, because both acetylcholine[38,39] and serotonin[8,40-42] also induce the release of a contracting factor, probably PGH_2, from SHR aorta, an additional effect of Ro 40-5967 on this pathway cannot be excluded. In fact, the experiments conducted with the thromboxane/PGH_2 receptor antagonist AH 23848[22] are in favor of this. Reduction of serotonin-induced contraction by AH 23848 was less in aortas from Ro 40-5967–

treated rats than in controls, suggesting that the contribution of PGH_2 to the contraction induced by serotonin was already partially inhibited by Ro 40-5967. Ro 40-5967 could inhibit PGH_2-induced contraction through an action in the smooth muscle either by directly inhibiting PGH_2-induced contraction or by amplifying the effect of NO. Inhibition of the contractile factor alone cannot account for the final effect of Ro 40-5967 on serotonin-induced contraction. Even in the presence of AH 23848, contractions elicited by serotonin were less in aortas from Ro 40-5967–treated than those from control SHR.

Thus, the favorable effect of Ro 40-5967 on serotonin-induced contraction probably results from enhancement of the relaxant effect of NO and concurrent inhibition of PGH_2-induced contraction. These improvements in endothelial function are most likely related to actions of Ro 40-5967 per se rather than being secondary to the blood pressure–lowering effect of the compound. In a previous study,[11] long-term treatment of SHR with the vasodilator hydralazine had no effect on acetylcholine-induced relaxation or serotonin-induced contraction.

As we have previously discussed (see above and Reference 43), it may be more accurate to describe endothelial dysfunction in SHR in terms of intimal dysfunction. We found that the technique of rubbing the intimal surface to remove the endothelium also resulted in removal of the monocyte-macrophages from the subendothelium. It cannot be ruled out that these cells also contribute to vascular dysfunction by producing contracting factors or factors that reduce the efficacy of EDRF. This also means that the amelioration of endothelial function brought about by Ro 40-5967 could be related to the accompanying structural changes of SHR aorta, characterized by reduction in the thickness of the subendothelium. Subendothelial thickening results from invasion of blood-borne monocyte-macrophages and increased synthesis of extracellular matrix[5] and basement membrane–like structures.[1] Prevention of monocyte-macrophage infiltration was shown by the reduction of cell nuclei counted in the subendothelium of Ro 40-5967–treated rats. The very marked decrease in subendothelial area in Ro 40-5967–treated SHR shows that Ro 40-5967 may have additionally interfered with noncellular elements of the subendothelium. The mechanisms responsible for arterial injury in response to hypertension remain to be defined. However, it has been suggested that basement membrane accumulation is an early complication of hypertension, with the artery responding to the increase in passive tensile stress by synthesis of tension-bearing proteins.[2] Prevention of morphological changes may therefore be due to the local action of Ro 40-5967 on arterial tone. In an earlier study,[12] long-term treatment with an antihypertensive dose of the angiotensin converting enzyme inhibitor cilazapril also prevented morphological changes in the subendothelium of SHR. However, reduction of blood pressure using the vasodilator hydralazine failed to change the relative composition of cerebral arterioles in stroke-prone SHR.[44] Likewise, lowering of blood pressure with felodipine had no effect on the structure of mesenteric arterioles from SHR.[45] Thus, the structural changes noted with Ro 40-5967 are probably related to more specific actions of the compound. For example, endothelial cells were shown to modulate granulocyte adhesion and chemotaxis; improvement of endothelial function by Ro 40-5967 could therefore contribute to reduction of cellular infiltration.[46]

In conclusion, our data suggest that facilitation of the effect of NO and slight inhibition of the effect of the constricting factor PGH_2 in addition to a direct vasodilator effect may contribute to the antihypertensive action of Ro 40-5967. The accompanying and perhaps related inhibition of subendothelial thickening and macrophage infiltration may also favor normal arterial function.

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