Comparison of Inhibitory Effects of Calcium Channel Blockers and That of a Calmodulin Antagonist in Strips of Mesenteric Arteries from Spontaneously Hypertensive and Normotensive Rats

Masahisa ASANO, Kaoru MASUZAWA, Masayoshi KOJIMA, Kyuzo AOKI and Tomohiro MATSUDA

Department of Pharmacology and 2nd Department of Internal Medicine, Nagoya City University Medical School, Mizuho-ku, Nagoya 467, Japan

Accepted May 26, 1988

Abstract—Effects of calcium channel blockers and of calmodulin antagonist on the contractile responses to norepinephrine (NE) were compared between strips of mesenteric arteries from 6- and 14-week-old spontaneously hypertensive rats (SHR) and age-matched, normotensive Wistar-Kyoto rats (WKY). The ratio of the maximum contraction developed by NE to that by 60 mM KCl was significantly increased in strips from 14-week-old SHR. Niludipine, verapamil and diltiazem antagonized the maximum NE contraction to a greater extent in strips from 14-week-old SHR than in those from the WKY. However, the antagonism by niludipine of the KCl- or caffeine-induced contraction was not significantly different between the strips from 14-week-old SHR and those from WKY. In strips from 6-week-old rats, there was no difference in the antagonism by niludipine of the maximum NE contraction. On the other hand, the effect of W-7 on the maximum NE contraction was not significantly different between the strips from 14-week-old SHR and those from WKY. Schild plot analyses demonstrated that \( \alpha_1 \)-adrenoceptors were the same for the two strains. These results suggest that the enhanced maximum NE contraction in the mesenteric artery from 14-week-old SHR reflects the increased transmembrane influx of calcium, and the activity of calmodulin seems to be the same for the two strains.

Functional alterations in vascular smooth muscle play a dominant role in increased total peripheral resistance in patients with essential hypertension. These alterations are initiated by abnormal functions of neurogenic or circulating humoral substances that regulate vascular resistance (1–3). McGregor and Smirk (4) observed that mesenteric arteries from both genetic and renal hypertensive rats contract to a greater extent than those from normotensive controls, when treated with either NE or 5-hydroxytryptamine. The maximum contractile response to NE has been reported to be significantly greater in the mesenteric artery from the adult SHR, as compared to age-matched WKY (5–8).

Lederballe Pedersen et al. (9) reported that the effect of nifedipine, a calcium channel blocker, on the contractile response of aorta to NE was significantly greater in the SHR than in the WKY. Consistent with these findings, differences between aortas from the two strains were found with regard to dependency of the extracellular calcium concentration for the contraction. As a possible explanation of the preferential antagonism by nifedipine in the SHR, they pointed out that the extracellular calcium influx through receptor-operated calcium channels may be increased in vascular smooth muscles from SHR. As there is little information concerning the effects of calcium channel blockers on arteries from the SHR, in
particular, those small enough to contribute to the peripheral resistance, we investigated the effects of three structurally different calcium channel blockers, niludipine, verapamil and diltiazem on the contractile response to NE in distal portions of mesenteric arteries from 6- and 14-week-old SHR, animals that are in an early stage of hypertension, and after the development of hypertension (8, 10).

The important role of calcium as a second messenger mediating excitation-contraction coupling is being given increasing attention (11). Biochemical and pharmacological studies of calcium-mediated cellular processes have increased following the recognition that calmodulin, an intracellular calcium receptor (12), is a ubiquitous and extraordinarily versatile calcium-binding protein. Calmodulin activates a large number of calcium-dependent enzymes and plays a role in modulating various physiological processes, including muscle contraction, cell motility, exocytosis, axonal flow and neurotransmitter release (12–15). Inasmuch as calmodulin can influence such a wide variety of cellular events, the modification of its activity by drugs might have profound pharmacological implications. A growing volume of evidence indicates that many diverse classes of drugs can modify the activity of calmodulin (16, 17). It is noteworthy that some calcium channel blockers can interact with calmodulin (16, 17). Among the calmodulin antagonists, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) has been reported to interact specifically with calmodulin in intact arterial preparations (16–19). Therefore, the inhibitory effect of W-7 was also compared between strips of mesenteric arteries from SHR and those from WKY.

Materials and Methods

Characteristics of SHR: Female SHR, 6 and 14 weeks of age, and age-matched WKY were inbred in our laboratory. Systolic blood pressure was measured in conscious rats by tail cuff plethysmography. The systolic blood pressure of rats in each group and their body weights have already been reported (8, 10). Briefly, SHR, 14 weeks of age had a marked increase in blood pressure (174±6 mmHg, N=29) when compared with age-matched WKY (119±5 mmHg, N=29). The blood pressure of 6-week-old SHR (127±5 mmHg, N=24) was significantly higher than that of age-matched WKY (113±4 mmHg, N=24), and it was significantly lower than that of 14-week-old SHR.

Preparation of arterial strips for recordings of mechanical activity: The rats were stunned and exanguinated. The distal portion of the superior mesenteric artery (0.6–0.8 mm outside diameter for the 14-week-old rats and 0.5–0.7 mm o.d. for the 6-week-old rats) was quickly excised. After removal of remaining fat and loosely adhering adventitia, the artery was helically cut into strips of 0.8 mm in width and 7 mm in length, according to the previously described method (20, 21).

Arterial strips were mounted vertically between hooks in water-jacketed (37±0.5°C) tissue baths containing 20 ml of Krebs-bicarbonate solution of the following composition (in mM): NaCl, 115.0; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 25.0; KH₂PO₄, 1.2; and dextrose, 10.0. Tissue bath solutions were maintained at 37±0.5°C and continuously bubbled with a mixture of 95% O₂ and 5% CO₂. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (TB-612T, Nihon Kohden Kogyo Co., Tokyo, Japan). The strips were stretched passively to optimal length by imposing the optimal resting tension, which resulted in the development of maximum isometric tension after stimulation with 60 mM KCl (20, 21). The resting tensions determined were as follows: 14-week-old SHR, 0.5 g; 14-week-old WKY, 0.5 g; 6-week-old SHR, 0.4 g; 6-week-old WKY, 0.4 g. These resting tensions were maintained throughout the experiments. After application of the resting tension, the strips were equilibrated for 90 min in oxygenated Krebs-bicarbonate solution.

After the 90-min equilibration period, a submaximally effective concentration of K⁺ (30 mM) as administered two or three times at 40-min intervals until the responses were reproducible. At the final response, 60 mM K⁺ was cumulatively added to obtain the maximum contraction of the strip.

Relaxation of arterial strips: To compare the
ability of antagonists which relax blood vessels, strips were contracted with either $10^{-5}$ M NE or 30 mM K⁺ before challenge with the antagonists (19). Dose-relaxation curves for the antagonists against the NE-induced contraction were determined noncumulatively, because $10^{-5}$ M NE produced the sustained contraction only for 60 min (Fig. 1). On the other hand, dose-relaxation curves for the antagonists against the K⁺-induced contraction were determined cumulatively. At the end of each series of experiments, $10^{-4}$ M papaverine was added to obtain the maximum relaxation of the strip.

Effects of antagonists on the contractile responses to NE and caffeine: Dose-response curves for the contractile effect of NE were determined by a cumulative addition of NE in the presence of $1 \times 10^{-6}$ M propranolol, a β-adrenoceptor antagonist. Effects of antagonists on the dose-response curve for NE were determined in the following way. Three sequential dose-response curves for NE were determined simultaneously on paired strips with an interval of 90 min between each determination. Usually the paired strips were subjected to different treatments. The antagonist applied to one strip of the pair: the first curve was taken as a control, and the effects of the antagonist were determined at the second and third curves. The antagonist was present from 40 min before NE was tested. The other strip of the pair was a control that served as an indicator of changes in tissue sensitivity to NE during the course of the experiment (19). Effects of the calcium channel blocker on the contractile response to 10 mM caffeine were also determined in a similar fashion. Preliminary experiments indicated that caffeine in concentrations ranging from 0.5 to 20 mM produced a dose-dependent contraction, and the maximum contraction was obtained when 10 mM caffeine was added in strips of both strains.

Determination of pA₂ values for α-adrenoceptor antagonists: To classify the subtype of α-adrenoceptors in strips of mesenteric arteries from SHR and WKY, effects of prazosin, a selective $α_1$-adrenoceptor antagonist; yohimbine, a selective $α_2$-adrenoceptor antagonist; and phentolamine, a non-selective α-adrenoceptor antagonist, on the dose-response curves for α-adrenoceptor agonists were determined. Each individual dose-response curve was plotted to obtain the ED50 values for the α-adrenoceptor agonist following the various antagonist treatments. Dose-ratio, i.e., ED50 in the presence of antagonist divided by the ED50 in the absence of antagonist, for each agonist was obtained at varying concentrations of the antagonist. Then the data were subjected to a Schild plot analysis according to the method of Arunlakshana and Schild (22), and the pA₂ value for the antagonist and the slope of the line were determined from the regression analysis.

Statistical analysis: When assessing the ED50 value for an agonist, responses to the agonist were calculated as % of the maximum response obtained with the agonist. The ED50 value was obtained visually from a plot of % response vs. log concentration of the agonist, and was expressed as a negative log (pD₂ value).

Unless specified, results shown in the text, tables and figures are expressed as the mean value±S.E. (N=number of preparations). Statistical analysis of the data was done by Student’s t-test for paired or unpaired data, or by completely randomized design, one-way analysis of variance followed by Newman-Keuls test for a significant F-ratio (P<0.05), depending on which test was statistically appropriate. Two groups of data were considered to be significantly different when P<0.05.

Drugs: The following drugs were used: /-norepinephrine bitartrate (NE; Sigma Chemical Co., St. Louis, MO, U.S.A.), /-phenylephrine hydrochloride (PE; Sigma), clonidine hydrochloride (Sigma), caffeine sodium benzoate (Iwaki Pharmaceutical Co., Tokyo, Japan), dl-propranolol hydrochloride (ICI Pharmaceuticals, England), prazosin hydrochloride (Taito Pfizer Co., Tokyo, Japan), yohimbine hydrochloride (Nakarai Chemicals, Kyoto, Japan), phentolamine mesylate (Ciba-Geigy, Takarazuka, Japan), niludipine (BAY a 7168; Bayer Yakuhin, Ltd., Osaka, Japan), verapamil hydrochloride (Eisai Co., Tokyo, Japan), diltiazem hydrochloride (Tanabe Pharmaceutical Co., Osaka, Japan) and W-7 (a kind gift of Prof. H. Hidaka, Nagoya
University School of Medicine). NE, PE and clonidine were prepared daily in Krebs-bicarbonate solution and kept on ice during the experiment. Niludipine was dissolved in a mixture of ethanol, polyethylene glycol 400 and distilled water (15:20:65) to make a stock solution of $10^{-3}$ M, and aliquots of this solution were then diluted to $10^{-6}$ M with distilled water before use. Aqueous stock solutions were prepared for other drugs. Concentrations of drugs are expressed as final molar concentrations in the tissue bath.

Results
Maximum NE- and K⁺-induced contractions in strips of mesenteric artery from SHR and WKY: To define the enhanced maximum NE contraction in strips of mesenteric artery from 14-week-old SHR, the strip weight, tension development by either $10^{-5}$ M NE or 60 mM K⁺, and the ratio of the NE-contraction to the K⁺-contraction were compared between the SHR and WKY at 14 and 6 weeks of age (Table 1). The strip weight was not significantly different between the two strains. The addition of $10^{-5}$ M NE produced a maximum contraction in all the strips from SHR and WKY. The contractile tension developed by $10^{-5}$ M NE expressed as mg in strips from 14-week-old SHR was significantly greater than that from age-matched WKY. The addition of 60 mM K⁺ produced a maximum contraction of this agonist in all the strips. The contractile tension developed by 60 mM K⁺ was also significantly greater in strips from 14-week-old SHR than those from the age-matched WKY. The ratio of the contraction developed by $10^{-5}$ M NE to that by 60 mM K⁺ (NE/K⁺ ratio) in strips from the SHR was significantly greater than the ratio in the WKY. At 6 weeks of age, contractile tensions developed by both $10^{-5}$ M NE and 60 mM K⁺ were significantly greater in strips from SHR than those from WKY. However, the NE/K⁺ ratios were not significantly different between the strips from 6-week-old SHR and those from the age-matched WKY (Table 1). The maximum contractions developed by $10^{-5}$ M NE were not significantly altered by $10^{-5}$ M propranolol, a β-adrenoceptor antagonist, in all the strips from SHR and WKY (data not shown). Thus, the enhanced maximum NE contraction (NE/K⁺ ratio) was observed only after the development of hypertension.

Arterial relaxant responses to calcium channel blockers: The maximum contraction developed by $10^{-5}$ M NE in strips from 14-week-old SHR was sustained for 60 min. Niludipine, a dihydropyridine calcium channel blocker, elicited a dose-dependent relaxation in the NE-contracted strips (Fig. 1A). Dose-relaxation curves for niludipine in the NE-contracted strips from 14-week-old SHR and WKY are shown in Fig. 2. The relaxant effect of niludipine was significantly greater in the SHR than in the WKY. The relaxant response to niludipine in NE-contracted

| Age          | Rat  | N  | Strip weighta (mg) | Tension developed by NE $10^{-5}$ M³ | KCI 60 mM³ | NE/K⁺ ratiob |
|--------------|------|----|-------------------|-----------------------------------|-----------|--------------|
| 14-week-old | SHR  | 48 | 0.72±0.03         | 489±16**                          | 312±11**  | 1.57±0.09**  |
|              | WKY  | 46 | 0.70±0.03         | 304±14                           | 243±10    | 1.24±0.04    |
| 6-week-old  | SHR  | 25 | 0.61±0.03         | 256±12*                          | 211±10*   | 1.21±0.06    |
|              | WKY  | 25 | 0.62±0.04         | 207±10                           | 176±9     | 1.19±0.04    |

aStrips of mesenteric arteries were contracted with 60 mM KCI and $10^{-5}$ M NE. At the end of the experiment, the strip was gently blotted and weighed. bThe ratio of the maximum contraction developed by $10^{-5}$ M NE to the contraction by 60 mM KCI in the strip was determined (NE/K⁺ ratio). N indicates the number of preparations used. Data are expressed as the mean±S.E. Asterisks indicate those points in SHR significantly different at P<0.05 (*) or P<0.01 (**) when compared with the value obtained in the age-matched WKY.
strips from 6-week-old SHR was significantly weaker than that from 14-week-old SHR and was similar to that from 14-week-old WKY. The dose-relaxation curve for niludipine in the NE-contracted strips was not significantly different between 6-week-old SHR and age-matched WKY (data not shown). Other structurally different calcium channel blockers, verapamil and diltiazem, also produced similar actions on these strips (Fig. 2). The addition of 30 mM K+ produced a sustained contraction in the strips of mesenteric arteries. Niludipine elicited a dose-dependent relaxation in the K+-contracted strips (Fig. 1B). Dose-relaxation curves for niludipine in the K+-contracted strips from 14-week-old SHR and WKY are shown in Fig. 3. The relaxant effect of niludipine on K+-contracted strips was not significantly different between the two strains. Verapamil and diltiazem also produced similar relaxations between the K+-contracted strips from 14-week-old SHR and those from the WKY (Fig. 3). Effective concentrations of the three calcium channel blockers in the K+-contracted strips (Fig. 3) were significantly lower than those in the NE-contracted strips (Fig. 2).

To rule out the possibility that the difference in the effects of calcium channel blockers depends on the different methods used between the NE- and K+-contracted strips, we attempted to determine the dose-relaxation curve for verapamil noncumulatively in the K+-contracted strips from 14-week-old SHR and WKY. pD2 values for the relaxant effects of verapamil determined noncumulatively were 7.64±0.06 (SHR, N=6) and 7.65±0.05 (WKY, N=6), respectively. On the other hand, pD2 values for verapamil determined cumulatively (Fig. 3) were 7.62±0.04 (SHR, N=9) and 7.63±0.04 (WKY, N=9), respectively.

Arterial relaxant response to W-7: The dose-relaxation curve for W-7, a calmodulin antagonist, determined in the NE-contracted strips was not significantly different between the strips from 14-week-old SHR and those from age-matched WKY (Fig. 4). pD2 values for W-7 in the NE-contracted strips from SHR and WKY were 4.34±0.02 (SHR, N=6) and 4.32±0.03 (WKY, N=8), respectively. W-7 also produced a similar relaxation in the K+-contracted strips from 14-week-old SHR and WKY. pD2 values for W-7 in the K+-contracted strips were 4.33±0.03 (SHR, N=6).
Fig. 2. Dose-relaxation curves for niludipine (left), verapamil (center) and diltiazem (right) in NE-contracted strips of mesenteric arteries from 14- and 6-week-old SHR and age-matched WKY. Experimental conditions were the same as in Fig. 1A. Mean values of the contractile tensions developed by $10^{-5}$ M NE in the strips were 487±9 mg (14-week-old SHR, N=10), 303±16 mg (14-week-old WKY, N=9) and 266±16 mg (6-week-old SHR, N=8), respectively. Relaxation induced by $10^{-4}$ M papaverine was taken as 100%. Vertical bars represent S.E. Numbers beside the dose-response curves indicate the number of preparations used. Asterisks indicate those points in 14-week-old SHR that are significantly different at P<0.05 (*) or P<0.01 (**) when compared with the value obtained in the age-matched WKY.

Fig. 3. Dose-relaxation curves for niludipine (left), verapamil (center) and diltiazem (right) in K+-contracted strips of mesenteric arteries from 14-week-old SHR and age-matched WKY. Experimental conditions were the same as in Fig. 1B. Mean values of the contractile tensions developed by 30 mM KCl in the strips were 262±17 mg (SHR, N=7) and 205±13 mg (WKY, N=7), respectively. Relaxation induced by $10^{-4}$ M papaverine was taken as 100%. Vertical bars represent S.E. Numbers beside the dose-response curves indicate the number of preparations used.
Effects of various antagonists on the dose-response curve for NE: The cumulative addition of NE in concentrations ranging from $10^{-9}$ to $10^{-4}$ M produced a dose-dependent contraction in strips from 14-week-old SHR and age-matched WKY. $pD_2$ values for NE in the strips were $6.87 \pm 0.11$ (SHR, $N=9$) and $7.31 \pm 0.08$ (WKY, $N=9$), respectively. This represents a small but significant ($P<0.05$) decrease in the sensitivity of the strips from SHR to NE. When the dose-response curve of the strips for the contractile effect of NE was determined in the presence of $2 \times 10^{-9}$ and $8 \times 10^{-9}$ M niludipine, a reduction of the maximum NE contraction was observed with a marked rightward displacement of the dose-response curve (Fig. 5). In the presence of $2 \times 10^{-9}$ M niludipine, the $pD_2$ value for NE in strips from 14-week-old SHR was decreased by $0.97 \pm 0.10$ ($N=9$) log units when compared with the control curve, while the $pD_2$ value in strips from age-matched WKY was decreased by $0.95 \pm 0.08$ ($N=9$). Thus, the change in the $pD_2$ value for NE was not significantly different between the SHR and WKY. Niludipine reduced the maximum contractions induced by $10^{-5}$ and $10^{-4}$ M NE in strips from 14-week-old SHR to a greater extent than in strips from age-matched WKY (Fig. 5 and Table 2). Verapamil at the concentration of $4 \times 10^{-8}$ M also exhibited a greater reduction of the maximum NE contraction in strips from 14-week-old SHR (Table 2). However, the extent of the reduction of the maximum NE contraction by the calcium channel blockers was the same between the strips from 6-week-old SHR and age-matched WKY (Table 2).

When W-7 was added to the bath in concentrations of $1 \times 10^{-5}$ and $3 \times 10^{-5}$ M, a typical noncompetitive antagonism was observed in the dose-response curve for NE (Fig. 6). The potency of W-7 to reduce the maximum NE contraction was the same for the strips from both 14-week-old SHR and age-matched WKY (Table 2). W-7 produced similar antagonisms on the strips from 6-week-old SHR and WKY (Table 2).

Effects of niludipine on the contractile response to caffeine: The addition of 10 mM caffeine produced a maximum contraction of this agonist in strips from both 14-week-old SHR and age-matched WKY. The ratio of the
Fig. 5. Effects of niludipine (Nil) on the dose-response curves for NE in strips of mesenteric arteries from 14-week-old SHR (left) and age-matched WKY (right). Mean values of the maximal contractile tensions developed by NE in the absence of niludipine in the strips were 487±23 mg (SHR, N=9) and 314±19 mg (WKY, N=9), respectively. Niludipine was added 40 min before the determination of the dose-response curve for NE, and it was present during the addition of NE. In each strip, the tension developed by 10^{-4} M NE in the absence of niludipine was taken as 100%. For details, see "Materials and Methods". Vertical bars represent S.E. Numbers beside the dose-response curves indicate the number of preparations used. Asterisks indicate those points in SHR that are significantly different at P<0.05 (*) when compared with the value obtained in WKY.

Table 2. Effects of niludipine, verapamil and W-7 on the maximum contractile response to NE in strips of mesenteric arteries from 14- and 6-week-old SHR and age-matched WKY

| Age of rats   | Antagonist  | % NE-contraction in the presence of antagonist | SHR       | WKY       |
|---------------|-------------|-----------------------------------------------|-----------|-----------|
|               |             |                                               |           |           |
| 14-week-old   | Niludipine  |                                               |           |           |
|               | 2×10^{-9} M | 76.3±3.4* (9)                                 | 84.6±3.3 (9) |
|               | Niludipine  |                                               |           |           |
|               | 8×10^{-9} M | 62.5±3.2* (9)                                 | 75.1±3.2 (9) |
|               | Verapamil   |                                               |           |           |
|               | 4×10^{-8} M | 80.4±2.5* (8)                                 | 89.6±2.6 (8) |
|               | W-7         |                                               |           |           |
|               | 1×10^{-8} M | 81.5±5.6 (6)                                  | 80.1±5.3 (6) |
|               | W-7         |                                               |           |           |
|               | 3×10^{-8} M | 29.7±5.0 (6)                                  | 32.7±5.6 (6) |
| 6-week-old    | Niludipine  |                                               |           |           |
|               | 2×10^{-9} M | 88.6±2.9 (8)                                  | 88.7±3.5 (8) |
|               | Niludipine  |                                               |           |           |
|               | 8×10^{-9} M | 84.1±2.6 (8)                                  | 86.2±4.5 (8) |
|               | Verapamil   |                                               |           |           |
|               | 4×10^{-8} M | 92.4±3.3 (8)                                  | 89.7±2.9 (8) |
|               | W-7         |                                               |           |           |
|               | 3×10^{-8} M | 26.9±5.1 (4)                                  | 29.7±5.5 (4) |

*aThree sequential dose-response curves for the contractile effect of NE were determined from a single strip. First the dose-response curve was taken as a control, and each antagonist was added before the second and third dose-response curve. For details, see "Materials and Methods". bContractile tensions developed by 10^{-4} M NE in the absence of the antagonists were taken as 100%. Numbers in parentheses indicate the number of preparations used. Data are expressed as the mean±S.E. *Significantly different when compared with age-matched WKY (P<0.05).
maximum contraction induced by caffeine to that by K+ (caffeine/K+ ratio) was significantly (P<0.001) greater in strips from 14-week-old SHR (0.355±0.013, N=14) than in strips from age-matched WKY (0.283±0.007, N=14) (Table 3). In the presence of 2×10^{-9} M niludipine, the caffeine-induced contractions were not significantly affected in strips of both SHR and WKY (Table 3). However, a relatively high concentration of niludipine (1×10^{-8} M) produced a weak inhibition against the caffeine-induced contraction, which was not significantly different between the SHR and WKY (Table 3).

Comparison of pA2 values for α-adrenoceptor antagonists in mesenteric arteries from SHR and WKY: In mesenteric arteries from 14- and 6-week-old SHR and age-matched WKY, prazosin in concentrations of 5×10^{-10}, 3×10^{-9} and 2×10^{-8} M produced a rightward displacement of the dose-response curve for phenylephrine (PE) without any significant reduction of the maximum response. pA2 values and slopes calculated
from the Schild plot for prazosin-induced antagonism of the response to PE are listed in Table 4. The pA2 values for prazosin were identical among the mesenteric arteries from 14 and 6-week-old SHR and age-matched WKY. The Schild plot for prazosin antagonism gave a regression line with a slope of unity and a pA2 value of approximately 9.5 in the four groups. The pA2 value for prazosin against clonidine was in good agreement with the pA2 value for this antagonist against PE. The pA2 values for yohimbine were in good agreement with the values for this antagonist reported for α1-adrenoceptors (23, 24) rather than α2-adrenoceptors (23). These pA2 values for yohimbine were the same for strips from 14-week-old SHR and age-matched WKY. Phentolamine also exhibited similar pA2 values among the mesenteric arteries from 14- and 6-week-old SHR and age-matched WKY (Table 4).

**Discussion**

The major conclusion of the present study is that the enhanced maximum NE contraction in the mesenteric artery from 14-week-old SHR reflected an increased transmembrane influx of calcium in response to NE. This conclusion is suggested from the following observations: 1) calcium channel blockers antagonized the maximum NE contraction to a greater extent in the mesenteric artery from 14-week-old SHR than that from age-matched WKY, 2) these blockers exhibited the similar antagonism against the K+-contraction in both the two strains, 3) only a high concentration of niludipine produced weak inhibitions against the caffeine-induced contractions in the two strains, 4) the extent of antagonism by the calcium channel blockers of the maximum NE contraction was the same for mesenteric arteries from 6-week-old SHR and WKY, 5) W-7 produced similar antagonisms against the NE- and K+-contractions in both strains, and 6) the Schild plot analyses demonstrated that α1-adrenoceptors were the same for the mesenteric arteries from SHR and WKY.

Many investigators have already demonstrated the enhanced maximum NE contraction in strips of mesenteric artery (4-8), tail artery (25) and femoral artery (10) from genetically hypertensive rats including SHR.
To define the functional alteration in the response to NE in SHR, we expressed the maximum NE contraction as a NE/K⁺ ratio (Table 1). It is well-known that an increased total peripheral resistance is related to an increased wall/lumen ratio in arterial resistance vessels (26). Direct measurement (27) indicates a reduced lumen and a thicker media in small mesenteric arteries from SHR. However, K⁺-induced contractions were the same in the mesenteric arteries from SHR and WKY (6, 7). Therefore, the NE/K⁺ ratio is one of the suitable parameters to define the functional alteration in the vascular tissue. In the present study, the NE/K⁺ ratio was increased in the mesenteric artery from 14-week-old SHR, as compared with age-matched WKY, but was not increased in the artery from 6-week-old SHR. Thus, the change in the NE/K⁺ ratio in SHR was in good agreement with the change in blood pressure of the SHR.

The main purpose of the present study was to determine whether the difference in the maximum NE contraction of mesenteric arteries could be explained by the difference in calcium mobilization. The NE-induced contraction depends on both extracellular and intracellular calcium. The initial rapid component of NE-induced contraction is related mainly to the intracellular calcium stores, whereas the maintained tonic component is associated with an increased calcium influx (28, 29). In the present study, the maintained tonic component of NE contraction was measured in all the experiments of relaxations and dose-response curves. Therefore, it is likely that the difference in the NE/K⁺ ratio between 14-week-old SHR and WKY may result from the difference in calcium influx induced by NE. A potentially useful approach for investigating the role of calcium in the enhanced maximum NE contraction is through the application of pharmacological agents that selectively modify calcium channel functions. Maximum NE contractions in mesenteric arteries from 14-week-old SHR and WKY were antagonized by three calcium channel blockers, namely niludipine, verapamil and diltiazem. The extent of antagonisms induced by calcium channel blockers was not observed when the arteries were contracted with either KCl or caffeine. The caffeine-induced contraction depends largely on intracellular calcium (30). The finding that only a high concentration of niludipine produced the weak inhibition against the caffeine-induced contraction which was comparable between the two strains, suggests that the difference in the release of intracellular calcium may not be responsible for the preferential effect of this blocker on the maximum NE contraction. At 6 weeks of age, niludipine produced a similar antagonism against the maximum NE contraction in the two strains. From results, it may be concluded that α-adrenoceptor-operated calcium influx induced by NE is significantly greater in the mesenteric artery from 14-week-old SHR than that from age-matched WKY, whereas calcium influx through the voltage-sensitive channels induced by KCl-depolarization is the same for mesenteric arteries from both strains. It has been demonstrated that the contraction of rat mesenteric artery induced by relatively high concentrations of NE was accompanied by membrane depolarization (31). Therefore, we can not exclude the possibility that the extent of depolarization induced by NE was different between the mesenteric arteries from SHR and those from WKY.

Synthetic compounds usually have multiple pharmacological effects. Some calcium antagonists are known to interact with calmodulin (16, 17). As described in the Introduction, calmodulin mediates many of the hormone messages relayed by intracellular calcium. Smooth muscle contractions is regulated by myosin phosphorylation (15). Myosin light chain kinase is absolutely dependent on calmodulin and catalyzes the phosphorylation of a 20,000-dalton myosin light chain. Phosphorylation of the light chain activates the actomyosin ATPase, leading to the hydrolysis of ATP and contraction of the myosin in the smooth muscle. Many diverse classes of drugs can modify the activity of calmodulin. Therefore, we compared the effect of a selective calmodulin antagonist, W-7, on NE-induced contraction and found that the antagonism by W-7 of NE-induced contraction was not significantly different.
between the mesenteric arteries from 14-week-old SHR and those from WKY. Thus, strain differences were not observed in the effect of the calmodulin antagonist on the response to NE. Therefore, the activity of calmodulin does not seem to be significantly different between the mesenteric arteries from SHR and those from WKY.

In a variety of vascular smooth muscles, calcium channel blockers have been demonstrated to antagonize contractile responses to \( \alpha_2 \)-adrenoceptor stimulation more preferentially than those to \( \alpha_1 \)-adrenoceptor stimulation (32, 33). Therefore, the difference in the effects of three calcium channel blockers on the maximum NE contraction between the mesenteric arteries from SHR and those from WKY may be explained by the difference in the subtype of \( \alpha \)-adrenoceptors in these arteries. However, this is unlikely, because the Schild plot analyses clearly demonstrated the existence of only \( \alpha_1 \)-adrenoceptors in the mesenteric arteries from the two strains (Table 4). Selective \( \alpha_2 \)-adrenoceptor agonists including clonidine have been reported to behave as partial agonists of \( \alpha_1 \)-adrenoceptors in several vascular tissues (23, 34, 35). Also, in the present study clonidine behaved as a partial agonist of \( \alpha_1 \)-adrenoceptors in several vascular tissues (23, 34, 35). The Schild plot analyses using prazosin, yohimbine and phentolamine as antagonists clearly demonstrated the existence of same \( \alpha_1 \)-adrenoceptors in mesenteric arteries from 14-week-old SHR and WKY. These \( \alpha_1 \)-adrenoceptors were also the same at the early stage of hypertension (6 weeks).

We have demonstrated that in the femoral artery from SHR, decrease in \( \beta \)-adrenoceptor activity was accompanied by an enhanced vasoconstriction induced by NE (10). However, in the case of the mesenteric artery, the presence of propranolol did not enhance the contractile tensions developed by NE. Therefore, the \( \beta \)-adrenoceptors of the mesenteric arteries from both SHR and WKY may be physiologically inactive or less active, as compared with those of the femoral arteries.

The findings of the present study suggest that in the mesenteric artery from 14-week-old SHR, \( \alpha_1 \)-adrenoceptor-operated calcium influx was increased in response to stimulation with NE, while the calcium influx through the voltage-sensitive channels induced by KCl-depolarization was the same as the mesenteric artery from WKY. This suggestion will have to be strengthened by the measurement of \(^{45}\text{Ca} \) influx in the same artery, and this is the subject of our current investigations.

Acknowledgments: Gifts of prazosin (Taito Pfizer), phentolamine (Ciba-Geigy), niludipine (Bayer Yakuhin), verapamil (Eisai) and diltiazem (Tanabe Pharmaceutical) are gratefully acknowledged. W-7 was kindly provided by Prof. H. Hidaka of Nagoya University. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 62570401) from the Ministry of Education, Science and Culture, Japan and a grant from The Ishida Foundation.

References

1. Latis, L.T., Shaffer, R.A. and Brody, M.J.: Neurogenic and humoral factors controlling vascular resistance in the spontaneously hypertensive rats. Circ. Res. 35, 764–774 (1974)
2. Collis, M.G. and Vanhoutte, P.M.: Vascular reactivity of isolated perfused kidneys from male and female spontaneously hypertensive rats. Circ. Res. 41, 759–767 (1977)
3. Webb, R.C. and Bohr, D.F.: Recent advances in the pathogenesis of hypertension: Consideration of structural, functional, and metabolic vascular abnormalities resulting in elevated arterial resistance. Am. Heart J. 102, 251–264 (1981)
4. McGregor, D.D. and Smirk, F.H.: Vascular response to 5-hydroxytryptamine in genetic and renal hypertensive rats. Am. J. Physiol. 219, 687–690 (1970)
5. Whall, C.W., Jr., Myers, M.M. and Halpern, W.: Norepinephrine sensitivity, tension development and neuronal uptake in resistance arteries from spontaneously hypertensive and normotensive rats. Blood Vessels 17, 1–15 (1980)
6. Mulvany, M.J. and Nyborg, N.: An increased calcium sensitivity of mesenteric resistance vessels in young and adult spontaneously hypertensive rats. Br. J. Pharmacol. 71, 585–596 (1980)
7 Nilsson, H. and Mulvany, M.J.: Prolonged exposure to ouabain eliminates the greater nor-
epinephrine-dependent calcium sensitivity of resistance vessels in spontaneously hypertensive
rats. Hypertension 3, 691–697 (1981)
8 Asano, M., Aoki, K. and Matsuda, T.: Quantitative changes of maximum contractile response to
norepinephrine in mesenteric arteries from spontaneously hypertensive rats during the develop-
ment of hypertension. J. Cardiovasc. Pharmacol. 6, 727–731 (1984)
9 Lederhalle Pedersen, U., Mickelsen, E. and
Anderson, K.E.: Effects of extracellular calcium on potassium and noradrenaline induced con-
tractions in the aorta of spontaneously hypertensive rats: Increased sensitivity to nifedipine.
Acta Pharmacol. Toxicol. 43, 137–144 (1978)
10 Asano, M., Aoki, K. and Matsuda, T.: Reduced
beta adrenoceptor interactions of norepinephrine
enhance contraction in femoral artery from
spontaneously hypertensive rats. J. Pharmacol.
 Exp. Ther. 223, 207–214 (1982)
11 Krestinger, R.H.: The informational role of calcium
in the cytosol. Adv. Cyclic Nucleotide Res. 11,
1–26 (1979)
12 Means, A.R. and Dedman, J.R.: Calmodulin—
an intracellular calcium receptor. Nature 285,
73–77 (1980)
13 Cheung, W.Y.: Calmodulin plays a pivotal role
in cellular regulation. Science 207, 19–27 (1980)
14 Weiss, B. and Wallace, T.L.: Mechanisms and
pharmacological implications of altering cal-
modulin activity. In Calcium and Cell Function.
Edited by Cheung, W.Y., Vol. 1, p. 329–379.
Academic Press, New York (1980)
15 Stull, J.T., Blumenthal, D.K. and Cooke, R.:
Regulation of contraction by myosin phos-
phorylation: A comparison between smooth and
skeletal muscles. Biochem. Pharmacol. 29,
2537–2543 (1980)
16 Asano, M. and Hidaka, H.: Biopharmacological
properties of naphthalenesulfonamides as potent
calmodulin antagonists. In Calcium and Cell Func-
tion. Edited by Cheung, W.Y., Vol. 5, p.
123–164, Academic Press, Orlando (1984)
17 Asano, M. and Stull, J.T.: Effects of calmodulin
antagonists on smooth muscle contraction and
myosin phosphorylation. In Calmodulin An-
tagonists and Cellular Physiology. Edited by
Hidaka, H. and Hartshorne, D.J., p. 225–260.
Academic Press, Orlando (1985)
18 Asano, M., Suzuki, Y. and Hidaka, H.: Effects of
various calmodulin antagonists on contraction of
rabbit aortic strips. J. Pharmacol. Exp. Ther.
220, 191–196 (1982)
19 Asano, M. and Hidaka, H.: Pharmacological
properties of N-(6-aminohexyl)-5-chloro-1-
naphthalenesulfonamide (W-7), a calmodulin
antagonist in arterial strips from rats and rabbits.
J. Pharmacol. Exp. Ther. 234, 476–484 (1985)
20 Asano, M., Masuzawa, K. and Matsuda, T.: Evidence for reduced β-adrenoceptor coupling to
adenylate cyclase in femoral arteries from spontaneously hypertensive rats. Br. J. Pharma-
col. 94, 73–86 (1988)
21 Asano, M., Aoki, K. and Matsuda, T.: Contractile
effects of Bay k 8644, a dihydropyridine calcium
agonist, on isolated femoral arteries from sponta-
neously hypertensive rats. J. Pharmacol. Exp.
Ther. 239, 198–205 (1986)
22 Arunlakshana, O. and Schild, H.O.: Some quan-
titative uses of drug antagonists. Br. J. Pharma-
col. 14, 48–58 (1959)
23 Ruffolo, R.R., Jr., Waddell, J.E. and Yaden, E.L.: Postsynaptic alpha adrenergic receptor subtypes
differentiated by yohimbine in tissues from the
rat. Existence of alpha-2 adrenergic receptors in
rat aorta. J. Pharmacol. Exp. Ther. 217, 235–240
(1981)
24 Agrawal, D.K., Triggle, C.R. and Daniel, E.E.: Pharmacological characterization of the post-
synaptic alpha adrenoceptors in vascular smooth
muscle from canine and rat mesenteric vascular
beds. J. Pharmacol. Exp. Ther. 229, 831–838
(1984)
25 Hermsemeyer, K.: Electrogenesis of increased	norepinephrine sensitivity of arterial vascular
muscle in hypertension. Circ. Res. 38, 362–367
(1976)
26 Folkow, B.: Physiological aspects of primary
hypertension. Physiol. Rev. 62, 347–503 (1982)
27 Mulvany, M.J., Hansen, P.K. and Aalkjaer, C.: Direct evidence that the greater contractility of
resistance vessels in spontaneously hypertensive
rats is associated with a narrowed lumen, a thicker
media, and larger number of smooth muscle cell
layers. Circ. Res. 43, 854–864 (1978)
28 Godfraind, T. and Kaba, A.: The role of calcium
in the action of drugs on vascular smooth muscle.
Arch. Int. Pharmacodyn. Ther. 196, Supp. 35–
49 (1972)
29 Deth, R. and van Breemen, C.: Relative contri-
butions of Ca2+ influx and cellular Ca2+ release
during drug induced activation of the rabbit
aorta. Pflugers Arch. 348, 13–22 (1974)
30 Loutzenhiser, R., Leyten, P., Saida, K. and van
Breemen, C.: Calcium compartments and mobil-
zation during contraction of smooth muscle. In
Calcium and Contractility: Smooth Muscle,
Edited by Grover, A.K. and Daniel, E.E., p. 61–82,
Human Press, Clifton (1985)

31 Mulvany, M.J., Nilsson, H. and Flatman, J.A.: Role of membrane potential in the response of rat small mesenteric arteries to exogenous nor-adrenaline stimulation. J. Physiol. (Lond.) 332, 363–373 (1982)

32 van Meel, J.C.A., de Jonge, A., Kalkman, H.O., Wilffert, B., Timmermans, P.B.M.W.M. and van Zwieten, P.A.: Vascular smooth muscle contraction initiated by postsynaptic α2-adrenoceptor activation is induced by an influx of extracellular calcium. Eur. J. Pharmacol. 69, 205–208 (1981)

33 Godfraind, T., Miller, R.C. and Lima, J.S.: Selective α1- and α2-adrenoceptor agonist-induced contractions and 45 Ca fluxes in the rat isolated aorta. Br. J. Pharmacol. 77, 597–604 (1982)

34 Beckeringh, J.J., Thoolen, M.J.M.C., de Jonge, A., Wilffert, B., Timmermans, P.B.M.W.M. and van Zwieten, P.A.: Differential effects of the calcium entry blocker D600 on contractions of rat and guinea-pig aortas elicited by various α1 adrenoceptor agonists. J. Pharmacol. Exp. Ther. 229, 515–521 (1984)

35 Beckeringh, J.J., Thoolen, M.J.M.C., de Jonge, A., Wilffert, B., Timmermans, P.B.M.W.M. and van Zwieten, P.A.: The contractions induced in rat and guinea-pig aortic strips by the α2-adrenoceptor selective agonist B-HT 920 and UK 14,304 are mediated by α1-adrenoceptors. Eur. J. Pharmacol. 104, 197–203 (1984)

36 Drew, G.M.: What do antagonists tell about α-adrenoceptors? Clin. Sci. 68, Supp. 10, 15s–19s (1985)