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Long-term effects of benidipine on cerebral vasoreactivity in hypertensive rats

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Abstract

We tested the hypothesis that long-term application of a Ca^{2+} channel blocker would ameliorate the functional and morphological deterioration of the cerebral arteries during hypertension. Male spontaneously hypertensive rats (SHR) were fed a standard rat chow, containing a low (3 mg/kg/day) or high dose (6 mg/kg/day) of benidipine, a Ca^{2+} channel blocker, for 2 months. Using a cranial window, we examined responses of the basilar artery to acetylcholine, sodium nitroprusside, (−)-(3*S*,4*R*)-4-(*N*-acetyl-*N*-hydroxyamino)-6-cyano-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol (Y-26763; an opener of ATP-sensitive K^+ channels), and (*R*)-(+)-*trans*-*N*-(4-pyridyl)-4-(1-aminoethyl)-cyclohexanecarboxamide (Y-27632; an inhibitor of Rho-associated kinase). Mean arterial pressure of the control group was 193±5 mm Hg (mean±S.E.M.), while that of the low-dose benidipine group was 183±5 mm Hg and that of the high-dose group was 159±4 mm Hg. Dilator responses of the basilar artery to acetylcholine and Y-26763 were impaired in SHR compared with those of normotensive Wistar–Kyoto (WKY) rats and treatment with benidipine enhanced the vasodilator responses to acetylcholine and Y-26763 in SHR. Y-27632-induced dilatation of the basilar artery was enhanced in SHR compared to that in WKY rats and the vasodilatation was reduced by benidipine in SHR. Sodium nitroprusside caused similar dilatation of the basilar artery, in both WKY rats and the SHR control group, and benidipine did not affect nitroprusside-induced dilatation of the artery in SHR. The wall of the basilar artery was significantly thicker in SHR than in WKY rats and benidipine treatment reduced the wall thickness of the artery in SHR. These findings suggest that chronic treatment with a Ca^{2+} channel blocker may enhance the dilator capacity and reduce contractility of the basilar artery during hypertension. Benidipine may also ameliorate the morphological changes of the basilar artery in hypertension.

Keywords: Cerebral artery; Hypertension; Nitric oxide (NO); K^+ channel; Y-27632; Rho-associated kinase

1. Introduction

A variety of morphological and functional changes appear to occur in cerebral blood vessels during chronic hypertension, which would contribute to the development of cerebrovascular disease (Heistad and Baumbach, 1992; Heistad et al., 1993). Endothelium-dependent dilatation of the cerebral arteries is shown to be markedly impaired in spontaneously hypertensive rats (SHR) (Mayhan et al., 1987; Yang et al., 1991; Kitazono et al., 1995b, 1996). Dilatation of the basilar artery (Kitazono et al., 1993b) and an increase in cerebral blood flow (Takaba et al., 1996) in response to activation of ATP-sensitive K^+ channels, both being independent of endothelial functions, are also impaired in chronic hypertensive rats. Moreover, it was reported recently that the activity of Rho-associated kinase, which increases the Ca^{2+} sensitivity of myofilaments and thereby mediates vasocontraction, is augmented in the basilar artery during chronic hypertension (Chrissobolis and Sobey, 2001). Together with these functional alterations of the vessels, morphological changes such as vascular wall hypertrophy and remodeling appear to contribute to increased resistance of the cerebral arteries during chronic hypertension (Baumbach and Heistad, 1989; Heistad and Baumbach, 1992; Heistad et al., 1993). These functional and morphological changes of the cerebral arteries may be beneficial to prevent an excessive increase in cerebral blood flow during severe hypertension; however, such changes may become a major cause of ischemic cerebrovascular disease.

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Benidipine hydrochloride, which is widely used in clinical practice for the treatment of hypertension, is a 1,4-dihydropyridine type Ca^{2+} channel blocker with a long-lasting action. It has been shown that chronic administration of this agent reduces the occurrence of cerebrovascular lesions in SHR (Ueno et al., 2000). Thus, we examined the long-term effects of benidipine on the reactivity and the wall thickness of the basilar artery of SHR. Regarding vasoreactivity, we especially focused on dilator responses mediated by nitric oxide (NO) and ATP-sensitive K^+ channels and the activity of Rho-associated kinase in the basilar artery.

2. Materials and methods

2.1. Animals

This experiment was reviewed by the Committee of the Ethics on Animal Experiment in Faculty of Medicine, Kyushu University, and carried out under the control of the Guidelines for Animal Experiment in Faculty of Medicine, Kyushu University and The Law (no. 105) and Notification (no. 6) of the Japanese Government.

Male SHR (4-month-old) were allocated at random to three groups (n=7, in each group). One group was fed standard rat chow, and the others received the same rat chow containing a low dose (3 mg/kg/day) or a high dose (6 mg/kg/day) of benidipine hydrochloride. Because the daily consumption of the chow by each rat (weight; 300 g) was about 30 g, the content of benidipine in the low- and high-dose groups was taken to be 0.03 and 0.06 mg/g chow, respectively. After feeding the animals with either of the chows for two months, we examined dilator responses of the basilar artery using a cranial window technique. We used male Wistar–Kyoto (WKY) rats of the same age (n=6) fed the standard rat chow as the normotensive control group.

2.2. Cranial window

The animals were anesthetized with amobarbital (100 mg/kg, i.p.). Catheters were placed in bilateral femoral arteries to measure systemic blood pressure and to obtain arterial blood. The right femoral vein was cannulated for infusion of supplemental anesthetic (20–25 mg/kg/h). The trachea was cannulated, and the animals were mechanically ventilated with room air and supplemental oxygen. Arterial blood gases were monitored and maintained within normal limits throughout the experiments in all animal groups. The animals were immobilized with d-tubocurarine chloride (2 mg/kg, i.v.). Depth of anesthesia was evaluated by applying pressure to a paw or the tail and by observing changes in heart rate or blood pressure. Additional anesthetic was administered when such changes occurred. Body temperature was maintained at 37 °C with a heating pad. A craniotomy was prepared over the ventral brainstem as previously described in detail (Faraci et al., 1987; Kitazono et al., 1998). In brief, after part of the dura was opened, the cranial window was suffused with artificial cerebrospinal fluid (CSF) [temperature, 37 °C; ionic composition (in mmol/l): 132 NaCl, 2.95 KCl, 1.71 CaCl2, 0.65 MgCl2, 24.6 NaHCO3, 3.69 D-glucose] that was bubbled continuously with appropriate gases to maintain normal levels of pH and pCO_2. After this preparation was completed, the window was suffused with CSF for at least 1 h before the experiment.

Diameters of the basilar artery were measured using a microscope equipped with a television camera coupled to an autowidth analyzer (C3161, Hamamatsu Photonics). We examined the responses of the basilar artery to topical application of acetylcholine (10^{-6} and 10^{-5} mol/l; Sigma, St. Louis, MO), sodium nitroprusside (10^{-8} and 10^{-7} mol/l; Sigma) as an NO donor, and (−)-(3*S*,4*R*)-4-(*N*-acetyl-*N*-hydroxyamino)-6-cyano-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol (Y-26763; 10^{-8} and 10^{-7} mol/l) as an opener of ATP-sensitive K^+ channels. We also tested the effects of (*R*)-(+)-*trans*-*N*-(4-pyridyl)-4-(1-aminoethyl)-cyclohexanecarboxamide (Y-27632; 10^{-6} and 10^{-5} mol/l), an inhibitor of Rho-associated kinase, on the baseline diameter of the basilar artery. Acetylcholine, sodium nitroprusside, and Y-27632 were dissolved in water. Y-26763 was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was less than 0.1 %, and at this concentration, DMSO did not cause any significant changes in diameter of the basilar artery (data not shown). The vasodilators were suffused over the craniotomy for 6 min. Internal diameters of the basilar artery were measured immediately before and during the last minute of application of each agonist. After application of a specific agonist, vessel diameters returned to the baseline level within a few minutes before subsequent application of an agonist. The application sequence of agonists was randomized. Because pretreatment of the basilar artery with N^G-nitro-L-arginine (10^{-5} mol/l) almost abolished acetylcholine-induced dilatation of the basilar artery (data not shown), acetylcholine-induced vasodilatation was mediated primarily by NO. Topical application of these agents did not

Table 1
Physiological variables

	WKY	SHR		
		Control	Benidipine (low dose)	Benidipine (high dose)
n	8	7	7	7
Weight, g	407±2	374±11[a]	384±6[a]	369±14[a]
MBP, mm Hg	106±3	193±5[a]	183±5[a]	159±4[a,b,c]
Hematocrit, %	44±1	48±1[a]	47±2[a]	51±1[a]
Glucose, mmol/l	9.71±0.78	8.55±0.78	8.72±1.78	8.33±0.44

Values are means±S.E.M.
MBP indicates mean blood pressure.
[a] $P<0.05$ vs. WKY.
[b] $P<0.05$ vs. Control.
[c] $P<0.05$ vs. Benidipine (low).

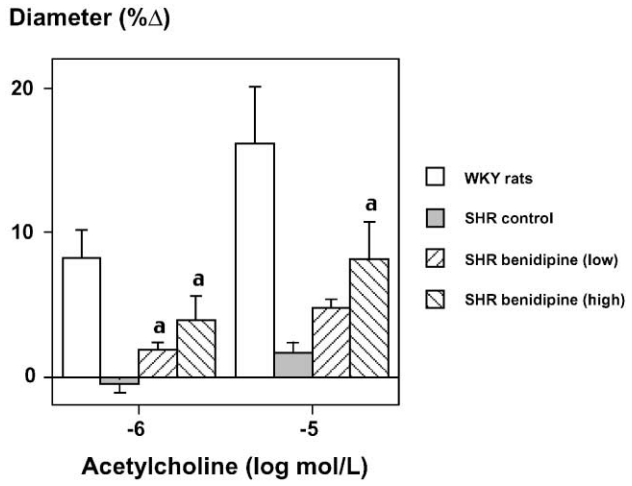

Fig. 1. Acetylcholine-induced dilatation of the basilar artery in WKY rats and SHR. Changes in diameter of the basilar artery in response to acetylcholine (10^{-6} and 10^{-5} mol/l) were measured in WKY rats, untreated SHR, and SHR treated with benidipine (3 and 6 mg/kg/day). Values are means±S.E.M. [a]$P<0.05$ vs. SHR control.

cause any changes in systemic arterial pressure (data not shown).

2.3. Data analysis

All values were expressed as means±S.E.M. One-way repeated-measures analysis of variance (ANOVA) was used to compare physiological variables, measurements of basilar artery, and concentration-dependent responses to vasodilators. Two-way repeated-measures ANOVA was used to compare responses under control conditions and those during interventions. When a significant F value was found, post hoc analysis was made with Wilcoxon's test. A value of $P<0.05$ was considered significant.

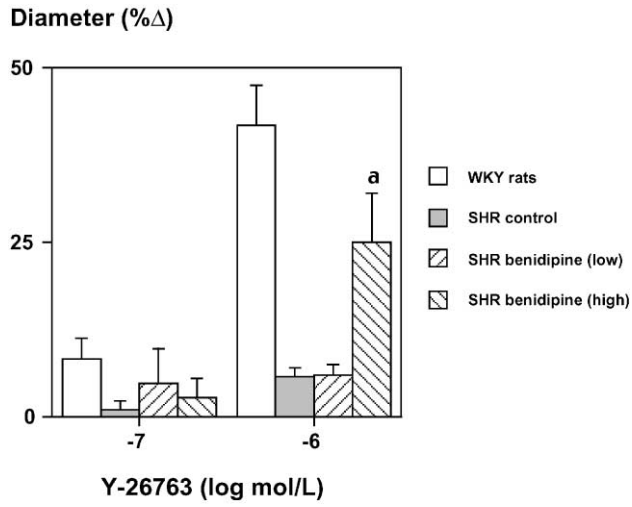

Fig. 3. Y-26763-induced dilatation of the basilar artery in WKY rats and SHR. Changes in diameter of the basilar artery in response to Y-26763 (10^{-7} and 10^{-6} mol/l) were measured in WKY rats, untreated SHR, and SHR treated with benidipine (3 and 6 mg/kg/day). Values are means±S.E.M. [a]$P<0.05$ vs. SHR control.

3. Results

Body weight was significantly lower in the SHR control group than in WKY rats (Table 1). Mean arterial pressure and hematocrit were significantly higher in the SHR control group than in WKY rats. Treatment with benidipine reduced mean arterial pressure but did not affect body weight, hematocrit, or the blood glucose concentrations.

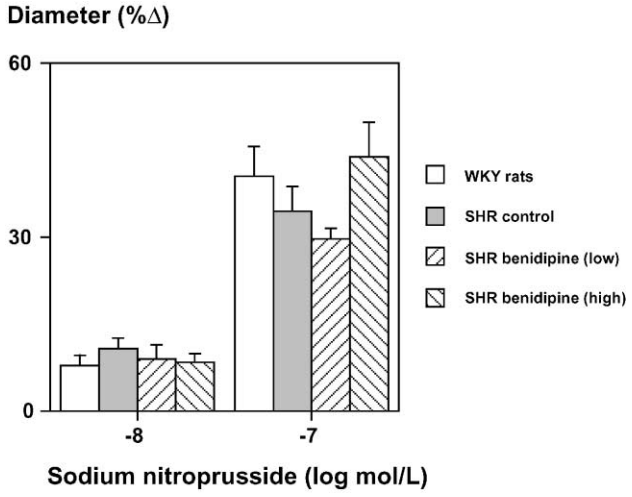

Fig. 2. Sodium nitroprusside-induced dilatation of the basilar artery in WKY rats and SHR. Changes in diameter of the basilar artery in response to sodium nitroprusside (10^{-8} and 10^{-7} mol/l) were measured in WKY rats, untreated SHR, and SHR treated with benidipine (3 and 6 mg/kg/day). Values are means±S.E.M.

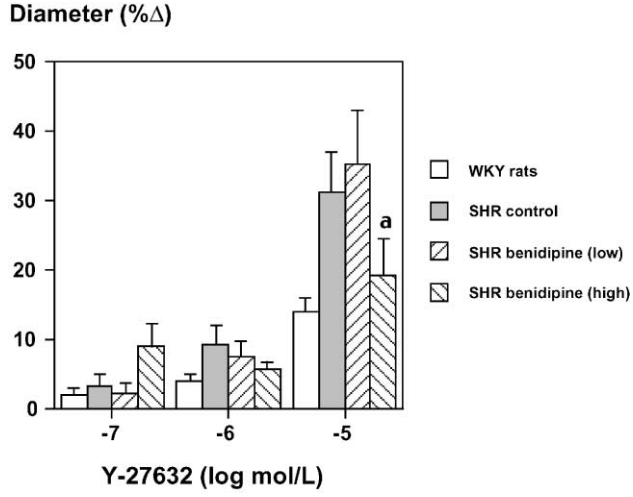

Fig. 4. Y-27632-induced dilatation of the basilar artery in WKY rats and SHR. Changes in diameter of the basilar artery in response to Y-27632 (10^{-7}, 10^{-6}, and 10^{-5} mol/l) were measured in WKY rats, untreated SHR, and SHR treated with benidipine (3 and 6 mg/kg/day). Values are means±S.E.M. [a]$P<0.05$ vs. SHR control.

Table 2
Measurements of basilar artery

	WKY	SHR		
		Control	Benidipine (low dose)	Benidipine (high dose)
External diameter, μm	304±10	272±6[a]	276±4[a]	267±8[a]
Internal diameter, μm	255±9	209±5[a]	226±4[a]	215±6[a]
Wall thickness, μm	24±2	31±1[a]	25±2[b]	26±2
Wall/internal diameter, %	9±1	15±1[a]	11±1[b]	12±1[b]

Values are means±S.E.M.
Wall thickness=(External diameter−Internal diameter)/2.
[a] $P<0.05$ vs. WKY.
[b] $P<0.05$ vs. Control.

In WKY rats, topical application of acetylcholine induced marked dose-dependent dilator responses of the basilar artery (Fig. 1). Acetylcholine-induced dilatation of the artery was significantly reduced in SHR (SHR control) compared with that in WKY rats. Chronic treatment with benidipine (3 and 6 mg/kg) significantly enhanced dilator responses of the basilar artery to acetylcholine (10^{-6} and 10^{-5} mol/l) (Fig. 1). Topical application of sodium nitroprusside caused similar dilatation of the basilar artery, in both WKY rats and the SHR control group, and treatment with benidipine had no significant effects on nitroprusside-induced vasodilatation in SHR (Fig. 2).

Topical application of Y-26763 caused marked dilatation of the basilar artery in WKY rats (Fig. 3). In contrast, the vasodilator responses to Y-26763 were almost abolished in the SHR control group. Treatment of SHR with high-dose benidipine produced a significant increase of the vasodilator responses to 10^{-5} mol/l Y-26763 (Fig. 3).

Y-27632, an inhibitor of Rho-associated kinase, increased the baseline diameter of the basilar artery in WKY rats and the vasodilatation was markedly enhanced in SHR (SHR control). Treatment of SHR with high-dose benidipine reduced the vasodilatation induced by 10^{-5} mol/l Y-27632 (Fig. 4).

The external and internal diameters of the basilar artery in the SHR control group were significantly smaller than those in WKY rats (Table 2). The wall of the basilar artery was significantly thicker in the SHR control group than in WKY rats. The internal diameter of the basilar artery in SHR treated with benidipine tended to increase (Table 2). The wall thickness to internal diameter ratio in both benidipine-treated groups was significantly smaller than that in the SHR control group (Table 2).

4. Discussion

Acetylcholine-induced dilatation of rat basilar artery is mediated primarily by endothelium-derived NO (Kitazono et al., 1993a; Kitayama et al., 2000). It has been shown that chronic hypertension causes marked deterioration of the endothelial function of cerebral blood vessels (Heistad and Baumbach, 1992; Heistad et al., 1993). Because nitroprusside-induced vasodilatation was preserved in SHR as shown in the present study, a reduced production of NO from endothelial cells rather than a decreased sensitivity of vascular muscle to NO may be responsible for the impaired acetylcholine-induced vasodilatation. It has also been reported that antihypertensive agents such as angiotensin-converting enzyme inhibitors and Ca^{2+} channel blockers have beneficial effects on endothelial function, which is impaired in hypertensive rats (Clozel et al., 1990; Yang et al., 1993; Tschudi et al., 1994). In the present study, chronic administration of benidipine ameliorated endothelial functions of the basilar artery in SHR. Dohi et al. (1996) have shown recently that antihypertensive treatment of SHR with benidipine also restored endothelium-dependent relaxation of the renal artery in vitro. These authors have also reported that ecarazine, another antihypertensive agent, reduced blood pressure to a similar extent as with benidipine, but did not restore the impaired vasorelaxation. Thus, it may be possible that besides reducing arterial pressure, Ca^{2+} channel blockers have some beneficial effects on the diseased arteries. In the present study, low-dose benidipine did not reduce arterial pressure but enhanced the acetylcholine-induced dilatation of the basilar artery. The findings also support the interpretation that benidipine, independently of its antihypertensive actions, may also have beneficial effects on the endothelial functions. Kobayashi et al. (1999) reported that the activity of NO synthase was decreased in renovascular hypertensive rats. After treatment with a subdepressor dose of benidipine, they found that the activity of NO synthase and eNOS mRNA expression were increased in the left ventricle of the animals. Benidipine may thus potentially increase NO production by the endothelium (Kobayashi et al., 1999). Beneficial effects on NO production similar to those of benidipine were observed with other Ca^{2+} channel blockers (Zhang and Hintze, 1998).

Activation of ATP-sensitive K^+ channels appears to be another important mechanism of dilatation of the cerebral arteries (Kitazono et al., 1995a). Dilatation of the basilar artery (Kitazono et al., 1993b) and an increase in cerebral blood flow (Takaba et al., 1996) in response to activation of ATP-sensitive K^+ channels, both being independent of endothelial functions, are also impaired in chronic hypertensive rats. In the present study, dilatation of the basilar artery in response to Y-26763, an opener of ATP-sensitive K^+ channels, was impaired in SHR compared with that in WKY rats. Moreover, benidipine partially restored the Y-26763-induced dilatation of the basilar artery in SHR. Only high-dose benidipine improved the vasodilatation, suggesting that the improvement of the reduced vasodilatation by Y-26763 may be essentially mediated by the antihypertensive actions of benidipine. Activation of the K^+ channel elicits vasodilatation through the inhibition of L-type Ca^{2+} channels in vascular muscle, which is the target of dihydropyridine type Ca^{2+} channel antagonists. In SHR, the expression of Ca^{2+} channels was shown to be enhanced compared with that in normoten-

sive rats (Wilde et al., 1994; Ohya et al., 1998). Since Ca^{2+} influx through the activation of Ca^{2+} channels is one of the major mechanisms of vasocontractile responses (Somlyo and Somlyo, 1994), the augmented activity of Ca^{2+} channels in vascular muscle may play an important role in the increased resistance of the cerebral arteries. Thus, chronic treatment of hypertension with a Ca^{2+} channel blocker may effectively decrease the contractile response of the artery, and thereby improve the vasodilator responses to an opener of ATP-sensitive K^+ channels.

Regulation of the Ca^{2+} sensitivity of myofilaments is another mechanism of vasocontractile responses. Rho-associated kinase inhibits myosin phosphatase and thereby increases the Ca^{2+} sensitivity of myofilaments. Chrissobolis and Sobey (2001) have shown that the activity of Rho-associated kinase is augmented in the basilar artery during chronic hypertension. In the present study, we also found that dilatation of the basilar artery in response to Y-27632, an inhibitor of Rho-associated kinase, was enhanced in SHR compared with that in WKY rats and that antihypertensive treatment with benidipine attenuated Y-27632-induced vasodilatation. The findings suggest that benidipine may reduce the increased activity of Rho-associated kinase and thereby attenuate the contractility of the basilar artery in vivo. Because only high-dose benidipine attenuated Y-27632-induced dilatation of the artery, antihypertensive effects of benidipine may be essentially involved in the reduction of the activity of the kinase in the basilar artery.

During chronic hypertension, morphological changes such as wall hypertrophy and remodeling appear to occur and contribute to increased resistance of the cerebral arteries (Baumbach and Heistad, 1989; Heistad and Baumbach, 1992; Heistad et al., 1993). In the present study, we found that the wall thickness of the basilar artery in SHR was greater than that in WKY rats. Thus, either hypertrophy or remodeling may also occur in the basilar artery in SHR. Benidipine reduced the increased thickness of the arterial wall in SHR, suggesting that antihypertensive treatment with the Ca^{2+} channel blocker may also improve such morphological changes during hypertension. Low-dose benidipine, which did not reduce arterial pressure significantly, reduced the wall thickness in SHR. Thus, both antihypertensive actions and other pressure-independent mechanisms may be involved in the improvement of the morphological changes of the basilar artery in SHR. Macroscopic and microscopic examinations of the artery may be useful to elucidate the precise mechanisms.

In summary and conclusion, we evaluated the effects of the long-acting Ca^{2+} channel blocker, benidipine, on hypertension-induced vascular dysfunction of the basilar artery. Benidipine improved dilator responses of the basilar artery to acetylcholine and a K^+ channel opener and reduced the increased activity of Rho-associated kinase in the artery of SHR. Moreover, benidipine treatment reduced the increased thickness of the basilar artery in SHR. Thus, antihypertensive treatment with the Ca^{2+} channel blocker may have beneficial effects on both functional and morphological changes of the basilar artery during chronic hypertension.

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