Disparate Effects of Calcium Antagonists on Renal Microcirculation

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Although calcium antagonists reduce systemic blood pressure, the effects of calcium antagonists on renal preglomerular and postglomerular microcirculation have been suggested to differ. In the present study we examined the vasodilator action of dihydropyridine-type calcium antagonists, including nifedipine, nicardipine, amlodipine, and efonidipine, on afferent and efferent arterioles during angiotensin II (A-II)- and norepinephrine (NE)-induced renal vasoconstriction. Isolated perfused hydronephrotic kidneys were used to directly visualize renal microcirculatory response to calcium antagonists. Both A-II and NE caused marked vasoconstriction of afferent (A-II, 27 ± 2% decrement; NE, 28 ± 2% decrement) and efferent arterioles (A-II, 25 ± 4% decrement; NE, 22 ± 2% decrement). The subsequent addition of nifedipine, nicardipine, and amlodipine reversed the afferent arteriolar vasoconstriction in a dose-dependent manner, and elicited complete vasodilation at 10⁻⁶ M. In contrast, efferent arteriolar vasoconstriction was relatively refractory to the dilator action of these calcium antagonists; maximal dilation observed at 10⁻⁶ M was 21 ± 1% (A-II) and 22 ± 3% (NE) for nifedipine, 25 ± 3% (A-II) and 20 ± 6% (NE) for nicardipine, and 39 ± 6% (A-II) and 37 ± 3% (NE) for amlodipine. In striking contrast, efonidipine dilated not only afferent arterioles, but also efferent arterioles in a dose-dependent manner. At 10⁻⁶ M, efonidipine completely inhibited the afferent (A-II, 89 ± 7% reversal; NE, 99 ± 8% reversal) and efferent arteriolar vasoconstriction (A-II, 93 ± 4% reversal; NE, 87 ± 9% reversal). These findings clearly demonstrate that calcium antagonists dilate the afferent arteriole. Unlike the effects on the afferent arteriole, efferent arteriolar responsiveness to calcium antagonists differ, depending on the type of calcium antagonist. The efonidipine-induced efferent arteriolar vasodilation is probably not related to voltage-operated calcium channels, and may act, in concert with blood pressure lowering effect, to ameliorate glomerular capillary hypertension. (Hypertens Res 1996; 19: 31-36)

Key Words: calcium antagonists, afferent arteriole, efferent arteriole, renal circulation, hypertension

Calcium antagonists are widely used in the field of cardiovascular medicine. Although they were initially used as coronary vasodilators, subsequent investigations have revealed that calcium antagonists reduce blood pressure by direct systemic vasodilator action (1), and thus accelerated the clinical use of calcium antagonists for antihypertensive therapy (2). In addition to blood-pressure-lowering effects, calcium antagonists also dilate the renal vasculature (3). Particular interest has been focused on the renal action of calcium antagonists, since the kidney plays an important role in the regulation of systemic blood pressure and sodium homeostasis (4) and is responsible for the initiation and/or development of hypertension. Conversely, hypertension causes renal hemodynamic changes and the subsequent renal injury. Thus, calcium antagonists may affect the renal outcome of exposure to systemic and glomerular hypertension by modifying systemic blood pressure and renal hemodynamics.

Calcium antagonists specifically inhibit voltage-operated calcium channels, reducing the calcium influx through these channels. It has been demonstrated that in large conduit arteries the ability of calcium antagonists to inhibit norepinephrine-induced vasoconstriction is diminished, as compared with that to reverse KCl-induced vasoconstriction (5). Furthermore, investigative attention was hitherto focused on the effects of calcium antagonists on large conduit vessels and recently expanded to the renal microvascular actions of calcium antagonists. Thus, several investigations have demonstrated that calcium antagonists, including nifedipine, diltiazem, and verapamil, dilate the renal afferent arteriole, but exert only modest action on the efferent arteriole (6-8). In contrast, a newly-developed compound, manidipine, has been reported to elicit efferent arteriolar dilation (9). Therefore, the vasodilator effects of calcium antagonists vary, depending on the type of vessels, underlying vasoconstric-
tor stimuli, and type of calcium antagonist.

The aim of the present study was to delineate the effects of diverse dihydropyridine-class calcium antagonists on renal afferent and efferent arterioles during stimulation with two important vasoconstrictors, angiotensin II and norepinephrine. We anticipated that the present study would help to clarify the role of calcium channels in mediating the vasoconstriction of renal pre- and post-glomerular microvessels.

Methods

Chronic hydronephrosis was established to facilitate subsequent visualization of the renal microcirculation in isolated perfused kidneys as described previously (10-12). Six-week-old male Sprague-Dawley rats were anesthetized with ether. The right ureter of each animal was ligated through a mid-abdominal incision. Following 8-10 weeks, at which time renal tubular atrophy had progressed to a stage that allowed direct microscopic visualization of renal microvessels (13), the kidneys were harvested for perfusion study. The animals had free access to water and regular chow throughout the study. All procedures involving this study were performed following the guidelines of the Animal Care Committee of Keio University.

Donor animals were anesthetized with ether, and the abdominal cavity was exposed by a midline incision. The renal artery of the hydronephrotic kidney was cannulated in situ across the aorta through the superior mesenteric artery. Warm oxygenated media was perfused throughout the cannulation procedure. The hydronephrotic kidney was excised and placed on the stage of an inverted microscope (IMT-2, Olympus, Tokyo, Japan) modified to accommodate a heated chamber equipped with a thin glass viewing port on the bottom surface. Kidneys were allowed to equilibrate for at least 30 min before initiating experimental manipulations.

Kidneys were perfused with media consisting of a Krebs-Ringer bicarbonate buffer containing 5 mmol/l D-glucose, 7.5% bovine serum albumin (Sigma, St. Louis, MO), and a complement of amino acids as described previously (14). The perfusion apparatus is illustrated in our previous publication (10). The perfusion media was saturated with a gas mixture of 95% O2/5% CO2 within a pressurized reservoir. The perfusion pressure, monitored at the level of the renal artery, was altered by adjusting the back-pressure-type regulator (Model 10BP, Fairchild Industrial Products Co., Winston-Salem, NC), which controlled the exit of gas from the media reservoir.

Vessel diameters were measured as described in a previous publication (10-12). In brief, video images from a video camera (model XC-77, Sony, Tokyo, Japan) were recorded with a video cassette recorder and transmitted to a computer (PS55/Model 5551, IBM Japan, Tokyo, Japan) equipped with a video acquisition and display board (Targa 16+, Truevision Inc., Indianapolis, IN). Vessel diameters were estimated with an automated program custom designed to permit determination of the mean distance between parallel edges of the selected microvessels (10-12). A segment of afferent and efferent arterioles approximately 50 μm in length was scanned at intervals of 1-3 seconds. Mean vessel diameter was determined by averaging all measurements obtained during the plateau of the response.

Experimental protocols

Renal microvascular effects of calcium antagonists were assessed during two different vasoconstrictor stimuli (i.e., angiotensin II and norepinephrine; Sigma Chemical Co., St. Louis, MO). Initially, either angiotensin II (0.3 nmol/l) or norepinephrine (0.3 μmol/l) were added to the perfusate to obtain basal vascular tone of the afferent and efferent arteriole. Following the determinations of baseline vasoconstrictor responses, the effects of calcium antagonists, including nifedipine (Sigma), nicardipine (Sigma), amlodipine (Pfizer Pharmaceuticals, Japan), or efonidipine (Nissan Chemical, Saitama, Japan) were assessed. To eliminate pressure-induced changes in vessel diameter, renal perfusion pressure was maintained constant at 80 mmHg, with the use of a back-pressure-type regulator. Nifedipine and nicardipine were dissolved in polyethylene glycol, and amlodipine and efonidipine were dissolved in dimethylsulfoxide (Wako Chemical, Osaka, Japan). The dose of the solvent (i.e., less than 0.01%) had no effects on either basal diameter or responsiveness to calcium antagonists.

Analysis of Data

All data are expressed as the mean ± standard error of the mean. Data were analyzed by analysis of variance followed by multiple comparison post hoc test. P values < 0.05 were considered to indicate statistically significance.

Results

Angiotensin II-Induced Vasoconstriction

Figure 1 depicts representative tracings illustrating the effects of nifedipine on angiotensin II-induced vasoconstriction of both afferent and efferent arterioles. The addition of 0.3 nmol/l angiotensin II elicited marked vasoconstriction of an afferent (from 19.1 to 12.6 μm) and an efferent arteriole (from 16.9 to 12.7 μm). The subsequent addition of nifedipine (from 10−9 to 10−6 mol/l) reversed the afferent arteriolar vasoconstriction in a dose-dependent manner, whereas efferent arteriolar constriction was refractory to the dilator action of nifedipine (Fig. 1).

In contrast to the preferential dilator action of nifedipine, efonidipine vasodilated not only afferent arterioles but also efferent arterioles (Fig. 2). Thus, efonidipine at concentrations of 10−8, 10−7, and 10−6 mol/l dose-dependently reversed the angiotensin II-induced vasoconstriction of both an afferent and an efferent arteriole.

Figure 3 summarizes the reversal by calcium antagonists of angiotensin II-induced vasoconstriction of afferent and efferent arterioles. To facilitate comparison of the effects of calcium antagonists on
Fig. 1. Representative tracings illustrating the reversal by nifedipine of angiotensin II-induced vasoconstriction of renal microvessels. Nifedipine inhibited the afferent arteriolar vasoconstriction in a dose-dependent manner. In contrast, efferent arteriolar vasoconstriction was relatively refractory to the action of nifedipine.

Fig. 2. Representative tracings illustrating the reversal by efonidipine of angiotensin II-induced vasoconstriction of renal microvessels. In contrast to the effect of nifedipine, efonidipine inhibited both afferent and efferent arteriolar vasoconstriction in a dose-dependent manner.

Fig. 3. Graphs showing the effects of calcium antagonists on angiotensin II-induced vasoconstriction of afferent and efferent arterioles. * p < 0.05 vs. afferent arteriolar response.

Norepinephrine-Induced Vasoconstriction

Fig. 4. Graphs showing the effects of calcium antagonists on norepinephrine-induced vasoconstriction of afferent and efferent arterioles. * p < 0.05 vs. afferent arteriolar response.

affluent and efferent arterioles, the results are expressed as the reversal by calcium antagonists of angiotensin II-induced vasoconstriction. Angiotensin II elicited marked vasoconstriction of afferent (from 19.0 ± 0.7 to 13.9 ± 0.7 μm, p < 0.001, n = 23) and efferent arterioles (from 16.2 ± 0.5 to 12.0 ± 0.8 μm, p < 0.001, n = 22). As depicted, nifedipine, nicardipine, and amlodipine dose-dependently reversed the angiotensin II-induced vasoconstriction of afferent arterioles. Thus, at a concentration of 10⁻⁹ mol/l, nifedipine, nicardipine, and amlodipine returned the afferent arteriolar diameter from 13.5 ± 1.1 to 14.6 ± 0.9 μm (p < 0.01, n = 6), from 14.1 ± 0.8 to 15.1 ± 0.6 μm (p < 0.01, n = 5), and from 14.0 ± 1.0 to 15.1 ± 0.8 μm (p < 0.01, n = 6), respectively. At 10⁻⁶ mol/l, these calcium antagonists completely reversed the afferent arteriolar vasoconstriction (nifedipine, 101 ± 3%; nicardipine, 103 ± 10%; amlodipine, 108 ± 7%).

In contrast, efferent arteriolar vasoconstriction was resistant to the vasodilator action of these calcium antagonists. Thus, 10⁻⁹ mol/l nifedipine, nicardipine, and amlodipine inhibited the efferent arteriolar constriction by only 21 ± 1% (n = 6), 25 ± 3% (n = 5), and 39 ± 6% (n = 5), respectively. In striking contrast, efonidipine induced similar vasodilatory effects on afferent and efferent arterioles. Thus, efonidipine dose-dependently restored the afferent arteriolar diameter, with 89 ± 7% reversal (n = 6) at 10⁻⁶ mol/l. Similarly, efonidipine markedly reversed efferent arteriolar vasoconstriction; 10⁻⁸, 10⁻⁷, and 10⁻⁶ mol/l efonidipine inhibited the efferent arteriolar vasoconstriction by 44 ± 12%, 83 ± 8, and 93 ± 4% (n = 6), respectively.

Norepinephrine-Induced Vasoconstriction
Norepinephrine elicited marked decreases in both afferent (from 18.8 ± 0.4 to 13.5 ± 0.4 μm, p < 0.01, n = 22) and efferent arteriolar diameter (from 17.9 ± 0.4 to 14.0 ± 0.6 μm, p < 0.01, n = 22) (Fig. 4). Similar to their effects on angiotensin II-induced vasoconstriction, nifedipine, nicardipine, and amlodipine all predominantly reversed the norepinephrine-induced vasoconstriction of afferent arterioles. Thus, 10⁻⁶ mol/l nifedipine, nicardipine, and amlodipine reversed the norepinephrine-induced vasoconstriction by 91 ± 1% (n = 6), 105 ± 7% (n = 5) and 97 ± 3% (n = 6), respectively, whereas efferent arterioles manifested only 22 ± 5% (n = 6), 20 ± 6% (n = 5), and 37 ± 3% reversal (n = 6) in response to 10⁻⁶ mol/l nifedipine, 10⁻⁶ mol/l nicardipine, and 10⁻⁶ mol/l amlodipine, respectively.

In contrast, the renal microvascular responses to efonidipine differed as compared with those of other calcium antagonists. Efonidipine reversed both afferent and efferent arteriolar vasoconstriction in a dose-dependent manner. Thus, 10⁻⁸, 10⁻⁷, and 10⁻⁶ mol/l efonidipine inhibited afferent arteriolar vasoconstriction by 40 ± 14%, 63 ± 10%, and 99 ± 8%, respectively (n = 5). Similarly, efonidipine reversed the efferent arteriolar vasoconstriction by 28 ± 3% (10⁻⁸ mol/l), 59 ± 7% (10⁻⁷ mol/l), and 87 ± 9% (10⁻⁶ mol/l), respectively (n = 5).

Discussion
Calcium antagonists are potent vasodilators, affecting not only the systemic resistance vessel but also the renal microvasculature (2, 3). Several lines of evidence have demonstrated that voltage-operated calcium channels, target channels for calcium antagonists, prevail in renal afferent arterioles (15-17). In contrast, these channels are sparse or functionally silent in efferent arterioles. As a consequence of these differences, it has been demonstrated that dihydropyridine-class calcium antagonists preferentially dilate the afferent arteriole, whereas the efferent arteriole is relatively refractory to the vasodilator action of calcium antagonists (6-8). Conceivably, the preferential dilation of the afferent arteriole by calcium antagonists could elevate the glomerular capillary pressure, with consequent adverse effects on the glomerulus (18-20). In this regard, it has recently been suggested that newly-synthesized dihydropyridine-type calcium antagonists, including manidipine and efonidipine, decrease filtration fraction (21, 22), suggesting that these calcium antagonists not only dilate the afferent arteriole but also reduce the efferent arteriolar tone. It has not been directly determined, however, whether these new calcium antagonists may elicit efferent arteriolar vasodilation.

The present study demonstrates that effects of calcium antagonists on the renal microvasculature differ, depending on the type of calcium antagonist used. Calcium antagonists markedly inhibit the afferent arteriolar vasoconstriction induced by both angiotensin II and norepinephrine. In accordance with previous reports (6-8), both nifedipine and nicardipine are much less potent in inhibiting efferent arteriolar vasoconstriction. Amlodipine mildly dilates the efferent arteriole, although the afferent arteriole is a predominant site of vasodilator action. In striking contrast, efonidipine inhibits both afferent and efferent arteriolar vasoconstriction during angiotensin II- and norepinephrine-induced vasoconstriction; the ability of efonidipine to reverse afferent and efferent arteriolar vasoconstriction was nearly identical. These findings support the hypothesis that the effects of calcium antagonists on the efferent arteriole vary, depending on the type of calcium antagonist. The disparate effects of calcium antagonist on the efferent arteriole suggest that the efonidipine-induced vasodilation of this vessel is attributable to non-class effects of calcium antagonists.

Voltage-operated calcium channels are widely distributed in the systemic vasculature. In the renal microcirculation, however, several lines of investigations failed to provide evidence for operation of voltage-operated calcium channels in the efferent arteriole (15-17). Loutzenhiser et al. (15) previously demonstrated that KCl-induced vasoconstriction was predominantly observed in the afferent arteriole, and was completely reversed by nifedipine. These findings suggest that KCl-induced depolarization elicits opening of voltage-operated calcium channels.
predominantly in the afferent arteriole. Furthermore, using calcium-sensitive dyes, Carmines et al. (16) demonstrated that KCl causes a preferential increment in intracellular calcium, whereas efferent arteriolar calcium concentration did not increase, but actually decreased, in response to KCl-induced depolarization. Additionally, Steinhausen et al. (17) reported that Bay K 8644, a calcium channel agonist, elicited a preferential preglomerular vasoconstriction. Collectively, these observations support the formulation that voltage-operated calcium channels prevail in the afferent arteriole, whereas these channels or their function are lacking in the efferent arteriole.

The role of voltage-operated calcium channels in angiotensin II- or norepinephrine-induced vasoconstriction may differ in preglomerular and postglomerular microvessels. Thus, the present study suggests that both angiotensin II- and norepinephrine-induced afferent arteriolar vasoconstriction is mediated by activation of voltage-operated calcium channels; the ability of nifedipine to reverse angiotensin II- and norepinephrine-induced afferent arteriolar vasoconstriction was similar to that of nifedipine to reverse KCl-induced vasoconstriction (15). In striking contrast, nifedipine, nicardipine, and amlopidine failed to reverse, or only modestly inhibited, angiotensin II-induced efferent arteriolar vasoconstriction. In contrast to these calcium antagonists, the present study also demonstrates that efonidipine completely inhibits both angiotensin II- and norepinephrine-induced vasoconstriction of the efferent arteriole, where voltage-operated calcium channels are sparse or functionally silent. It is reasonable, therefore, to postulate that efonidipine not only inhibits voltage-operated calcium channels but also affects other mechanisms for smooth muscle contraction. In this regard, Nishijima et al. (23) have recently suggested in a preliminary report that efonidipine, by affecting intracellular calcium release mechanism, reduced intracellular calcium concentration. Regardless of whether this mechanism is operative in renal microvessels, it is obvious that efonidipine possesses potent vasodilator action that is independent of voltage-operated calcium channels.

The long-term renal protective effects of calcium antagonists in hypertension remain to be elucidated (24). Because calcium antagonists elicit predominant dilation of the afferent arteriole and reduce systemic blood pressure, the net effects of calcium antagonists on glomerular microcirculation depend on the balance between blood pressure reduction and the afferent arteriolar vasodilation. The altered glomerular hemodynamics would modify the glomerular barotrauma induced by systemic hypertension. In addition, calcium antagonists may reduce glomerular hypertrophy, resulting in a decrease in capillary wall tension (25). Thus, our laboratory reported that amlopidine preserved renal function in subtotally nephrectomized SHR (26). Conversely, in patients with diabetic nephropathy, nifedipine has been reported to accelerate the progression of renal injury (20, 27), suggesting that selective afferent arteriolar vasodilation could exacerbate intraglomerular hypertension. It has not been determined fully, therefore, whether calcium antagonists may ameliorate or aggravate the hypertensive renal injury. Nevertheless, the present study reveals that efonidipine, a novel calcium antagonist, dilates both afferent and efferent arterioles. It is possible, therefore, that efonidipine could lower blood pressure without increments in glomerular capillary pressure. This premise is consistent with the observations that efonidipine reduces filtration fraction in vivo (22) and prominently suppresses increases in proteinuria and progressive renal injury despite partial amelioration of hypertension (28, 29). Clearly, further investigations are required to elucidate the long-term effects of calcium antagonists on hypertensive renal injury.

In conclusion, the present study demonstrates that all calcium antagonists studied dilate angiotensin II- and norepinephrine-induced afferent arteriolar vasoconstriction. In contrast to consistent effects on the afferent arteriole, the effects of calcium antagonists on the efferent arteriole differ, depending on the type of antagonist. Such differences in the efferent arteriolar action of efonidipine may offer favorable renal protective effects in hypertensive kidneys in addition to the beneficial effects induced by lowering blood pressure (28, 29).

References