Neural Effects on Renal Blood Flow During Acute Hypotension Vary With Antihypertensive Drugs

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Abstract

To examine the neural effects of antihypertensive drugs on renal blood flow, we measured blood flow and renal sympathetic nerve activity simultaneously in conscious spontaneously hypertensive rats aged 13 to 15 weeks. One to two days after surgery, intravenous administration of manidipine (calcium antagonist, n=10), doxazosin (α₁-adrenergic receptor antagonist, n=9), and clonidine (n=7) lowered mean arterial pressure by at least 20% from baseline levels. Manidipine initiated a reduction of renal flow when mean pressure decreased by 20±2 mm Hg. At the maximal decrease in renal blood flow (mean pressure, −33±2 mm Hg), percent decrease in flow (−27±2%) significantly correlated with percent increase in renal nerve activity (+205±40%, r=-.878). Doxazosin began to decrease renal blood flow at a level of arterial pressure similar to that in manidipine treatment, whereas the maximal decrease in flow (−19±2%; mean pressure, −33±2 mm Hg; nerve activity, +225±44%) was significantly less than that in manidipine treatment. Although clonidine decreased arterial pressure and renal nerve activity, renal blood flow did not decrease even at the maximal decrease in mean pressure of 29±1 mm Hg. The addition of clonidine to manidipine treatment suppressed reflexly enhanced renal nerve activity and restored blood flow to the pretreatment level despite pronounced hypotension. These results clearly demonstrate that antihypertensive drugs with blocking action on renal nerve activity are capable of maintaining renal blood flow and that those associated with reflex-induced enhancement of nerve activity exert deteriorating effects on renal blood flow. Furthermore, a decrease in renal blood flow induced by calcium antagonists is mainly attributed to reflexly enhanced renal nerve activity. (Hypertension. 1994;23[suppl I]:I-97-I-101.)

Key Words • renal circulation • clonidine • adrenergic α receptor blocker • calcium • rats • sympathetic nervous system • antihypertensive agents

Enhancement of renal sympathetic nerve activity (RSNA) leads to a variety of abnormalities of renal function through changing renal vascular resistance, renal blood flow (RBF), renin release, and urinary sodium and water excretions.1-3 Treatment with antihypertensive drugs that have vasodilating actions is known to enhance RSNA reflexly, which may in turn adversely affect renal function.

To examine the neural effects of antihypertensive drugs on RBF directly and quantitatively under physiological conditions, we measured RBF and RSNA simultaneously in conscious and unrestrained spontaneously hypertensive rats (SHR) during acute hypotension induced by clonidine (central α₂-adrenergic receptor agonist), manidipine (calcium antagonist), and doxazosin (α₁-adrenergic receptor antagonist).

Methods

Male 8-week-old SHR were purchased from Charles River Japan Inc (Kanagawa, Japan). All procedures were in accordance with institutional guidelines. In SHR at 13 to 15 weeks of age and under pentobarbital sodium anesthesia (200 μmol/kg [50 mg/kg] IP and 40 to 60 μmol [kg⁻¹ · h⁻¹] [10 to 15 mg · kg⁻¹ · h⁻¹] IV as a supplemental dose), polyethylene catheters were placed in a femoral artery and vein for pressure measurement with a pressure transducer (P23ID, Gould, Oxnard, Calif) and administration of drugs, respectively. RBF and RSNA were recorded as described previously.7 Briefly, the right renal artery was exposed via a retroperitoneal approach, and a miniature pulsed Doppler flow probe (DBF 1.0 gauge, Crystal Biotech, Hopkinton, Mass) was placed around the artery, with great care taken to not damage the renal nerves.6 After closure of the right flank incision, a branch of the left renal nerves was dissected retroperitoneally and placed on a bipolar silver-wire electrode (AGST, Medwire, Mount Vernon, NY). The electrode was fastened to the nerve and insulated with a small amount of silicone gel (Silgel 604, Wacker, Munich, Germany). Catheters and lead wires from the recording electrode and flow probe were exteriorized through the dorsal skin of the neck and then fixed to the skin.

Changes in blood flow velocity were measured as the Doppler shift in kilohertz by a pulsed Doppler flow/dimension system (VF-I, Crystal Biotech). Assessment of blood flow velocity with the pulsed Doppler system has been shown to be directly and linearly related to volume flow.6 Although volume flow is not derived quantitatively, percent changes in flow can be calculated accurately. Therefore, changes in RBF were expressed as percent changes from basal mean Doppler shift in kilohertz. Relative vascular resistance (RVR) was derived as follows: RVR (mm Hg/kHz) = Mean Arterial Pressure (MAP) / Doppler Shift, and percent changes in renal vascular resistance were calculated using this resistance value. For measurement of RSNA,7 original renal nerve signals were amplified (DPA-100E, Dia Medical System, Tokyo, Japan) and filtered (100 to 1000 Hz). The output from the amplifier was fed into a spike counter (DSE-325, Dia Medical System), which identified spikes exceeding a preselected level. The renal neurogram and Doppler shift of flow signal along with arterial

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pressure pulse were stored on a magnetic tape recorder (RD-100 T, TEAC, Tokyo, Japan) for later analysis. The cutoff level of the spike counter was set to filter out the background noise that persisted after injection of phenylephrine hydrochloride (24.5 nmol [5 µg]) or after euthanasia by overdose of pentobarbital. The number of nerve spikes per 1 or 2 seconds was continuously displayed on a chart recorder (RJG-4128, Nihon Kohden, Tokyo, Japan) together with pulsatile pressure and MAP, heart rate triggered by arterial pressure pulse, and RBF as the Doppler shift. Changes in RSNA were expressed as percent changes from basal spike counts. At least 18 hours after surgery, experiments were carried out on each conscious and unrestrained rat placed into a plastic bowl with a diameter of 18 cm. After a stabilization period of at least 30 minutes, the following drugs were administered intravenously in doses sufficient to lower MAP by at least 20% from the pretreatment value: clonidine hydrochloride (Sigma Chemical Co, St Louis, Mo; 19 to 56 nmol/kg [5 to 15 µg/kg], 7 rats), manidipine hydrochloride (2.9 to 29 nmol·kg⁻¹·min⁻¹ [2 to 20 µg·kg⁻¹·min⁻¹], 10 rats), and doxazosin mesylate (0.9 to 1.8 µmol/kg [0.5 to 1 mg/kg], 9 rats). When two or more kinds of drugs were applied to one rat, the administration of drugs was separated by 24 hours. In some of the rats given manidipine or doxazosin, clonidine (19 nmol/kg [5 µg/kg]) was added while RBF was reduced by a preceding drug.

Values are expressed as mean±SEM. The data were analyzed by either Student’s t test or repeated-measures analysis of variance where appropriate. Linear regression was calculated by the least-squares method. A value of P<.05 was considered statistically significant.

Results

Representative tracings of RSNA and pulsatile RBF measured by Doppler shift are shown before and after manidipine infusion in Fig 1.

After clonidine injection, MAP rose transiently and then decreased gradually from the preinjection value of 135±4 to 106±4 mmHg in association with a pronounced decrease in RSNA (−84±2% maximally). There was no significant reduction of RBF (Fig 2) associated with a 26±2% reduction in RVR at the maximal decrease in MAP. In fact, RBF significantly increased by 6±2% at a 20% reduction in MAP. At the maximal decrease in MAP, the percent decrease in RSNA (−70±7%) significantly correlated with a decrease in heart rate (−30±7 beats per minute; r=.920, P<.005) but not with that in MAP.

Manidipine infusion at a dosage of 2 to 20 µg·kg⁻¹·min⁻¹ decreased MAP from 132±3 to 98±2 mmHg, increased heart rate from 342±19 to 469±15...
Manidipine

MRBF (kHz)

beats per minute, and enhanced RSNA by 205±40%. Although RBF increased initially by 4±1%, it began to decline when MAP fell by a mean of 20±2 mm Hg associated with enhancement of RSNA by 124±23% (Fig 2). At this point, RVR was decreased by 19±2%. Thereafter, RBF gradually decreased: -10±2% at a 20% reduction of MAP and -25±3% at the nadir of MAP. At the maximal decrease in RBF (MAP, -33±2 mm Hg from preinfusion value), RVR was not altered (3±2%), and percent decrease in RBF (-27±2%) significantly correlated with percent increase in RSNA (r=-.879, P<.001). After cessation of the infusion around the nadir of MAP, an additional injection of clonidine suppressed the enhanced RSNA and decreased further both MAP and RBF (Fig 3). When MAP was restored to the level at which RBF was maximally reduced by manidipine alone, RBF recovered to the premanidipine level. bpm, beats per minute.

Doxazosin decreased MAP rapidly from 133±4 to 97±4 mm Hg, increased heart rate from 333±12 to 430±19 beats per minute, and augmented RSNA by 234±44%. An increase in heart rate during a decrease in MAP was significantly less than that in manidipine treatment (F=5.428, P<.05). On the other hand, an enhancement of RSNA was greater than that in manidipine treatment (F=9.177, P<.01, Fig 2). RBF began to decrease when MAP decreased by a mean of 19±2 mm Hg. At the maximal decrease in RBF (MAP, -33±2 mm Hg), the percent decrease in RBF (-19±2%) was significantly less than that obtained through manidipine infusion (P<.05) and did not correlate with percent increase in RSNA (r=.136). In contrast to manidipine infusion, RVR was significantly reduced (-13±2%, P<.005 versus that with manidipine treatment). An addition of clonidine restored RBF by 11±2% (n=6). This level of RBF was 8±2% less than that before doxazosin treatment, resulting in a 17±1% reduction in RVR.

Discussion

The results of this study using the direct and physiological approach demonstrate that drug-induced augmentation of RSNA decreases RBF after a critical reduction of arterial pressure and that suppression of RSNA restores RBF despite pronounced hypotension. An intravenous administration of clonidine depressed RSNA markedly. RBF, however, did not decrease despite a reduction of MAP to the degree at which RBF declined in both manidipine and doxazosin treatment. This finding, that maintenance of RBF is associated with a reduction of RVR during acute hypotension, is relevant to the work of Smits and Struyker-Boudier10 conducted in conscious SHR. Concerning renal a-adrenergic receptors in SHR, a2-receptor density is increased more than a1-receptors.11 The renal vascular responses to renal nerve stimulation, however, have been reported to be mediated by a1-receptors but not to any significant extent by a2-receptors.12 Although clonidine has minimal renal vasoconstrictor activity through a1- and a2-adrenergic receptors when injected into the renal artery,12 its systemic administration elicits suppression of sympathetic nerve activity as shown in the present study through activation of central a2-adrenergic receptors.13 Therefore, preservation of RBF during acute hypotension may be mainly attributed to this sympatholytic action. The addition of clonidine to the preceding drug treatment might be adequate in evaluating the effects of enhanced RSNA on RBF or renal vasoconstriction through a1-adrenergic receptors in SHR. On the other hand, the possibility that clonidine may exert vasodilating effects in addition to its inhibitory action on sympathetic outflow to resistant vessels could not be excluded. Stimulation of presynaptic a2-receptor of sympathetic nerves, selective reduction of sympathetic tone to the veins,14 and nitric oxide released through activation of a2-receptor15 of the
endothelium might contribute to preserving RBF during acute hypotension. However, experimental data on these possible actions of clonidine in the renal vasculature have been scarce. Studies would be needed to clarify the issues.

Manidipine, a new dihydropyridine derivative, has been reported to preserve RBF in an animal model and humans with hypertension. In accordance with our preliminary report, intravenous administration of manidipine elicited a slight increase in RBF in the initial phase of the infusion and decreased RBF after MAP was reduced by a mean of 20 mm Hg from the preinfusion level. RSNA progressively increased in parallel with a progressive reduction of MAP. With respect to the role of an enhancement of RSNA in renal hemodynamics, Persson et al. documented that even modest sympathetic activation by bilateral carotid occlusion that did not induce changes in RBF itself shifted the lower limits of autoregulatory response of RBF to the upper level of renal perfusion pressure in conscious dogs. Thus, any reduction of arterial pressure under these conditions would decrease RBF. In the present study, a gradual reduction of arterial pressure and increasing enhancement of RSNA conceivably surpassed the vasodilating effects of manidipine, resulting in a progressive decrease in RBF. At the nadir of RBF in the present study, renal RVR was not altered, indicating the lack of renal vasodilation at this point. A decrease in RBF during acute hypotension induced by calcium antagonists could be attributed to an enhancement of RSNA through arterial baroreceptor reflex. This assumption was supported by the work of Brody et al. conducted in rats with sinoaortic denervation. They demonstrated that in the sinoaortic-denervated group, nisoldipine, another dihydropyridine derivative, significantly reduced renal RVR, whereas in the baroreceptor reflex-intact group, the drug decreased RBF and had no effect on resistance. Furthermore, a highly significant correlation was revealed between percent decrease in RBF and percent increase in RSNA at the nadir of RBF attained in the present study. The addition of clonidine restored RBF to the control level in association with suppression of reflexly enhanced RSNA elicited by manidipine treatment. A possible contribution of angiotensin II to a decrease in RBF during acute hypotension should be considered. However, its contribution might be small because calcium antagonists have been reported to counteract angiotensin II-induced renal vasoconstriction more effectively than a norepinephrine-induced one. Collectively, these findings suggest that a decrease in RBF during the treatment with calcium antagonists is mainly attributed to reflexly enhanced RSNA.

Doxazosin, a highly selective α1-adrenergic receptor antagonist, showed less tachycardiac response despite greater enhancement of RSNA when compared with that in manidipine treatment. This may be explained by its lack of blocking activity of prejunctional α2-adrenergic receptors, which inhibit norepinephrine release. Greater enhancement of RSNA might be due to faster reduction of arterial pressure, resulting in more activation of baroreceptor reflex. Doxazosin began to decrease RBF at a level of reduction of MAP similar to that with manidipine treatment. However, the maximal decrease in RBF was significantly less than that with manidipine treatment. Although α1-adrenergic receptor blockade with doxazosin may explain this less deteriorating effect on RBF, a modest contribution of reflexly augmented RSNA to a decrease in RBF was suggested by an additional injection of clonidine, indicating insufficient blockade in the kidney in the face of marked augmentation of RSNA in the present study. In addition, a contribution of angiotensin II to a decrease in RBF with doxazosin treatment may be possible because doxazosin has no effects on pressor responses to angiotensin II.

In conclusion, after a critical reduction of MAP by approximately 20 mm Hg, reflex-induced enhancement of RSNA played a more important role in decreasing RBF in manidipine treatment than in doxazosin treatment. Clonidine itself did not decrease RBF, and its addition to manidipine treatment restored RBF to the pretreatment level despite pronounced hypotension. These results demonstrate that during acute reduction of blood pressure, antihypertensive drugs with blocking action on RSNA are capable of maintaining RBF, and those associated with reflex-induced enhancement of RSNA exert deteriorating effects on RBF. A decrease in RBF during the treatment with calcium antagonists appears to be mainly due to reflexly enhanced RSNA.

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References
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