Neurotransmitter Release and Vascular Responsiveness in Mesenteric Vasculatures from Two-Kidney, One-Clip Goldblatt Hypertensive Rats

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SUMMARY

In order to investigate the peripheral neuroeffector functions of renal hypertension, norepinephrine release from the sympathetic nerve endings and vascular responsiveness were evaluated in the mesenteric vasculatures from two-kidney, one-clip Goldblatt hypertensive rats (2K, 1C-HT). Norepinephrine release and pressor responses during periarterial nerve stimulation (40 V, 5 msec rectangular pulses for 1 min, 5, 10 and 15 Hz) were unchanged during the acute phase (3 weeks after surgery), and were rather reduced during the chronic phase (7–8 weeks after surgery) in 2K, 1C-HT compared to sham-operated normotensive control rats. By contrast, the vasoconstrictor responses to exogenously applied norepinephrine were significantly greater in 2K, 1C-HT than in the normotensive controls. From these observations, it seems unlikely that peripheral adrenergic function in the blood vessels plays an important role in the pathogenesis of 2K, 1C-HT. However, the increased sensitivity of vascular smooth muscle cells to norepinephrine may contribute to the maintenance of high blood pressure in the face of reduced vascular sympathetic tone in this form of hypertension.

Additional Indexing Words:

Two-kidney, one-clip hypertensive rats Norepinephrine release
Sympathetic nervous system Vascular responsiveness Mesenteric vasculature

IT is well known that adrenergic neurotransmission in blood vessels has an important role in the regulation of vascular tone both in vitro and in vivo. Much evidence has indicated that functional changes in the neuro-muscular junctions could contribute to an increase in vascular resistance in hyperten-

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sion.¹⁾⁻⁵⁾ We also reported that neurotransmitter release from the adrenergic nerve endings was increased in spontaneously hypertensive rats (Okamoto and Aoki strain) and DOCA-salt hypertension.⁶⁾⁻⁹⁾ However, few studies have been carried out to clarify the alterations of neurotransmitter release in renal hypertension. This study was performed to examine the norepinephrine release and vascular responsiveness in the mesenteric vasculatures from 2K, 1C-HT, and to evaluate the role of adrenergic neurotransmission in the pathogenesis of this form of renal hypertension.

MATERIALS AND METHODS

All studies were performed on male Wistar rats (150–170 g of body weight). Rats were anesthetized with pentobarbital (intraperitoneal injection, 40 mg/kg), and a silver clip (2×8 mm) was placed across the left renal artery with the right kidney untouched. Normotensive control rats did undergo sham treatment. Experiments were carried out 3 weeks (acute phase) and 7–8 weeks (chronic phase) after surgery.

Rats were anesthetized with pentobarbital (40 mg/kg, intraperitoneal injection), and isolated mesenteric vasculatures were prepared according to the modified method of Castellucci et al.^{10),11)} The superior mesenteric artery was perfused through a cannula inserted into the artery at its origin from the aorta. In each preparation of mesenteric vasculatures, only four main branches from the mesenteric artery trunk were used. All other branches were ligated.

The isolated mesenteric preparations were placed in a glass chamber (maintained at 37°C) and gently laid on a wire gauze so as to avoid any traction on the cannulated artery. The preparations were perfused with a modified Ringer-Locke solution (mmol/l: NaCl 120.7, KCl 5.9, CaCl₂ 2.5, MgSO₄ 1.3, NaHCO₃ 15.5, NaH₂PO₄ 1.2 and glucose 11.5, pH 7.4, 37°C), bubbled with a 95% O₂ and 5% CO₂ mixture. The flow rate of the perfusion was maintained constant by means of a peristaltic pump (Harvard Apparatus, Model 1200) at 0.8 ml/min. Changes in the perfusion pressure were monitored by a pressure transducer connected to a polygraph (Nihon Kohden, Model 620G) (Fig. 1).

Periarterial nerve stimulation was performed with bipolar platinum electrodes around the proximal end of the mesenteric artery. The stimulation was applied at supramaximal voltage (40 V) with rectangular pulses of 5 msec duration for 1 min at 5, 10 and 15 Hz using an electrical stimulator (Nihon Kohden, Model SEN-3201). Exogenous norepinephrine (0.10–3.3 µg in 0.1 ml of buffer) was given as a bolus injection through the arterial can-

Fig. 1. Schematic drawing of the perfusion system of the mesenteric preparation and the procedure of norepinephrine measurement in the perfusate.

nula. Pressor responses to electrical nerve stimulation or exogenous norepinephrine were determined as the increase in the perfusion pressures of the preparations. A 30 min-equilibration period was allowed for the basal perfusion pressure to stabilize before electrical nerve stimulation or exogenous norepinephrine application.

In order to measure the norepinephrine release from the sympathetic nerve endings in vascular beds, the perfusate through the mesenteric preparation was collected into a tube containing EGTA (90 mg/ml) and glutathione (60 mg/ml) in the ratio of 20 μ l/ml of the perfusate. The collecting periods were (A) a period of 3 min duration before the electrical nerve stimulation and (B) a 1 min stimulation period followed by a 2 min period after the stimulation. Norepinephrine release during electrical nerve stimulation was defined as the difference of norepinephrine overflow into the perfusate during (A) and (B), and was normalized as ng/g of wet tissue weight for each preparation. The samples were stored at -85°C , and the measurement of norepinephrine in the perfusate was performed according to the following procedure. Fifty mg of alumina was added into each sample, which was stirred with 500 μ l of Tris-HCl buffer (pH 8.6) for 5 min. After washing the alumina with distilled water, the norepinephrine was extracted in 200 μ l of 0.1 mol/l perchloric acid, and was assayed by high performance liquid chromatography (HPLC) with an electrochemical detector (Bioanalytical

System, Model LC-4A, carbon electrode, 700 mV).¹²⁾ The HPLC unit was equipped with a delivery system (Waters Assoc., Model 510, flow rate 1.0 ml/min), an automatic injector (Waters Assoc., Model 710B, injected volume 100 μ l) and a Chemosorb 3-ODS-H column (Chemco Scientific Co., Ltd., size 4.6 $\times$ 125). The mobile buffer for the separation of norepinephrine was 0.1 mol/l of monochloroacetic acid with 2.0 mmol/l of EDTA, 300 mg/l of sodium octyl sulfate and 9% (v/v) of acetonitrile (pH 3.02 at room temperature).

Previously, we showed that the pressor responses and norepinephrine release were completely blocked by guanethidine, which provided evidence that the periarterial stimulation was neuronal in nature.¹³⁾ Further, these responses were stable during at least seven repeated stimuli in the same preparation.¹³⁾ The studies were performed during this stable period.

Values are expressed as means $\pm$ SEM. Statistical significances were determined by Student's t-test. Differences of $p < 0.05$ were considered significant.

All drugs were obtained from Sigma Chemical Co., Ltd. (USA).

RESULTS

Systolic blood pressure, measured by the tail-cuff method (programmed electro-sphygmomanometer, Narco Biosystem Inc., Model PE-300) was 158.0 ± 3.8 mmHg ($n=8$) during the acute phase and 167.6 ± 4.7 mmHg ($n=7$) during the chronic phase for 2K, 1C-HT. The values in the sham-operated control rats were lower, reaching 115.7 ± 5.8 mmHg ($n=8$) during the acute phase and 124.8 ± 3.9 mmHg ($n=7$) during the chronic phase. There were no significant differences in the basal perfusion pressure between 2K, 1C-HT and sham-operated controls (acute phase: 2K, 1C-HT 27.9 ± 0.4 mmHg, $n=8$, sham controls 27.7 ± 1.0 mmHg, $n=8$; chronic phase: 2K, 1C-HT 25.0 ± 1.0 mmHg, $n=7$, sham controls 23.0 ± 1.0 mmHg, $n=7$).

Figure 2 illustrates a typical pressor response to electrical nerve stimulation (5, 10, 15 Hz) and exogenously applied norepinephrine (0.10, 0.25, 1.0, 3.3 μ g/0.1 ml of buffer, bolus injection). As summarized in Table I, the pressor responses to electrical nerve stimulation were unaltered during the acute phase in 2K, 1C-HT compared to sham-operated controls. Similarly, no significant difference between 2K, 1C-HT and control responses to electrical nerve stimulation appeared during the chronic period. By contrast, the pressor responses to intermediate to high doses of exogenous norepinephrine were significantly greater in 2K, 1C-HT than in the sham-operated control rats, during both acute and chronic stages. These differences were significant for

Fig. 2. Typical vasoconstrictive responses to (a) electrical nerve stimulation (5, 10, 15 Hz) or to (b) exogenous norepinephrine in the mesenteric vasculatures from two-kidney, one-clip hypertensive rats (2K, 1C-HT) and age-matched sham-operated normotensive control rats (acute: 3 weeks after surgery; chronic: 7-8 weeks after surgery).

1.0 and 3.3 μg doses during both stages; a significantly elevated pressor response in 2K, 1C-HT was also observed for a 0.25 μg norepinephrine dose during the chronic phase. This implies an increased sensitivity to norepinephrine in the 2K, 1C-HT group.

The spontaneous norepinephrine output (determined as the norepinephrine content of perfusate during the unstimulated period) was not significantly different between 2K, 1C-HT and the normotensive controls during both acute and chronic phases (acute phase: 2K, 1C-HT 0.31 ± 0.04 ng/g of wet tissue weight, $n=8$, sham-operated controls 0.28 ± 0.04 ng/g of wet tissue weight, $n=6$; chronic phase: 2K, 1C-HT 0.24 ± 0.04 ng/g of wet tissue weight, $n=7$, sham-operated controls 0.21 ± 0.05 ng/g of wet tissue weight, $n=6$). However, differences were observed in norepinephrine release into the perfusate during the stimulation period (1 min stimulation and following 2 min rest period). Figure 3 shows the norepinephrine release during electrical nerve stimulation in 2K, 1C-HT and the sham-operated controls. During

Table I. Pressor Responses to (a) Electrical Nerve Stimulation or (b) Exogenous Norepinephrine in Mesenteric Vasculatures from 2K, 1C-Hypertensive Rats (2K, 1C-HT) and Normotensive Sham-Operated Rats (acute: 3 weeks after surgery, chronic: 7-8 weeks after surgery)

(a)	5 Hz	10 Hz	15 Hz
(Acute phase)			
2K, 1C-HT	3.1 ± 0.7 mmHg (n=8)	21.8 ± 5.3 mmHg (n=8)	30.9 ± 5.9 mmHg (n=8)
Sham control	3.0 ± 0.5 mmHg (n=5)	18.4 ± 5.7 mmHg (n=8)	33.5 ± 8.8 mmHg (n=5)
(Chronic phase)			
2K, 1C-HT	3.6 ± 0.5 mmHg (n=7)	35.7 ± 4.7 mmHg (n=7)	45.4 ± 7.7 mmHg (n=6)
Sham control	3.8 ± 1.4 mmHg (n=6)	41.7 ± 5.2 mmHg (n=6)	55.8 ± 6.8 mmHg (n=6)

(b)	0.10 μ g	0.25 μ g	1.0 μ g	3.3 μ g
(Acute phase)				
2K, 1C-HT	3.9 ± 1.0 mmHg (n=7)	14.3 ± 2.9 mmHg (n=7)	48.9 ± 7.2 mmHg* (n=7)	72.5 ± 6.6 mmHg* (n=7)
Sham control	2.5 ± 0.8 mmHg (n=5)	4.5 ± 0.9 mmHg (n=5)	17.0 ± 1.2 mmHg (n=5)	34.5 ± 8.4 mmHg (n=5)
(Chronic phase)				
2K, 1C-HT	3.8 ± 1.2 mmHg (n=4)	14.3 ± 1.5 mmHg* (n=4)	77.5 ± 5.9 mmHg* (n=6)	102.5 ± 5.3 mmHg* (n=6)
Sham control	1.3 ± 0.6 mmHg (n=6)	5.3 ± 1.1 mmHg (n=7)	38.5 ± 14.1 mmHg (n=5)	45.0 ± 9.9 mmHg (n=6)

Pressor responses are determined as the increase in the perfusion pressure of the preparation. Significant differences are indicated between 2K, 1C-HT and sham-operated controls (means $\pm$ SEM, * $p < 0.05$).

the acute phase, the norepinephrine release during electrical nerve stimulation was not significantly different between 2K, 1C-HT and the sham-operated control rats. During the chronic phase, though, the amount of endogenous norepinephrine release was significantly decreased in 2K, 1C-HT rats (5 Hz: 2K, 1C-HT 0.35 ± 0.10 ng/g of wet tissue weight, $n=6$, sham-operated controls 0.53 ± 0.09 ng/g of wet tissue weight, $n=6$; 10 Hz: 2K, 1C-HT 0.50 ± 0.07 ng/g of wet tissue weight, $n=7$, sham-operated controls 0.99 ± 0.17 ng/g of wet tissue weight, $n=6$, $p < 0.05$; 15 Hz: 2K, 1C-HT 0.43 ± 0.18 ng/g of wet tissue weight, $n=6$, sham-operated controls 1.07 ± 0.14 ng/g of wet tissue weight, $n=5$, $p < 0.05$). Thus, the increased sensitivity to norepinephrine appears to be offset by a decrease in stimulation-evoked norepinephrine release in the 2K, 1C-HT group during the chronic phase.

Fig. 3. Endogenous norepinephrine release from the sympathetic nerve endings in the mesenteric vasculatures of 2K, 1C-HT and the sham-operated control rats (during 5, 10 and 15 Hz electrical nerve stimulation). Norepinephrine release is expressed as nanogram per gram of wet tissue weight of each preparation.

DISCUSSION

Much evidence has suggested that enhanced adrenergic function is involved in the pathogenesis of hypertension.¹¹⁻⁹⁾ It has been reported that administration of sympatho-depressive drugs results in a significant blood pressure decrease during acute and chronic stages of renal hypertension, suggesting the possible involvement of sympathetic nerve activity in the initiation and maintenance of this disorder.^{14),15)} By contrast, Reid et al have reported that the plasma norepinephrine concentration is increased in 1K, 1C-HT, but not in 2K, 1C-HT.¹⁶⁾ Thus, the relationship between adrenergic function and 2K, 1C-HT is unclear.

The present study demonstrates that vascular sensitivity to norepinephrine was enhanced in the mesenteric vasculatures from 2K, 1C-HT, as evidenced by increased pressor responses to exogenously applied norepinephrine. However, the pressor responses evoked by sympathetic nerve stimulation were not statistically different between 2K, 1C-HT and sham-operated controls. This lack of a difference in pressor responses to electrical nerve stimulation may partially reflect the fact that the electrical stimulation-induced release of endogenous norepinephrine was reduced significantly in 2K, 1C-HT during the chronic phase of hypertension.

Webb et al have reported that the tail arteries of 2K, 1C-HT have depleted catecholamine stores, suggesting a reduced adrenergic function in this

type of renal hypertension.¹⁷⁾ Our results suggest that a decreased catecholamine content in blood vessels of 2K, 1C-HT is not due to the enhanced norepinephrine release from the nerve endings. On the other hand, Ljungqvist and Ungerstedt have observed the sympathetic innervation in kidney juxtaglomerular cells, and proposed that increased sympathetic nerve activity may not contribute strongly to the pathogenesis of renal hypertension.¹⁸⁾ This finding is compatible with our observations that stimulation-induced norepinephrine release from the sympathetic nerve endings was unchanged or reduced in 2K, 1C-HT compared to the normotensive controls.

It is also generally accepted that presynaptic angiotensin II receptors could facilitate the neurotransmitter release from the nerve endings.^{19),20)} Toda et al have proposed that locally generated angiotensin II may stimulate these receptors and induce increased sympathetic nervous activity in 2K, 1C-hypertensive dogs.²¹⁾ Isolated mesenteric vasculature preparations are independent of levels of angiotensin II, so that potentiating effects on norepinephrine release through presynaptic angiotensin II receptors are unlikely to be a factor in our experiments. This feature, though, may partly explain the discrepancy between *in vitro* and *in vivo* studies.

We have previously observed that both pressor responses and norepinephrine release of the mesenteric vasculatures were enhanced in spontaneously hypertensive rats and DOCA-salt hypertensive rats compared with age-matched normotensive controls.⁶⁾⁻⁹⁾ These suggest that adrenergic transmission in the blood vessels may be altered differentially in different stages and types of hypertension. By contrast, vascular smooth muscle sensitivity to norepinephrine was increased in 2K, 1C-HT with a reduction in norepinephrine release during the chronic phase. Webb et al have also reported an increased sensitivity to catecholamines in isolated tail arteries from 2K, 1C-HT rats.¹⁷⁾ The reason for this change is not clear. It may reflect an up-regulatory mechanism for decreasing norepinephrine release from the presynaptic nerve endings, or hypertrophy of the smooth muscle layer with encroachment of the lumen in 2K, 1C-HT.^{22),23)}

In conclusion, the present results demonstrate that norepinephrine release during electrical nerve stimulation was decreased during the chronic phase in 2K, 1C-HT, whereas the postsynaptic vascular smooth muscle contractions were enhanced in this form of hypertension during both acute and chronic phases. It is unlikely that decreased adrenergic function plays a key role in the initiation and pathogenesis of hypertension of 2K, 1C-HT. However, the enhanced vascular sensitivity may contribute, at least partially, to the maintenance of high blood pressure in 2K, 1C-HT when vascular adrenergic function is diminishing.

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