Renovascular hypertension impairs formation of endothelium-derived relaxing factors and sensitivity to endothelin-1 in resistance arteries

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1 Endothelium-dependent vascular regulation was investigated in mesenteric resistance arteries of Goldblatt two kidney-one clip (2K1C) renovascular hypertensive rats.

2 Third order branches of mesenteric arteries were dissected free and mounted on glass cannulae in an organ chamber. Changes in vascular diameter were measured in pressurized and perfused arteries with a video dimension analyzer.

3 Acetylcholine evoked endothelium-dependent relaxations that were much more pronounced with intraluminal than with extraluminal application. In 2K1C rats the relaxation induced by intraluminal, but not extraluminal acetylcholine was decreased compared to normotensive Wistar Kyoto rats (WKY). Increased duration of hypertension further decreased the response to intraluminal but not extraluminal acetylcholine.

4 Endothelin-1 and noradrenaline caused contractions which were augmented by removal of the endothelium. This augmentation was reduced in 2K1C rats compared to WKY; the difference was small with noradrenaline but more pronounced with endothelin-1.

5 In arteries without endothelium the sensitivity, but not the maximal contraction to endothelin-1 was lower in 2K1C rats, while the response to noradrenaline was not different in 2K1C rats and WKY. The sensitivity to the peptide was not further affected by increasing the duration of hypertension.

6 Thus, renovascular hypertension leads to an impaired intraluminal, but not extraluminal activation of the release of endothelium-derived relaxing factor and a decreased inhibitory effect of the endothelium against endothelin-1- and noradrenaline-induced contractions in mesenteric resistance arteries. Furthermore, the sensitivity, but not the maximal response of vascular smooth muscle to endothelin-1 was reduced.

Keywords: Acetylcholine; endothelin-1; endothelium-derived relaxing factor; noradrenaline; Wistar Kyoto rats; renovascular hypertension; resistance arteries

Introduction

Vascular smooth muscle tone is locally regulated by the endothelium (Lüscher, 1988; Lüscher & Vanhoutte, 1990). The endothelium releases endothelium-derived relaxing factor(s) [EDRF(s)] or endothelium-derived contracting factors in response to vasoactive substances and physical stimuli (Lüscher, 1988; Lüscher & Vanhoutte, 1990). The endothelium can reduce the effects of vasoconstrictor substances either through basal or stimulated release of relaxing factors (Lüscher, 1988; Lüscher & Vanhoutte, 1990; Dohi *et al.*, 1990). Alterations in this modulating function of the endothelium may play an important role in the development or maintenance of hypertension, particularly if occurring in small arteries which take part in the regulation of peripheral vascular resistance and organ blood flow (Lüscher, 1988; 1989; Lüscher & Vanhoutte, 1990). Indeed, endothelium-dependent relaxation to acetylcholine is decreased in isolated arteries of spontaneously hypertensive rats (Lüscher, 1988; 1989; Lüscher & Vanhoutte, 1990). However, in the resistance circulation it remains uncertain whether these changes observed in the spontaneously hypertensive rat are truly related to blood pressure. Thus, more information about endothelium-dependent responses in a secondary form of hypertension appears appropriate.

Besides the relaxing factors, the endothelium produces the potent vasoconstrictor endothelin (Yanagisawa *et al.*, 1988).

The peptide evokes a strong and long-lasting contraction in isolated vascular tissue including that obtained from man (D'Orleans-Juste *et al.*, 1989; Lüscher *et al.*, 1990; Dohi & Lüscher, 1990a). However, the role of endothelin in hypertension is not clear since the responsiveness of arteries obtained from hypertensive animals to the peptide is still controversial. Increased (Tomobe *et al.*, 1988; Miyauchi *et al.*, 1989; Criscione *et al.*, 1990), decreased (Dohi & Lüscher, 1990b; Auch-Schweik & Vanhoutte, 1989) and normal responses (Diederich *et al.*, 1989; Criscione *et al.*, 1990) to endothelin-1 have been reported in vessels from spontaneously hypertensive rats. The responsiveness of resistance arteries to endothelin-1 in renovascular hypertension would be of pathophysiological interest, since this type of hypertension is characterized by an increased activity of the renin-angiotensin system (Brunner *et al.*, 1962; Fourcade *et al.*, 1971; Ploth *et al.*, 1977; Huang *et al.*, 1981; Miyazaki *et al.*, 1986; Wilson *et al.*, 1989) and because angiotensin II stimulates the production of endothelin in cultured endothelial cells (Emori *et al.*, 1989).

The present study was designed to investigate (1) the modulatory function of the endothelium and (2) the response to endothelin-1 in mesenteric resistance arteries of renovascular hypertensive rats.

Methods

Animals

Male Wistar Kyoto rats (WKY) were obtained from IFFA CREDO (L'Arbresle, France). At the age of 4 weeks the left

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renal artery of the rat was clipped under anaesthesia with ether. The rats were maintained on a standard rat chow with free access to drinking water and used for experiments at the age of 12 or 16 weeks, respectively. Systolic blood pressure was measured in conscious rats by the tail-cuff method. Systolic blood pressures averaged 233 ± 7 mmHg and 266 ± 6 mmHg in 12 and 16 week old two kidney-one clip (2K1C) rats, respectively ($P < 0.01$) and 122 ± 5 mmHg in 12 week old WKY ($P < 0.0001$ versus age-matched 2K1C rats).

Experimental set-up

The mesentery was removed from rats anaesthetized with pentobarbitone (50 mg kg^{-1} , i.p.) and placed into cold buffer solution of the following composition (mm): NaCl 118.6, KCl 4.8, CaCl_2 2.5, MgSO_4 1.2, KH_2PO_4 1.2, NaHCO_3 25.1, disodium calcium edetate 0.026, glucose 10.1. A small segment (3 mm in length) of the third order branch of the mesenteric artery was isolated under a dissection microscope. The artery was transferred to an arteriograph chamber (Halpern *et al.*, 1984; Dohi *et al.*, 1990; Dohi & Lüscher, 1990a) filled with buffer solution (95% O_2 : 5% CO_2 , 37°C).

The chamber contained two glass microcannulae. The proximal end of the artery was cannulated with the afferent cannula and secured with a surgical nylon suture. The distal end was attached to the inside of the efferent cannula. The artery was perfused with buffer solution containing 1% bovine serum albumin (supplied from a 500 ml reservoir) and equilibrated under an optimal transmural pressure (i.e. that pressure at which contractions to KCl were maximal) of 30 mmHg (Dohi *et al.*, 1990; Dohi & Lüscher, 1990a) for 1 h before experiments. A polyethylene catheter (internal diameter $50 \mu\text{m}$) was placed in the afferent cannula and connected to a pressure transducer for measurement of transmural pressure or for intraluminal application of drugs. Transmural pressure was adjusted by changing the height of the reservoir supplying the perfusate.

The arteriograph was placed on a stage of a microscope which had a TV camera attached to the viewing tube. The signal derived from the video image of the vessel was processed by an electronic system (Living Systems Instrumentation, Burlington, VT, U.S.A.) for continuous measurement and recording of intraluminal diameter.

Endothelium removal

To remove the endothelium, 0.5% 3-[(3-cholamidopropyl)-dimethylammonio]1-propanesulphonate (CHAPS) was infused intraluminally for 30 s (Tsfamariam & Halpern, 1988; Dohi *et al.*, 1990; Dohi & Lüscher, 1990a). The resting intraluminal diameter and the contraction to KCl (100 mM) were not affected by endothelial removal. The presence or absence of the endothelium was confirmed by the presence or absence of the response to acetylcholine ($1 \times 10^{-6} \text{ M}$).

Vascular dimension in normotensive and hypertensive rats

In 12 week old WKY, the intraluminal diameter and wall thickness of the studied vessels were $199 \pm 5 \mu\text{m}$ and $30 \pm 1 \mu\text{m}$, respectively. The wall thickness was greater in 12 week old 2K1C rats ($38 \pm 1 \mu\text{m}$; $P < 0.005$) than in age-matched WKY, whereas the intraluminal diameter of 2K1C rats ($196 \pm 4 \mu\text{m}$) was not significantly different. In 16 week old 2K1C rats, neither the wall thickness ($30 \pm 1 \mu\text{m}$) nor the intraluminal diameter ($197 \pm 5 \mu\text{m}$) differed from those of 12 week old 2K1C rats.

Protocols

Concentration-response curves to extraluminal noradrenaline and endothelin-1 were obtained in arteries with and without endothelium by cumulative application of the drug. To study endothelium-dependent relaxations to intraluminal and

extraluminal acetylcholine, arteries were precontracted half-maximally with extraluminal noradrenaline. Flow through the vascular segment was repeatedly determined by counting the drops at the efferent cannula. In this experimental set-up, 20 drops corresponded to 1 ml; accordingly the volume of 1 drop equalled 0.05 ml. Depending on the contractile state of the blood vessel, the intraluminal flow rates ranged from 0.03 – 0.6 ml min^{-1} . The concentrations of intraluminally applied drugs required to construct a concentration-response curve were calculated for the actual level of flow under each experimental condition and the infusion rate (through the polyethylene catheter) of the solution containing the drugs was adapted accordingly (Syringe infusion pump 22, Harvard Apparatus, Boston, Mass., U.S.A.; infusion rates: 1 – $30 \mu\text{l min}^{-1}$). Contractions or relaxations were expressed as percent decrease or increase in intraluminal vascular diameter, respectively. The diameter at the resting level was taken as 100%.

Drugs

The following drugs were used (unless otherwise stated, from Sigma Chemicals, St. Louis, Mo., U.S.A.): acetylcholine hydrochloride, 3-[(3-cholamidopropyl) dimethylammonio]1-propanesulphonate (CHAPS), endothelin-1 (Novabiochem AG, Switzerland), (–)-noradrenaline. Endothelin-1 was dissolved in distilled water containing 0.05% albumin and all other drugs in distilled water. The concentrations are expressed as final molar concentration.

Calculations and statistics

Data are given as mean $\pm$ s.e.mean. The concentration of an agonist causing half-maximal contraction (ED_{50} value) or half-maximal inhibition of a preceding contraction (IC_{50} value) was calculated for each experiment and expressed as negative log molar (pD_2 value). The shift of concentration-response curves was expressed as concentration shift at pD_2 values. In each set of experiments, n equals the number of animals studied. Statistical evaluation was done by unpaired Student's t test. Means were considered to be significantly different when P values were less than 0.05.

Results

Endothelium-dependent relaxation to acetylcholine

Intraluminal activation: Intraluminal acetylcholine (10^{-9} – 10^{-6} M) evoked concentration-dependent relaxations in arteries with endothelium (Figure 1a). In 12 week old 2K1C rats both the pD_2 value and the maximal response were smaller than in age-matched WKY ($P < 0.05$; Table 1).

In 16 week old 2K1C rats the relaxation to $3 \times 10^{-8} \text{ M}$ ($54 \pm 7\%$) and 10^{-7} M of acetylcholine ($80 \pm 3\%$) were further reduced as compared to 12 week old 2K1C rats ($78 \pm 8\%$ and $92 \pm 3\%$, respectively; $P < 0.05$), while the pD_2 value and the maximal response were identical in the two groups (Table 1).

Extraluminal activation: In 12 week old rats, extraluminal acetylcholine was less effective in causing relaxation than intraluminal acetylcholine (Figure 1b). In WKY, the pD_2 value, but not the maximal response was smaller with extraluminal application of acetylcholine than with intraluminal application (concentration shift: 6.6 fold; $P < 0.005$; Table 1). In 2K1C rats the concentration shift comparing intra- and extraluminal acetylcholine was smaller (3.7 fold; $P < 0.05$); the maximal response also was similar to that seen with intraluminal activation (Table 1). With extraluminal application of acetylcholine, neither the pD_2 value nor the maximal response differed in WKY and 2K1C rats.

In 16 week old 2K1C rats, the relaxation to extraluminal acetylcholine was not different from that in 12 week old 2K1C

Figure 1 Concentration-response curves to intraluminal (a) and extraluminal acetylcholine (b) in mesenteric resistance arteries obtained from 12 week old Wistar Kyoto rats (control; ○) and age-matched two kidney-one clip hypertensive rats (2K1C; ●) which were clipped at the age of 4 weeks. Note that the relaxation induced by intraluminal, but not extraluminal acetylcholine was impaired in 2K1C. Each point represents the mean value from five experiments for control; 6 experiments for 2K1C; s.e.mean shown by vertical bars.

rats and similar to the relaxation induced by intraluminal acetylcholine (Table 1).

Endothelin-1

In arteries with endothelium obtained from 12 week old rats, extraluminal endothelin-1 (10^{-11} – 3×10^{-8} M) evoked contractions in a concentration-dependent manner (Figure 2a). The pD_2 value was smaller in 2K1C rats than in WKY ($P < 0.005$), while the maximal response did not differ in 2K1C and WKY (Table 2).

Removal of the endothelium enhanced the sensitivity to the peptide in both groups (Figure 2). The shift at the pD_2 value was strikingly smaller in 2K1C rats (2.7 fold; NS) than in WKY (150 fold; $P < 0.05$), but the enhancement of the maximal response was identical in 2K1C rats and WKY (Table 2). In preparations of 2K1C rats without endothelium, the pD_2 value ($P < 0.05$), but not the maximal response was markedly reduced as compared to WKY (Table 2).

In 16 week old 2K1C rats, the maximal response, but not the pD_2 value was different in the presence and absence of the endothelium (Table 2). Neither the pD_2 value nor the maximal response both in preparations with and without endothelium were different between 12 and 16 week old 2K1C rats.

Noradrenaline

Noradrenaline (3×10^{-8} – 10^{-4} M) caused concentration-dependent contractions in WKY and 2K1C rats (Figure 3). In WKY, removal of the endothelium augmented both the pD_2 value ($P < 0.05$) and the maximal response to noradrenaline

Figure 2 Concentration-response curves to extraluminal endothelin-1 in mesenteric resistance arteries with (○) and without endothelium (●) obtained from 12 week old Wistar Kyoto rats (control; a) and age-matched two kidney-one clip hypertensive rats (2K1C; b). Note that the sensitivity but not the maximal response to endothelin-1 is reduced in 2K1C. Each point represents the mean value from five experiments; s.e.mean shown by vertical bars.

($P < 0.005$; Figure 3a and Table 2), while in 2K1C rats only the maximal response ($P < 0.005$), but not the pD_2 value was increased by the removal of the endothelium (Figure 3b and Table 2). The contractions to the agonist did not differ between WKY and 2K1C rats in preparations with or without endothelium.

Similarly, in 16 week old 2K1C rats the maximal response, but not the pD_2 value was different in the presence and absence of the endothelium (Table 2). The contraction to noradrenaline was identical in 12 and 16 week old 2K1C rats both in arteries with and without endothelium.

Potassium chloride

The contraction induced by KCl (100mM) in arteries with endothelium was identical in 12 week old WKY ($75 \pm 1\%$) and age-matched 2K1C rats ($73 \pm 1\%$) and 16 week old 2K1C rats ($73 \pm 1\%$).

Discussion

The major findings of the present study are reduced endothelium-dependent relaxation to intraluminal, but not extraluminal acetylcholine and an impaired inhibitory function of the endothelium against contractions induced by endothelin-1 and noradrenaline in mesenteric resistance arteries of renovascular hypertensive rats. In addition, renovascular hypertension is associated with a decreased response to endothelin-1 in the mesenteric resistance circulation.

Acetylcholine evoked potent endothelium-dependent relaxation in mesenteric resistance arteries of normotensive rats, confirming previous reports from other investigators

Table 1 The pD_2 values and maximal responses to intraluminal and extraluminal acetylcholine in mesenteric resistance arteries obtained from 12 week old Wistar Kyoto rats (WKY) and two kidney-one clip hypertensive rats (2K1C) at ages of 12 and 16 weeks

		12 week old				16 week old	
		WKY		2K1C ^a		2K1C ^a	
		pD ₂ ^b	Max ^c	pD ₂	Max	pD ₂	Max
Acetylcholine	Intraluminal	8.2 ± 0.1	100 ± 0	7.9 ± 0.1*	98 ± 1*	7.6 ± 0.1	94 ± 2
	Extraluminal	7.4 ± 0.1††	99 ± 1	7.3 ± 0.2†	92 ± 4	7.4 ± 0.1	91 ± 1

^a The renal arterial clipping was performed at the age of 4 weeks.
^b Negative logarithm IC_{50} value of acetylcholine.
^c Maximal relaxation to acetylcholine (%).
^{*} $P < 0.05$ as compared to WKY.
[†] $P < 0.05$ as compared to intraluminal application; ^{††} $P < 0.005$ as compared to intraluminal application.

Table 2 The pD₂ values and maximal responses to endothelin-1 and noradrenaline in arteries with and without endothelium obtained from 12 week old Wistar Kyoto rats (WKY) and two kidney-one clip hypertensive rats (2K1C) at ages of 12 and 16 weeks

		12 week old				16 week old	
		WKY		2K1C ^a		2K1C ^a	
		pD ₂ ^b	Max ^c	pD ₂	Max	pD ₂	Max
Endothelin-1	Endothelium with	9.4 ± 0.1	67 ± 2	8.6 ± 0.1**	60 ± 3	8.6 ± 0.1	59 ± 3
	without	11.6 ± 0.7†	82 ± 1††	9.0 ± 0.3*	82 ± 2††	8.9 ± 0.1	80 ± 1†††
Noradrenaline	with	5.8 ± 0.1	69 ± 1	5.9 ± 0.1	69 ± 2	5.9 ± 0.1	64 ± 1
	without	6.1 ± 0.1†	82 ± 1††	6.1 ± 0.1	84 ± 1††	6.1 ± 0.1	82 ± 1†††

^a The renal arterial clipping was performed at the age of 4 weeks.

^b Negative log ED₅₀ value of agonists.

^c Maximal contraction to agonists (%).

* $P < 0.05$ as compared to WKY; ** $P < 0.005$ as compared to WKY.

† $P < 0.05$ as compared to with endothelium; †† $P < 0.005$ as compared to with endothelium; ††† $P < 0.0001$ as compared to with endothelium.

(Tefamariam & Halpern, 1988; Watt & Thurston, 1989) and our own group (Dohi *et al.*, 1990; Diederich *et al.*, 1990). Intraluminal acetylcholine caused a more powerful relaxation than did extraluminal acetylcholine. In renovascular hypertensive rats, as in spontaneously hypertensive rats (Dohi *et al.*, 1990), the relaxation induced by intraluminal, but not extraluminal acetylcholine was decreased as compared to control rats. This indicates that high blood pressure primarily alters the intraluminal activation of the endothelium with the muscarinic agonist regardless of the cause or type of hypertension. This is of particular interest as renovascular hypertension is a secondary form of hypertension where, in contrast to genetic hypertension of the rat, blood pressure is primarily maintained by an increased activity of the renin-angiotensin system (Brunner *et al.*, 1962; Fourcade *et al.*, 1971; Ploth *et al.*, 1977; Huang *et al.*, 1981; Miyazaki *et al.*, 1986; Wilson *et al.*, 1989). Although under these conditions the vascular renin-angiotensin system is also activated, this does not appear to be associated with a more pronounced inhibition of endothelium-dependent relaxations than in spontaneous hypertension.

Increased duration of hypertension (from 8 to 12 weeks) further decreased the relaxation induced by intraluminal acetylcholine (although this further deterioration of endothelium function was very small), while the relaxation induced by extraluminal acetylcholine remained unaltered. Although intraluminal activation of the endothelium with acetylcholine was more effective than extraluminal activation in normotensive and hypertensive rats, this difference was significantly smaller in the animals exposed to renovascular hypertension for 8 weeks as compared to age-matched nor-

motensive rats, and after 12 weeks of hypertension intra- and extraluminal acetylcholine were similarly effective. This indicates that the functional alterations of the intraluminal surface of the endothelium become more pronounced with increasing duration of hypertension. On the other hand, the response of the extraluminal surface of the endothelium to acetylcholine is well preserved.

Endothelin-1 is a very potent vasoconstrictor in mesenteric resistance arteries of normotensive rats (Dohi & Lüscher, 1990a, b; Criscione *et al.*, 1990). In preparations without endothelium the sensitivity, but not the maximal response to endothelin-1 was decreased in 2K1C rats as compared to WKY. In WKY, the biphasic shape of the concentration-response curve to endothelin-1 in arteries without endothelium suggests that two receptor subtypes might mediate the contractions, one with a high affinity and low efficacy and another one with a low affinity and higher efficacy. The decreased sensitivity in 2K1C rats was specific for endothelin-1, since the response of mesenteric resistance arteries without endothelium to noradrenaline did not differ in 2K1C rats and WKY. In the spontaneously hypertensive rat, the responsiveness of vascular smooth muscle to endothelin-1 relative to its normotensive controls is still controversial; increased (Tomobe *et al.*, 1988; Miyauchi *et al.*, 1989; Criscione *et al.*, 1990), decreased (Dohi & Lüscher, 1990b; Auch-Schweik & Vanhoutte, unpublished observations) and normal responses (Diederich *et al.*, 1989; Criscione *et al.*, 1990) to endothelin-1 have been reported. Although some of the differences may be explained by the differences in the experimental conditions or the anatomical origin of the arteries used, the present study further supports the notion, at least in mesenteric resistance arteries, that hypertension is associated with a decreased sensitivity to the peptide. This decreased sensitivity may be due to downregulation of endothelin receptors on vascular smooth muscle particularly in the presence of increased vascular levels of the peptide. Indeed, downregulation of endothelin receptors occurs in cultured smooth muscle cells after exposure to the peptide (Hirata *et al.*, 1988; Roubert *et al.*, 1990). If so, it would appear that that receptor mediating the response to endothelin at lower concentrations is particularly affected. This could occur due to greater vascular endothelin production or pressure *per se*. Angiotensin II, which is elevated in renovascular hypertension (Brunner *et al.*, 1962; Fourcade *et al.*, 1971; Ploth *et al.*, 1977; Huang *et al.*, 1981; Miyazaki *et al.*, 1986), is known to stimulate the production of endothelin in cultured endothelial cells (Yanagisawa *et al.*, 1988; Emori *et al.*, 1989). However, plasma concentrations of endothelin are increased only in certain hypertensive patients (Cernacek & Stewart, 1989; Saito *et al.*, 1990; Shichiri *et al.*, 1990; Kohno *et al.*, 1990) and appear normal or decreased in hypertension in the rat (Suzuki *et al.*, 1990). Alternatively, the circulating levels of endothelin may not reflect the local concentration of the peptide within the blood vessel wall (particularly since greater amounts of endothelin are released abluminally;

Figure 3 Concentration-response curves to extraluminal noradrenaline in mesenteric resistance arteries with (○) and without endothelium (●) obtained from 12 week old Wistar Kyoto rats (control; a) and age-matched two kidney-one clip hypertensive rats (2K1C; b). Note that the response to noradrenaline was not different between control and 2K1C. Each point represents the mean value from five experiments; s.e.mean shown by vertical bars.

Yoshimoto *et al.*, 1990) or the catabolism of endothelin-1 by endogenous proteases may be increased in 2K1C rats.

Removal of the endothelium enhanced the sensitivity (pD_2 value) to the peptide in WKY. In 2K1C rats, the enhancement in the sensitivity to endothelin-1 was blunted, indicating a reduced inhibitory capacity of the endothelium in renovascular hypertension. This contrasts with the spontaneously hypertensive rat where the inhibitory function of the endothelium against contractions to extraluminal endothelin-1 is increased as compared to control rats (Dohi & Lüscher, 1990b). The contraction induced by extraluminal endothelin-1 both in arteries with and without endothelium was identical in 12 and 16 week old 2K1C rats. This indicates that, in contrast to the release of EDRF stimulated by acetylcholine, the inhibitory function of the endothelium against endothelin-1-induced contraction does not further deteriorate with prolonged renovascular hypertension.

The contraction induced by extraluminal noradrenaline in arteries without endothelium was identical in 2K1C rats and WKY, confirming previous data from other investigators (Duarte *et al.*, 1990). The presence of the endothelium slightly, but significantly reduced the sensitivity in WKY, but not in 2K1C rats though the maximal response was increased after endothelial removal in both groups. Thus, as in spontaneously hypertensive rats (Dohi *et al.*, 1990), the inhibitory function of the endothelium against the contraction induced by nor-

adrenaline is decreased in renovascular hypertensive rats, although this difference between the two strains was rather small. Since noradrenaline does not stimulate the release of EDRF in this preparation (Dohi *et al.*, 1990), this indicates that renovascular hypertension reduces the basal release of EDRF in the resistance circulation. The contraction induced by extraluminal noradrenaline both in arteries with and without endothelium was identical in 12 and 16 week old 2K1C rats, indicating that basal release of EDRF does not further decrease during this period. Since this alteration in endothelial function was small, it is uncertain whether it contributes to the hypertensive process.

In conclusion, the intraluminal, but not extraluminal activation of the release of endothelium-derived relaxing factors in response to acetylcholine is impaired in 2K1C rats. The inhibitory function of the endothelium against extraluminal endothelin-1 and to a lesser degree against noradrenaline is decreased in 2K1C rats. These functional changes of the endothelium in hypertensive resistance arteries may play a role in the maintenance of increased peripheral vascular resistance that occurs in established hypertension.

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