The Supersensitivity of Vascular Smooth Muscle Following Surgical Renal Denervation in the Dog

NAOHIRO OHGUSHI and HIRONOBU SHINOHARA

The first Department of Surgery, School of Medicine, Ehime University
Received for Publication, June 6, 1984.

Introduction

In 1939 Cannon formulated "A Law of Denervation"[4] for internal organs and their autonomic nerves.   Since then a number of reports have been published concerning post-denervation supersensitivity in various visceral smooth muscles.   Very few of them, however, refer to the kidney, in spite of the increased number of cases of denervation of this organ, as a result of surgical operations including kidney transplantation, kidney bench surgery, and synthetic vascular graft transplantation as a remedy for aneurysm of the abdominal aorta.

We attempted to investigate the effects of post-denervation supersensitivity in the renal vascular smooth muscle on the renal circulation.   The following two studies were made using renal interlobar arteries removed from dogs which had their left kidney denervated.

1)   Reinnervation after renal denervation.

2)   The time course of changes in the response of the renal vascular smooth muscle to catecholamines after denervation.

Materials and Methods

Fifty-five mongrel dogs of 6 to 15 kg body weight (mean $\pm$ SD : 9.5 $\pm$ 2.4 kg) and of both sexes were used.   Forty-eight of them underwent unilateral renal denervation on the left side, while the other 7 were left unoperated to serve as controls.   The denervated dogs were anesthetized by either intravenous or intramuscular injection of 25 mg/kg sodium pentobarbital and laparatomy was performed.   After sectioning the left renal artery it was reanastomosed under a surgical microscope and using interrupted suture with 7–0 nylon thread.   Additionally section and anastomosis of the left renal vein were performed on the dogs from which both kidneys were to be removed after 6 months.

In order to investigate the time course of the changes in the effects of denervation on the renal blood vessels, the denervated dogs were further divided into subgroups of 6,6,6, 8,6,8 and 8 and both kidneys were removed 2,7 and 14 days and 1,3 and 6 months after the denervation of the respective group.

All the dogs including those used as controls were sacrificed by exsangination from the common carotid artery under anesthesia with sodium pentobarbital prior to the removal of the kidney, which were then immediately placed in a nutrient solution described later.

To study the density and pattern of innervation, the glyoxylic acid fluorescence histochemical method for monoamines was used.

The interlobar artery obtained from each of the removed kidneys was promptly incubated for at least one hour in 2% glyoxylic acid in 0.1 M phosphate buffer solution (pH 7.0) at room temperature.

After stripping away the adventitia in the solution, each artery was dissected longitudinally, blotted, stretched on a fluorescence-free glass plate and desiccated. The speciments were then heated to 100°C for 4 minutes and prepared for assessment by microscopy and microphotography. For fluorescence microscopy a Zeiss microscope was used with BG 12 and BG 50 primary and secondary filters, respectively. Using the interlobar arteries obtained from the unoperated kidneys as controls, reinnervation of the adrenergic nerves was graded into five classes according to fluorescence intensities as shown in Table 1. In the present study, it took about 15 minutes after removal to place the interlobar arteries in the buffer solution. It was previously proved by preliminary experiments using femoral vein of rats that no significant reduction of the intensity in monoamine fluorescence was produced within the period of 15 minutes.

Reactivity of renal blood bessels.:

Immediately after the removal of both kidneys, the interlobar artery was isolated from each kidney and cleaned of the connective tissue under a dissecting microscope. The artery was then made into a 1–2 mm by 20 mm strip by spiral dissection at an angle of 45°.

The preparation was then suspended in 20 ml organ bath and 1.5 g resting tension was applied. The composition of the nutrient solution which was maintained at 37 ± 0.5°C and gassed with 95% oxygen and 5% carbon dioxide, was as follows: (in mEq/l): Na^+: 145, K^+: 5.4, Mg^{++}: 2.1, Ca^{++}: 2.9, Cl^-: 135, HCO_3^-: 20.0, and glucose: 5.6: pH 7.4–7.6.

Tension was measured using an isometric strain gauge (model, SB-1t-H.) and polygraph (model U-425 M, Nippon Koden).

After allowing one hour for stabilization, the samples were exposed to drugs.

Norepinephrine (NE) or dopamine (DA) were cumulatively added directly to the bath to reach the concentration range of 10^{-9}–5×10^{-5} (mEq/l) or 2×10^{-8} (mEq/l), respectively. For these experiments only one dose-response curve was obtained with each sample. The response of each interlobar artery from the denervated kidney to drugs was compared with its own counterlateral control and evaluated by using Student's test.

Results

1) Fluorescence histochemical studies based on monoamines.

A dense fluorescent nerve plexus was noted in the control interlobar artery (Fig. 1–A), but the fluorescence became blurred on the 2nd day after denervation, and then completely disappeared on the 4th day as shown in Fig. 1–B. The fluorescence, although sparse, reappeared 3 months

　　　　　日外宝　第53巻　第5号（昭和59年9月）
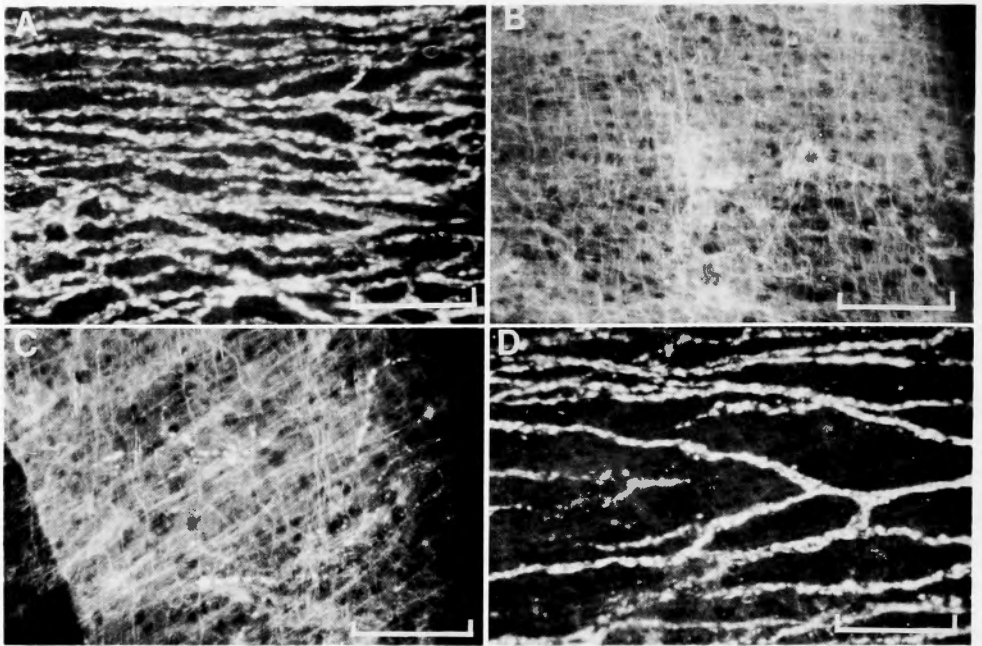
Fig. 1.　Photomicrographs of stretch preparation of the canine interlobar artery mag
×160 Scale bar 100 μ m, nerves demonstrated by glyoxylic acid fluorescence
histochemistry.
A: Control.　B: 1 month after denervation.　C: 3 months after denervation.
D: 6 month after denervation.

after denervation (Fig. 1–C).　Six months after denervation, a meshwork of fluorescent nerve
fibers as shown in Fig. 1–D, was observed and graded into five classes depending on fluorescence
intensity, and presented in Table 1.

2)　Reactivity of the renal interlobar artery.

Fig. 2 illustrates the effect of denervation on the dose-response curves to NE. and DA in

Table 1.　Reinnervation of adrenergic nerves in the interlobar
artery of the kidney.

| Grade	Time after denervation (months)		
	1	3	6
0	14		
1		11	
2		3	2
3		2	17
4			4

This table shows the number of specimens.
Grade 0: Adrenergic nerves not observed.
Grade 1: Observed in part of specimen without a network pattern.
Grade 2: Observed in part of specimen and form a network pattern.
Grade 3: Observed in the major part of specimen with network pattern.
Grade 4: Almost impossible to discriminate from control.
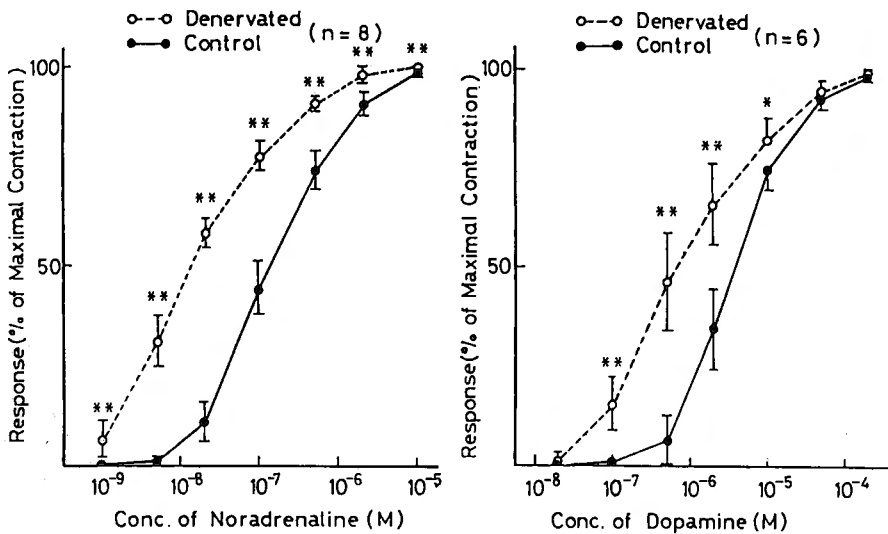
Fig. 2. Dose-response curves of interlobar artery to noradrenaline and dopamine 14 days after unilateral renal denervation in the dog. Vertical bars represent standard deviation.;
*p<0. 05, **p<0. 01 vs. control response using Student's t-test.

control and the contralaterally denervated samples. Here the samples were prepared from kidneys removed on the 14th day after denervation.

In control tissues, the contractile response to NE is started at a concentration of 2×10^{-8} mEq/l and reaches to its maximum at 10^{-5} mEq/l, whereas on the denervated side, it started at as low as 10^{-9} mEq/l and the maxium response was attained at 2×10^{-6} mEq/l. The dose-response curve of the denervated is shifted in an almost parallel fashion to the left of the control case. Comparing levels of ED 50, the degree of supersensitivity for NE produced by denervation is 10.6-fold.

Similar results occur with DA, although the leftward shift at higher concentrations is less remarkable than that in lower ones. The differences in artery contractile response to NA and DA at the level of ED 50 are 26.6 fold on the control side and 48.4-fold on the denervated side.

Depending on invididual cumulative dose response curves in Fig. 3, the difference in ED 50 for both NE and DA between unoperated and denervated interlobar arteries were plotted against time after denervation. As early as 2 days post denervation, the sensitivity to NE and DA was

Table 2. Maximal contractions of interlobar artery to noradrenaline and dopamine 14 days after unilateral renal denervation in the dog. Using Student's t-test there is no significant difference between denervated and control side.

	Noradrenalin (n=15)	Dopamine (n=10)
Denervated	3. 61±0. 92	3. 49±1. 15
Control	3. 60±1. 17	3. 32±1. 17

Value expressed as mean±S.D. (g)
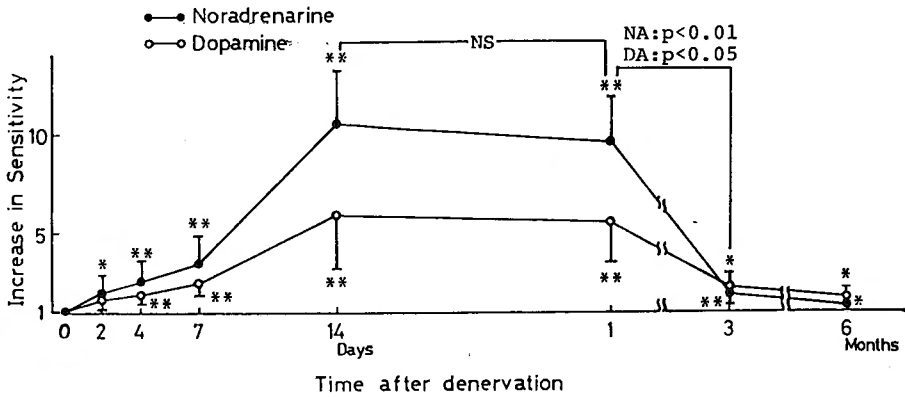
Fig. 3. Development of supersensitivity of the interlobar artery to noradrenaline and dopamine after unilateral renal denervation in the dog. Ordinate shows increate shows increase in sensitivity to noradrenaline and dopamine, expressed as ratio ED$_{50}$ control/ED$_{50}$ denervated side. Vertical bars represent standard deviation. *p<0.05, **p<0.01 vs. time zero using Student's t-test.

increased 2.1±0.9 (SD) and 1.8±0.8 (SD) times respectively. The supersensitivity gradually increased thereafter and reached a maximum 2 weeks after denervation, when the sensitivity to NE and DA had been increased 10.6±2.7 (SD) and 6.0±2.7 (SD) times, respectively. No significant difference was observed in the sensitivity during the period from 14 to 30 days after denervation, but the maximum sensitivity noted one month after denervation was significantly decreased three months after denervation when reinnervation was demonstrated in the denervated interlobar arteries by the fluorescence histochemistry. The supersensitivity was markedly reduced together with the progress of reinnervation and 6 months after denervation, the sensitivity to NE and DA was 1.5±0.3 (SD) and 1.8±0.3 (SD) times, respectively.

Discussion

1) Reinnervation in the kidney.

Using the glyoxylic acid technique it was demonstrated that, in the renal interlobar artery, reinnervation started at three months after denervation and progressed gradually, and a meshwork of the regenerated nerves was noted almost over the whole surface of the blood vessel after denervation.

The renal nerves originating from sympathetic ganglia at Th$_{12}$ to L$_3$ levels pass through the greater or lumbar splanchnic nerves, celiac or superior mesenteric ganglion, and the renal plexus surrounding renal artery, and enter the kidney along the renal artery. In the kidney, they run along the adventitia to the interlobar, arcuate, interlobar, and then afferent arteries or arterioles. Most intrarenal nerves are found in the cortex or among convoluted tubules and terminate between tubular cells and in basal lamina. There are few nerves in the medulla. The nerve fibers are nonmedullated and sympathetic vasoconstriction nerve fibers.[11,17] According to NOMURA et al.[14], who examined interlobar and arcuate arteries for reinnervation by the Falck-Hillarp method for catecholamine fluorescence, no reinnervation appeared one and three months after denerva-

tion, but reinnervation became apparent 6 months post-operatively, although the inensity of fluorescence was less intense than in the unoperated condition. NORVELL[15] measured NE concentrations in the kidney together with a histological study using Falck-Hillarp method, and reported that reinnervation started 3 to 6 mouths after denervation, but a complete recovery of the nervous system was not achieved even 26 months after denervation. He also mentioned that the reinnervation progressed differently depending on the site and NE concentration in the kidney 26 months after denervation was about 75% of the normal concentration.

Based on this evidence, in addition to the present findings, it is concluded that, after renal denervation, reinnervation appears first in the proximal arteries such as the renal and interlobular arteries within 3 months and then in the arcuate and interlobar arteries after about 6 months and thereafter. As shown in Table 1, innervation in the interlobar arteries recovered to almost the normal state about 6 months after denervation, but the renal reinnervation on the whole seemed to be considerably inferior to the normal state. Although the small diameter arcuate and interlobular arteries could not be examined for reinnervation in the present investigation by the glyoxyilic acid method, as shown in Table 1, reinnervation of various degrees up to meshwork formation could be demonstrated by the method over a wide area within an isolated artery.

2) Supersensitivity of vascular smooth muscle in the kidney.

In the present experiments carried out in vitro, supersensitivity appeared from as early as the 2nd day after denervation and increased to reach the maximum 2 weeks after denervation, when the sensitivity to NE and DA was increased 10.6 and 6.0 times without a change in the maximum contraction force. The supersensitivity at the maximum level was maintained without a significant change until reinnervation appeared. It decreased markedly thereafter with the progress of reinnervation, but the sensitivity to NE and DA was still increased 1.5 and 1.7 times, respectively, and the denervated arteries were significantly supersensitive over control one ($p > 0.01$) even 6 months after denervation, although there had been a considerable degree of innervation.

The abundant blood supply relative to its weight is a feature of the kidney.[7] It accounts for about 20% of the cardiac output. The kidney is regulated by humoral factors such as catecholamines in the blood as well as neural factors. For example, blood vessels in the kidney constrict when the greater splanchnic nerve is excited, but the neural regulation of blood flow scarcely functions in the normal state and the renal denervation does not affect renal circulation. In shock, the nerves reduce renal blood flow and a blocking of renal nerves inhibits renal vascular resistance[3]. The changes in renal circulation in shock were attributed to increased concentration of catecholamines in the blood[8,13,23]. However, as these experiments on renal circulation in shock were carried out with the kidney under neural rasgulation or immediately after denervation, no consideration was given to supersensitivity after renal denervation. Essentially no report described the morphology and function over a long period of time after the denervation.

According to MIYAZAKI[13], who measured both NE in the blood and renal blood flow in various pathological state, catecholamines exceeding 10 ng/ml in the blood flow in the cortex and reported that the sensitivity of cortical vascular system to NE was gradually increased in renal allografts within a few days after grafting. Based on these findings, as well as the findings ob-

tained from the present experiments, the supersensitive kidney before reinnervation is considered to be prepared to reduce blood flow and, in an emergency state, such as shock, the reduction of renal blood flow will be easily achieved by intrinsic catecholamines increased in the blood.

DA has been increasingly used therapeutically in shock, and after various types of surgery, because it increases renal blood flow.[12] Treatment with DA in large doses, however, requires circumspection because of its α-adrenergic action which elevates blood pressure and causes vascular constriction in the kidney. Consequently, after operations which produce renal denervation and then a supersensitive condition, treatment with DA causes a reduction of renal blood from even if DA is given in a dose as low as one which does not produce a harmful effect on normal renal circulation.

The contraction of the interlobar artery by both NE and DA was inhibited by phenoxybenzamine, suggesting that the contraction was α-adrenergic. The distribution of α- and β-receptors in the renal vascular beds has been estimated by measuring renal blood flow under anesthesia[1,5,10], but here is some range in the results obtained. The differences in the strained condition of the renal blood vessels[19] and in doses of catecholamines[10] may account for such a diversity of reactivity noted in the renal vascular beds. Therefore renal vascular responses tō catecholamines should be evaluated depending on the intensity of the vascular tension and doses of catecholamines. The interlobar artery was loded with a static tension of 1.5 g so that the contraction due to NE could become maximum[21]. Arteries in the body are constricted to some extent, but in general isolated arteries are fully dilated in a nutrient solution[2]. Consequently, results obtained in vitro cannot always be equated to the response in the body.

Although the present experiments were carried out with only the interlobar arteries to obtain information on vascular supersensitivity, the response of renal blood vessels to catecholamines usually varies in different sites and veins may also develop such supersensitivity[16]. Taking it into consideration that the response of renal blood vessels varies depending on the intensity of tension and on concentrations on catecholamines, and there are some differences between in vivo and in vitro conditions, further studies on renal circulation in vivo are necessary to clarify the clinical significance of the changes in renal circulation due to supersensitivity of vascular smooth muscles after denervation.

References

1) Ahlquist RP: A Study of adrenotropic receptors. Am J Physiol, **153** (1948) 586–600.
2) Boar DF, Goulet PL, et al: Direct tension recording from smooth muscle of resistance vessels from various organs. Angiology, **12** (1961) 478–485.
3) Broad J: The Kidney, 66–70, Butterworths, London, 1973.
4) Cannon WB: A law of denervation. Am J Med Sci, **198** (1939) 737–750.
5) Corcoran AC and Page IH: Renal hemodynamic effects of adrenaline and "Isuprel": Potentiation of effects

of both drugs by tetraethylammonium.   Proc Soc exp biol med, **66** (1947) 148–151.

6) Cowen T and Burnstock G: Quantitative analysis of the density and pattern of adrenergic innervation of blood vessels.   Histochemistry, **66** (1980) 19–34.

7) Fujimoto M: The physiology of metabolism.   Production and excretion of urine.   A comprehensive treatise on physiology, Vol. *IV*, part 2, 307 (1972) Igaku-shoin, Tokyo.

8) Grandchamp A, Veyrat R et al: Relationship between renin and intrarenal hemodynamics in hemorrhagic hypotension.   J Clin Invest, **50** (1971) 970–978.

9) Lindvall O and Björklund A: The glyoxylic acid fluorescence histochemical method: A detailed account of the methodology for the visualization of central catecholamine neurons.   Histochemistry, **39** (1974) 97–127.

10) Mark AL, Eckstein JW, et al: Renal vascular responses to Isoproterenol.   Am J Physiol, **217** (1969) 764–767.

11) McKenna OC and Angelakos ET: Adrenergic innervation of the canine kidney.   Circulation Research, **22** (1968) 345–354.

12) McNay JL, McDonald RH, et al: Direct renal vasodilation produced by dopamine in the dog.   Circulation Research, **16** (1965) 510–517.

13) Miyazaki M: Renal pathophysiology in shock and chemical mediators.   J Jpn Surg Soc, **80** (1979) 1580–1584).

14) Nomura G, Kurosaki M, et al: Reinnervation and renin release after unilateral renal denervation in the dog.   J Appl Physiol, **33** (1972) 649–655.

15) Norvell JE and Banes RT: Histofluorescence and fluorometric analysis of adrenergic reinnervation in the dog kidney following autotransplantation.   Transplantation, **27** (1979) 69–70.

16) Ohgushi N, Sato K, et al: The supersensitivity of vascular smooth muscle after sympatnectomy.   Myakkan-Kenkyu, **3** (1978) 39–41.

17) Ohgushi N, Tsunekawa K, et al: A study on adrenergic innervation of the kidney.   Myakkan-Gaku, **9** (1969) 143–148.

18) Ohnishi H: Serial measurement of the cortical blood flow in the canine renal allografts during the rejection crisis.   Arch Jap Chir, **38** (1969) 372–393.

19) Spencer MP: The renal vascular response to vasodepressor sympathomimetics.   J Pharmacol exp Thr, **116** (1956) 237–244.

20) Tanaka C: The fluorescence histochemistry of monoamines.   New histocytochemical method, edited by Hamajima, Y. and Fujita, T. 159–169, The Japanese Association of Histochemistry, Kyoto, 1977.

21) Toda N, Hatano Y, et al: Modifications by stretches of the mechanical response of isolated cerebral and extra-cerebral arteries to vasoactive agents.   Pflügers Arch Eur J Physiol, **374** (1978) 73–77.

22) Trendelenburg U.:   Time course of changes in sensitivity after denervation of the nictating membrane of the spinal cat.   J Pharmacol exp Ther., **142** (1963) 335–342.

23) Watts DT and Westfall V: Studies on peripheral blood catecholamine levels during hemorrhagic shock in dogs.   Proc soc exp biol med, **115** (1964) 601–604.

24) Westfall DP, McClure DC, et al: The effects of denervation, decentralization and cocaine on the response of the smooth muscle of the Guinea-Pig vas deferens to various drugs.   J Pharmacol exp Ther, **181** (1972) 328–338.

25) Winquist RJ and Bevan JA: The effect of surgical sympathetic denervation upon the development of intrinsic myogenic tone and the Alpha and Beta adrenergic receptor-mediated responses of the rabbit facial vein.   J Pharmacol exp Ther, **211** (1979) 1–6.

和文抄録

犬における片側外科的除神経後の腎血管
平滑筋過敏性に関する研究

愛媛大学医学部第一外科
大串　直太，篠原　洋伸

外科的除神経後の過敏性の研究を犬に用いて 1. 除神経後の神経再生について，2. 腎除神経後の血管平滑筋のカテコーラミンに対する過敏性について行った．

方法：雌雄雑犬を用いて，片側腎神経切断及び腎動脈離断再吻合を施行し除神経腎を作製した．除神経後2，4，7，14日，1，3，6ケ月後に両側腎を摘出し以下の実験を行った．

結果：1）リモノアミン螢光：腎葉間動脈における螢光は，除神経後4日目には完全に消失し，その後2，14日，1ケ月目に摘出した標本では全く観察されなかった．しかし除神経後3ケ月目になるとわずかであるが認められるようになり，さらに6ケ月後には形態学的には不完全であるが，かなり正常に近くまで回復しているモノアミン螢光が観察された．

2）腎血管反応性：腎葉間動脈摘出標本には，Norepinephrine (NE) と Dopamine (DA) に対し，ともに収縮反応がみられた．（magnus 法）用量収縮曲線を各症例ごとに作製し，NE 及び DA に対する過敏性を effect dose 50%（ED_{50}）で比較すると除神経後2日目からすでに NE に対し2.11 ± 0.89倍，DA に対し1.75 ± 0.77倍と過敏性の出現を認めた．その後時間の経過とともに過敏性が増大し，除神経後約2週間でNAに対し10.58 ± 2.68倍，DA に対して6.00 ± 2.74倍とそれぞれ最大値に達する．さらに除神経後1ケ月目に摘出した症例では過敏性に優位の変化はみられなかった．葉間動脈に神経再生が認められた除神経後3ケ月目になると過敏性は著明に減少し，その傾向は6ケ月目では一段と減少しNAに対し1.48 ± 0.27倍，DA に対し1.67 ± 0.39倍に低下している．

結語：以上の結果より腎除神経が行われた際は，腎血管平滑筋の除神経過敏性のため NE, DA の使用に十分な配慮が必要である．