Original

Changes of Renal Blood Flow Following Various Renal Denervation Procedures in Intact Kidneys of Rabbits Using Colored Microspheres

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Abstract: We investigated effects of acute renal denervation procedures on renal hemodynamics in intact kidneys of rabbits. Fifty-one Japanese white male rabbits were used. Renal blood flow (RBF) was measured by colored microspheres. In basic studies, first, colored microspheres 15μm in diameter were histologically trapped within glomerular capillaries, indicating measurement of glomerular blood flow. Secondly, we used right kidney as control and left kidney as experimental side in each rabbit because there was no bilateral difference in normal RBF (n=15). Renal ischemia (n=8) was produced by clamping left renal artery for 10 min. Renal denervation procedures were produced by end-to-end anastomoses of renal artery (n=10), those of renal vein (n=10), and renal sympathectomy (n=8) by stripping the adventitia of left renal artery. Following reperfusion, bilateral RBF was measured by colored microspheres. At 5 min after reperfusion, RBF of renal ischemia increased to 146.2±9.4 % of normal RBF(p=0.0018). At 5 min, RBF of end-to-end anastomoses of renal artery and renal sympathectomy decreased to 53.6±6.7 % ($p<0.0001$) and 64.7±12.5 % (p=0.0258) of normal RBF, respectively. At 35 min, RBF of renal ischemia, end-to-end anastomoses of renal artery, and renal sympathectomy all recovered to normal RBF. Section of renal vein did not affect RBF responses. In summary, RBF is transiently decreased due to section of renal artery and renal sympathectomy, and acute renal denervation and/or vascular spasm may influence RBF. RBF in intact denervated kidneys all recovers to normal RBF at 35 min. These findings indicate that the renal sympathetic regulation of RBF is overruled by renal autoregulatory mechanism in initial renal hemodynamics after acute renal denervation.

Keywords: renal blood flow, renal denervation, renal ischemia, colored microspheres, and rabbit
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Introduction

Renal blood flow (RBF) is controlled by the renal sympathetic nerve, renal autoregulation, hormones and autoacids[1-3]. The renal sympathetic nerves enter the hilum of the kidney in association with the renal artery and renal vein and thereafter are distributed along the renal arterial segments in the renal cortex and outer medulla. In response to normal physiological stimuli, changes in efferent renal sympathetic nerve activity contribute to homeostatic regulation of RBF and glomerular filtration rate[1-4]. Current evidence supports that renal sympathetic nerve activity does not play a significant role in the mechanisms involved in RBF autoregulation[1-5].

Schroeder HA and Cohn AE demonstrated that partial occlusion of the renal artery produced only a transient decrease in RBF followed by a rapid return to the control levels[6]. This phenomenon has been observed in denervated kidney, during blockade of intrarenal ganglia, and in isolated perfused kidney[3]. From these observations, RBF is normally determined by an autonomous intrinsic activity of the renal arterioles, and is independent upon tonic activity in the sympathetic pathways[1-3]. It is now generally agreed that under normal physiologic conditions basal renal nerve activity is generally too low to influence renal hemodynamics[2,7]. In the preliminary study, we encountered that acute renal denervation induced by section of the renal artery produced a transient decrease in RBF, followed by a

return to the normal levels at 35 min after perfusion using colored microspheres[8].

In 1985 Shell et al [9] developed nonradioactive, colored microspheres (CMS), which are chiefly applied to measurement of regional myocardial blood flow[10] As this procedure uses microspheres labeled with various colors instead of radioactive material, it has the advantage of not requiring special facilities and apparatuses. CMS injected into the left ventricle are distributed in each organ proportional to regional blood flow in the organs. As CMS 15μm in diameter are unable to pass through capillaries, they are retained in arterioles and thus they are suitable in measuring regional blood flow in the glomeruli[1,3]. Tsuchida, Y et al have evaluated that two measurements of jejunal blood flow and RBF by changing the color of CMS in the same rabbit are quantitative and useful[8,11].

In the present study, renal ischemia for model of innervated kidneys was employed. Three renal denervation procedures in the renal hilum of intact kidneys were produced[8,12-14]. First, end-to-end anastomoses of the renal artery were produced after section of the renal artery. Secondly, end-to-end anastomoses of the renal vein were produced in order to elucidate the effects of density difference of the renal nerves on RBF[4]. Thirdly, renal sympathectomy was produced by stripping the adventitia of the renal artery. At 5 and 35 min after reperfusion, bilateral RBF was measured by CMS[8-11]. Difference in the initial renal hemodynamics in acute intact innervated and denervated kidneys was analyzed.

Materials and Methods

Fifty-one Japanese white male rabbits (2.5 to 2.9 kg, mean 2.7 kg) were used. The care of the rabbits complies with the principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals (DHHS publication No. [NIH] 85-23, revised 1985). The protocols of the present study were approved by the Animal Care Committee of Saitama Medical Center, Saitama Medical School.

Animal preparation All rabbits were anesthetized with intramuscular ketamine hydrochloride (40 mg/kg). After intravenous injection of tubocuranine chloride (1 mg/kg) mechanical ventilation was commenced via tracheostomy with room air. Following thoracotomy, a 20-gauge angiocatheter was inserted in the left ventricle, to which a three-way stopcock was connected. One side was used for injection of CMS and the other side for monitoring left ventricular systolic pressure, which was constant within the range of 80 to 90 mmHg throughout the duration of the experiments. A polyethylene tube (0.40 mm I.D., 0.8 mm O.D., Dural Plastics, Australia) was inserted in the femoral artery for collection of arterial reference blood sample.

Basic studies Trapping of blue and yellow CMS in the normal kidneys was histologically examined by hematoxylin and eosin stain. In the present study, as the right kidney was employed as the control side and the left kidney of the same rabbit as the experimental side, the bilateral normal RBF (n=15) was determined by CMS. The bilateral difference in normal RBF was analyzed.

Experimental groups Operative procedure was not conducted on renal vessels of the right intact kidney of the rabbit which was employed as control side, while the left intact kidney was used as experimental side. Following midline laparotomy and incision of retroperitoneum, left renal artery and renal vein in the hilum of the intact kidney were dissected under the operating microscope. The rabbits were classified into four groups.

Group 1 Renal ischemia Renal ischemia for model of intact innervated kidney was used in 8 rabbits. The left renal artery with all renal nerves running to the left kidney kept intact was clamped for 10 min with micro-clip. Clamp time of the left renal artery for 10 min was established so that it would correspond to renal circulation blockage time of 10 min in the following renal denervation procedures. The bilateral RBF was measured at 5 min and 35 min after reperfusion.

Renal denervation procedures All visible renal nerves running to left intact kidney were sectioned under the operating microscope and the rabbits were classified into three groups. The caliber of the renal artery and that of the renal vein were uniform, being 1.5 mm and 2 - 3 mm, respectively. Mechanical anastomosis (Precise microvascular anastomotic system, 3M, St. Paul, MN) was employed in end-to-end anastomoses of the renal artery and those of the renal vein because the operative time required was as short as 5 - 9 min. Furthermore, as there was no leakage of RBF from the anastomosis site even after reperfusion, RBF was not impaired. Patency in all vessels was assessed by an “empty-refill” test performed distally on the renal artery and renal vein[15,16]. At 5 min and 35 min after reperfusion, bilateral RBF was measured.

Group 2: End-to-end anastomoses of renal artery After applying 2 % lidocaine hydrochloride for relief of spasm to the renal artery in 10 rabbits, a double

approximator clamp was applied to the left renal artery and the central region was sectioned with microsurgical scissors. The lumen was irrigated with 2 % lidocaine hydrochloride[15,16] and a solution of physiological heparinized saline (1,000 IU/L). End-to-end anastomosis of left renal artery was made under the operating microscope using 1.5 mm device (Fig. 1). Clamp of left renal artery was released at 10 min after cession of left renal artery circulation. RBF was measured at 5 min after reperfusion in order to effect topical application of 2 % lidocaine hydrochloride on renal vessels.

Group 3: End-to-end anastomoses of renal vein After renal circulation of left renal artery was first blocked with micro-clip, the same procedure in 10 rabbits was conducted as end-to-end anastomoses of renal artery, and end-to-end anastomoses of left renal vein were performed using 2 mm device. Clamp of left renal artery and renal vein was released at 10 min after cession of left renal artery circulation.

Group 4: Renal sympathectomy Without applying 2 % lidocaine hydrochloride to left renal vessels, the adventitia of renal artery in 8 rabbits was stripped with the media kept intact, and left renal artery was simultaneously clamped for 10 min with micro-clip.

Renal blood flow measurement Colored microspheres (CMS) with a diameter of $15 \pm 0.3 \mu m$ (E-Z TRAC, Inc, Los Angeles, CA) were employed at 100×10^4 per kg body weight. In each rabbit, bilateral RBF was measured at 5 min after reperfusion with blue CMS and at 35 min after reperfusion with yellow CMS. CMS were injected in one shot in the left ventricle, followed by washout with 1 mL of physiological saline. A reference blood sample was collected from the femoral artery at the rate of 6 mL/min by a Minipuls 3 peristaltic pump (Gilson Medical Electronics, S.A., France), which was started at 15 sec before injection of CMS and continued for 60 sec after injection. All the rabbits were sacrificed by an overdose of pentobarbital sodium. Right and left kidneys were harvested and weighed.

Blue and yellow CMS of right and left kidneys and reference blood samples were collected according to the E-Z TRAC procedures. Each kidney was placed in a 50 mL polypropylene centrifuge tube, to which 45 mL of tissue/blood digest reagent I was added. The tube was placed in a water bath heated to 70℃ for alkaline hydrolysis for 12 hours and vigorous vortex mixing was made for 30 sec. Tissue/blood digest reagent II was added to the tissue suspension to bring the total liquid volume to 15 mL and the centrifuge tube was centrifuged for 30 min. Each sediment was resuspended in 10 mL of CMS counting reagent by vortex mixing and thereafter centrifuged for 15 min. Moreover, CMS counting reagent was added to each sediment to bring the solution meniscus to an accurately measured total volume of 0.40 mL. Following vortex mixing, the sample preparation was ready for counting.

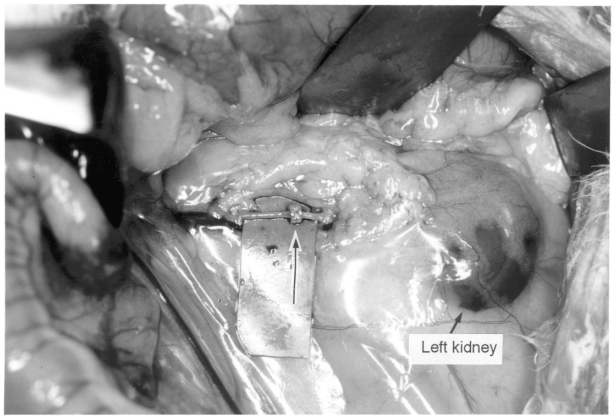

Fig. 1. Photograph showing end-to-end anastomosis of left renal artery using 1.5 mm device. Arrow indicates site of anastomosis.

Immediately after collection of reference blood, about 7.5 mL of the blood was mixed with EDTA Na_2 in a 50 mL centrifuge tube. Blood hemolysis agent was added to each blood sample to bring the total volume to 50 mL, followed by centrifugation for 30 min. The red supernate was aspirated down to a volume of 5 mL. The procedures thereafter were identical to those of kidney samples.

Four aliquots of the final kidney and reference blood solutions in each rabbit were respectively placed in four Fuchs-Rosenthal hemacytometers. Blue and yellow CMS could be recovered without color change after digesting with strong alkaline hydrolysis. The number of blue and yellow CMS in each kidney and reference blood sample was counted in a hemacytometer chamber under a microscope. RBF value (mL/ min/g) was calculated from the following equation:[8-11]

Renal blood flow (mL/min/g) $= (C_m \times Q_r)/C_r$ where C_m is the microsphere count per gram of kidney sample, Q_r is the withdrawal rate of the reference blood sample (6 mL/min), and C_r is the microspheres count in the reference blood sample.

Statistical analysis All data are expressed as means ± SEM. Regression analysis and paired t test were used to compare right and left normal RBF. When bilateral RBF after reperfusion was measured twice in each rabbit, the right RBF of the control on each occasion was regarded to be 100 % and left RBF of the experimental

group was expressed as percentage of the control value. Within-group comparison was made by analysis of variance and Fisher's PLSD post hoc test was used to test statistical significance. $P < 0.05$ was evaluated to be significant.

Results

Histology of kidney The histological findings in the kidney show that blue and yellow CMS 15 μm in diameter are mostly trapped within glomerular capillaries (Fig. 2). Thus, the relative number of CMS recovered from the kidneys should indicate regional glomerular blood flow.

Bilateral difference in normal RBF The mean right RBF in 15 rabbits was 4.77 ± 0.41 mL/min/g and the mean left RBF was 4.71 ± 0.41 mL/min/g, demonstrating no bilateral difference in normal RBF ($p=0.923$). Regression line formula of left RBF (Y) and right RBF (X) is:
$Y(mL/min/g) = 0.39 + 0.91 \times X$ ($r=0.923$, $p < 0.0001$, Fig. 3)
A close correlation was demonstrated between left RBF and right RBF. The foregoing results showed that in each rabbit the kidney on one side can be used as control side and the kidney on the contralateral side as experimental side.

Changes in RBF after reperfusion in renal ischemia In 8 rabbits, RBF of renal ischemia group at 5 min after reperfusion showed a remarkable increase, being 146.2 ± 9.4% of RBF of the control ($p=0.0018$). RBF at 35 min after reperfusion decreased to 112.1 ± 6.1 % of RBF of the control (NS, Fig. 4).

Changes in RBF after reperfusion in end-to-end anastomoses of renal artery In 10 rabbits, RBF of end-to-end anastomoses of renal artery at 5 min after reperfusion significantly decreased to 53.6 ± 6.7 % of the RBF of the control group ($p < 0.0001$). RBF of end-to-end anastomoses of renal artery group at 35 min after reperfusion recovered to 106.4 ± 0.6 % of RBF of the control group (NS, Fig. 5).

Changes in RBF after reperfusion in end-to-end anastomoses of renal vein In 10 rabbits, RBF of end-to-end anastomoses of left renal vein at 5 min after reperfusion decreased without statistical significance to 81.5 ± 10.5 % of RBF of the control group. RBF of end-to-end anastomoses of renal vein at 35 min after reperfusion recovered to 101.5 ± 10.1 % of the RBF of the control group (NS, Fig. 6). Though RBF of end-to-end anastomoses of renal vein did not show any significant difference from RBF of the control, it presented a same recovery pattern of RBF of end-to-end anastomoses of renal artery.

Changes in RBF after reperfusion in renal sympathectomy In 8 rabbits, RBF of renal sympathectomy at 5 min after reperfusion significantly decreased to 64.7 ± 12.5 % of RBF of the control group ($p=0.0258$). RBF of renal sympathectomy at 35 min after reperfusion recovered to 101.9 ± 13.0 % of RBF of the control group (NS, Fig. 7).

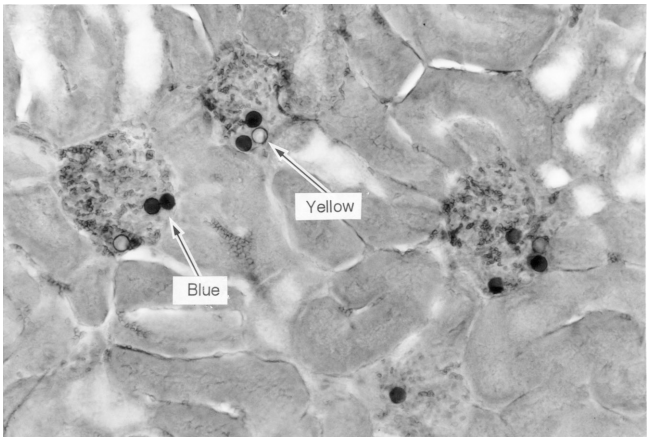

Fig. 2. Photomicrograph of blue and yellow colored microspheres in the kidney. (Hemtoxyline and eosin stain, magnification X 50).

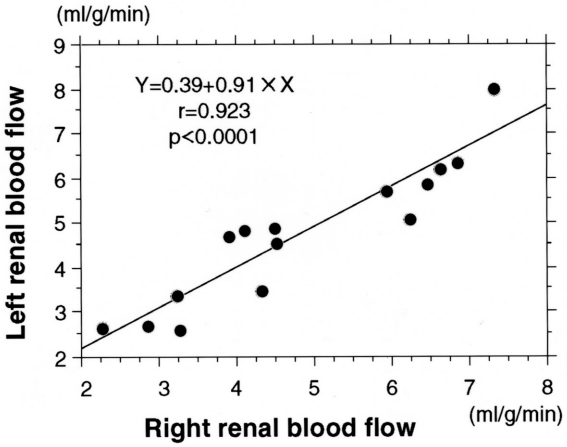

Fig. 3. Bilateral difference in normal renal blood flow.

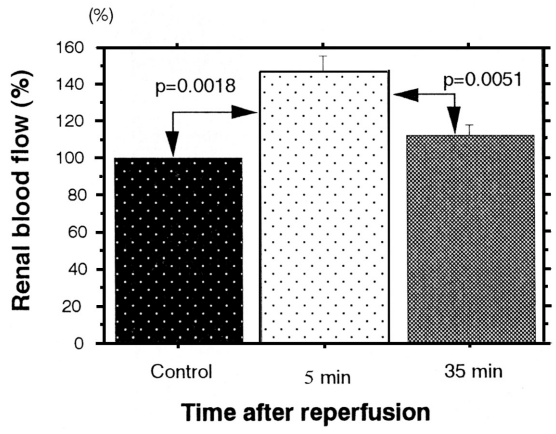

Fig. 4. Percentage of changes in renal blood flow after reperfusion in renal ischemia.

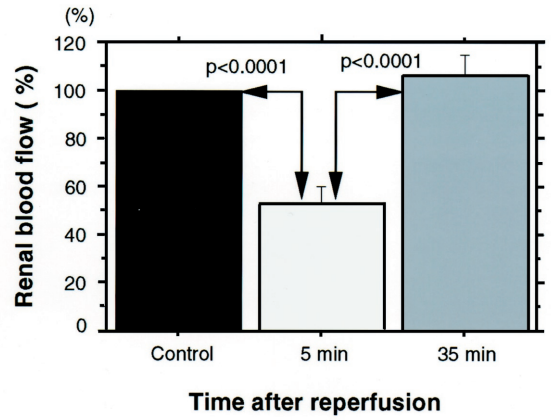

Fig. 5. Percentage of changes in renal blood flow after reperfusion in end-to-end anastomoses of renal artery.

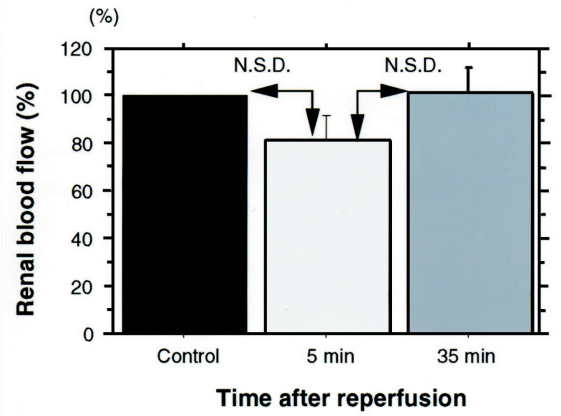

Fig. 6. Percentage of changes in renal blood flow after reperfusion in end-to-end anastomoses of renal vein.

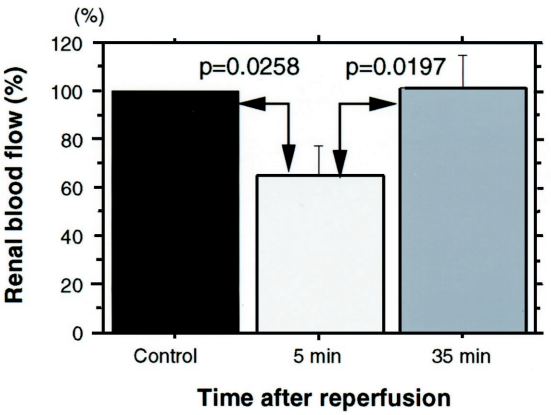

Fig. 7. Percentage of changes in renal blood flow after reperfusion in renal sympathectomy.

Discussion

This study aimed to elucidate the effects of acute renal denervation procedures on initial renal hemodynamics in intact kidneys of the rabbits. Our interesting observations are that first, renal blood flow (RBF) was damaged due to renal denervation induced by section of the renal artery and renal sympathectomy, and were transiently decreased to a level lower than normal RBF. Secondly, RBF in these denervated kidneys recovered to normal levels at 35 min after reperfusion. Thirdly, RBF was not affected by acute renal denervation induced by section of the renal vein.

Our data indicate that RBF of renal ischemia immediately after reperfusion showed a remarkable increase compared to normal RBF. This mechanism can be explained by reactive hyperemia in the innervated kidneys[17]. On the other hand, RBF at 5 min after reperfusion was transiently decreased due to acute renal denervation induced by section of the renal artery and renal sympathectomy, while it was not affected by section of the renal vein. These differences in RBF after reperfusion reflect that the distribution density of renal sympathetic nerves is higher around the renal artery than around the renal vein[4,18]. These findings may indicate that renal sympathetic nerve activity plays a role in the mechanisms involved in RBF responses.

Vascular spasm occurs immediately after renal artery or renal vein is cut, and results from nervous reflexes, local myogenic spasm, and local humoral factors. Most of vascular spasm probably results from local myogenic contraction of the renal vessels[19]. In the present study, 2 % lidocaine hydrochloride for relief of spasm was topically applied around renal vessels in the renal hilus, and the lumen was irrigated with 2 % lidocaine hydrochloride[15,16]. It remains unclear how extent vascular spasm influenced a transient decrease in RBF after section of renal artery or renal vein.

End-to-end anastomoses of the renal artery produce complete renal denervation by cutting both the adventitia and media[4,5]. On the other hand, renal sympathectomy involving only stripping of the adventitia is incomplete renal denervation, as perivascular neural plexuses around the media are kept intact[14,20-22]. Our results showed that there was no difference in recovery of RBF after reperfusion between end-to-end anastomoses of the renal artery and renal sympathectomy. This may indicate that RBF responses are more strongly controlled by renal nerve bundles in the adventitia than by the remaining perivascular plexus around the media. These results also suggested that initial renal hemodynamics in end-to-end anastomoses of the renal artery may be not strongly damaged by endothelial cell injury induced immediately after section of the renal artery[1,23].

It is an important finding of this study that RBF responses in end-to-end anastomoses of the renal artery and renal sympathectomy is completely reset to normal values at 35 min after reperfusion. Iversen B. J. et al[14] have produced renal sympathectomy in spontaneously hypertensive rats, and reported that the time course of readjustment in autoregulatory mechanism is within 10 min when the blood pressure acutely dropped within the pressure range of autoregulation. In our present study, as the second measurement time of RBF after reperfusion was late, the possibility is high that time for resetting normal RBF in end-to-end anastomoses of the renal artery and renal sympathectomy is shorter than 35 min.

It is considered that renal autoregulation is mediated by tubuloglomerular feedback (TGF) loop involving the juxtaglomerular apparatus and myogenic response of vascular smooth muscles in the interlobular arteries and afferent arterioles[1,2,24,25]. Normal TGF responses were observed in isolated perfused kidneys [26] and kidneys subjected to renal sympathectomy[4,21,22,27]. However, TGF responses were late being within 2 - 3 hours after renal sympathectomy, and consecutive measurements of TGF responses indicate that the process of TGF responses is time dependent[22]. Kurkus, J et al [27] have reported that resetting of TGF responses that developed 2-3 hours after renal sympathectomy are partially restored by 20 min of renal nerve stimulation at a frequency of 2 Hz. These results imply that renal nerves have an important impact on resetting of sensitivity of TGF mechanism.

TGF activity is often directly proportional to concentration of angiotensin II, especially in high renin state[23]. Iversen, B. M. et al[14] reported that acute normalization of the pressure range of RBF autoregulation in hypertensive rats is dependent on the degree of pressure reduction of the renal arterial pressure, whereas renal innervation and plasma renin concentration do not play a major role. Though our present study has a disadvantage that renin-angiotensin system, renal vascular resistance, and renal perfusion pressure were not analyzed, current evidences suggest that TGF mechanism in acute renal denervation is not a major mediator in initially resetting RBF autoregulation[28-30].

In conclusion, our results demonstrate that RBF in the intact kidneys was transiently decreased due to acute renal denervation induced by section of the renal artery and renal sympathectomy, while RBF in intact innervated kidneys was rapidly increased immediately after reperfusion. RBF in these denervated kidneys is completely restored to normal RBF at 35 min after reperfusion. RBF is not affected due to acute renal denervation induced by section of the renal vein. We conclude that renal sympathetic nerve activity partially controls renal blood flow responses, and that the renal sympathetic regulation of RBF is overruled by renal autoregulatory mechanism in initial renal hemodynamics after acute renal denervation.

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家兎正常腎を用いて各種の腎脱神経処置における腎血流量の変動colored microspheresによる腎血流量測定での検討

三浦洋靖，土田幸英，小山直基*，原科孝雄

目　的

我々は，各種の腎脱神経処置による腎血流動態への影響，および正常腎血流量に回復する時間を研究した．

実験方法

今回の研究において，日本白色ウサギ雄51羽を用いた．腎血流量測定は，カラーマイクロスフェアー（CMS）法で行った．全身麻酔下で気管切開し挿管し，GOFで維持した．開胸後，左心室に血管留置針を刺入し心筋に固定した．これに三方活栓を接続して一方は左心室圧をモニター，他方はCMS注入用とした．CMSを左心室にワンショットで注入した．屠殺後，手順に従って基準血液と腎臓のCMSを回収し，公式により腎血流量を計算した．

基礎研究から，CMS直径15 μ mは，糸球体毛細血管内に貯留していることを組織学的確認したので，本法は糸球体局所血流量を測定している．次に，正常腎血流量に左右差なかったので各兎において右腎臓をコントロール群，左腎臓を実験群として使用した．

開腹後左腎臓へ走行する交感神経を顕微鏡下に切断し，下記のように実験を 4 群に分類した．

(1) 腎虚血群（n＝8）：腎交感神経支配のモデルとして，左腎動脈を10分間マイクロクリップでクランプすることによって作製した．
(2) 腎動脈端端吻合群（n＝10）：左腎動脈を切断し血管吻合器（口径1.5 mm）で端端吻合を行った．
(3) 腎静脈端端吻合群（n＝10）：左腎静脈を切断し血管吻合器（口径2 mm）で端端吻合を行った．
(4) 腎交感神経切断群（n＝8）：左腎動脈外膜を顕微鏡下で剥離した．

腎血流量測定：腎動脈流を10分間遮断して，再灌流5分後に青色CMS，再灌流35分後に黄色CMSで左右腎血流量を測定した．

統計学的分析

数値を平均値±標準誤差で表示した．正常腎血流量を100％とし実験群腎血流量を百分率で表示した．数値を分散分析で検定しp＜0.05以下を有意差ありとした．

研究結果

(1) 腎虚血における腎血流量（RBF）：再灌流5分後の腎血流量は，正常RBFの146.2±9.4％まで増加した（p＝0.0018）．再灌流35分後では，腎血流量は正常値に回復した（NSD）．
(2) 腎動脈端端吻合における腎血流量：再灌流5分後の腎血流量は，正常RBFの53.6±6.7％まで減少した（p＜0.0001）．再灌流 35 分後では，腎血流量は正常値に回復した（NSD）．
(3) 腎静脈端端吻合における腎血流量: 再灌流5分後の腎血流量は，正常RBFの81.5±10.5％まで減少した（NSD）．再灌流35分後では，101.5±10.1％と正常値に回復した．再灌流後の腎血流量は，腎動脈端端吻合群の腎血流量と同じ経過を示したが有意差はなかった．
(4) 腎交感神経切断: 再灌流5分後の腎血流量は，正常RBFの64.7±12.5％まで減少した（p＜0.0001）．再灌流35分後では，腎血流量は正常値に回復した（NSD）．

結　論

腎血流量は，腎動脈切断と腎交感神経切断による腎脱神経で一過性に障害され,再灌流35分以内に正常腎血流量に回復した．腎脱神経における初期の腎血行動態は，腎交感神経よりも腎自己調節機構により強く支配されていることが今回の研究から示唆された．

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