Effect of Renal Denervation on the Compensatory Renal Growth Following Nephrectomy in the Cat

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Abstract: The purpose of this study was to clarify the effect of denervation on the mass of the remaining kidney with or without unilateral nephrectomy using adult cats. The animals were divided into 4 groups: (1) control group, the weights of the right and left kidneys were measured intact in 5 cats; (2) nephrectomy group (Nx, $n=5$ cats), the right kidney was removed and the left kidney was weighed 3–5 d after nephrectomy; (3) nephrectomy and denervation group (Nx+Dx, $n=7$ cats), the left kidney was weighed on the 7th day after surgery in which the left kidney was denervated and the right kidney was removed; and (4) denervation group (Dx+Dx, $n=5$ cats), both kidneys were weighed on the 7th day after denervation of the kidneys. In

the control group, the left and right kidney weights per body weight (LKW and RKW) were the same (LKW, $0.74 \pm 0.06\%$; RKW, $0.74 \pm 0.07\%$). In the Nx group, LKW increased to $0.90 \pm 0.03\%$ 3–5 d after nephrectomy, although RKW of the removed kidney was $0.66 \pm 0.01\%$. In the Nx+Dx group, LKW increased to $0.97 \pm 0.15\%$, which was similar to that of the Nx group. In the Dx+Dx group, LKW ($0.56 \pm 0.05\%$) and RKW ($0.54 \pm 0.05\%$) were significantly less than those in the control group. We conclude that the renal nerves may contribute to maintaining the renal mass and that the neural effect on compensatory growth following nephrectomy may be covered by other growth factors. [Japanese Journal of Physiology, 49, 373–377, 1999]

Key words: renal sympathetic nerve activity, reno-renal reflex, compensatory renal growth, unilateral nephrectomy.

Surgical removal of one kidney in experimental animals and humans has led to the observation that the remaining kidney subsequently undergoes compensatory growth [1]. The mass of the remaining kidney increases approximately 40% of the control value after nephrectomy in the rat [2] and mouse [3]; the occurring within 1–2 d after nephrectomy. Similar changes in the renal mass have been reported using human donors in renal transplantation [4]. Although the mechanisms accounting for this compensatory growth remain unknown, several hypotheses have been proposed [5]. One of the hypotheses is that the increment in workload of the remaining kidney, such

as increased glomerular filtration rate, enhanced reabsorption of sodium, bicarbonate and tubular fluid, may induce the compensatory renal growth [6, 7]. Another hypothesis is that a humoral growth factor may induce renal hypertrophy. Generally, it is considered that these local autoregulatory and humoral mechanisms mainly contribute to renal hypertrophy following nephrectomy [8–10]. On the other hand, a neural factor seemed to play no role in the compensatory renal growth because renal denervation did not affect renal hypertrophy in young rats [11, 12]. This previous study involved a potential problem of developmental renal growth, which might affect the result [13, 14].

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Although the mass of the denervated kidney increased following nephrectomy, the masses of sham-denervated and sham-nephrectomized kidneys also increased [11]. Therefore, whether the renal nerves may participate in renal growth following nephrectomy remains to be solved. To avoid this problem of developmental renal growth and examine the effect of denervation on renal mass following nephrectomy, it is necessary to use a mature animal.

Recently, it has been assessed whether renal sympathetic nerve activity to the remaining kidney is modified by nephrectomy in anesthetized rats [15]. When renal sympathetic nerve activity was recorded on 3, 7, 14, and 28 d after nephrectomy, renal sympathetic nerve activity was more enhanced than that in the sham-operated group. From this result, it is undeniable that an increase in renal sympathetic nerve activity is required for the renal compensatory responses including renal growth following nephrectomy. This neural hypothesis is supported by a previous study showing that vagotomy diminished compensatory renal growth after nephrectomy [16]. In addition, we have observed that sympathetic nerve activity to the hypertrophied contralateral kidney was increased following nephrectomy in conscious adult cats [17]. The mass of the remaining kidney also increased following nephrectomy, but the body weight remained the same. Therefore, if the effect of denervation on renal mass following nephrectomy is investigated using adult cats, the potential problem of developmental renal growth can be avoided. In this study, we compared the effect of denervation on renal mass with and without nephrectomy in adult cats.

METHODS

Preparation. The experiments were performed on 22 cats weighing between 1.7 and 4.3 kg according to the Guiding Principles for the Care and Use of Animals in the Fields of Physiological Sciences approved by the Physiological Society of Japan. Surgery was conducted for implantation of catheter, for nephrectomy, and denervation. Atropine sulfate (0.5 mg) was intramuscularly given as preanesthetic medication to reduce salivation and bronchial secretions. The anesthesia was induced by inhalation of a mixture of halothane (4%), N₂O and O₂, and then an endotracheal tube was inserted. The cats breathed spontaneously during surgery and electrocardiogram (ECG), heart rate, and respiration were continuously monitored. To maintain the level of surgical anesthesia, the concentration of halothane was increased in a range of 1.5–2.0% if an increase in heart rate and/or respiration

and/or withdrawal of the limb in response to noxious pinch of the paw and/or surgical procedure was observed. A polyvinyl catheter was inserted into the left external jugular vein for administering drugs. Rectal temperature was maintained at 37–38.5°C with a heating pad. The venous catheter was tunneled subcutaneously and exteriorized at the back of the neck. The cats were warmed with a heating pad until waking up. After recovery from the anesthesia, the cats were housed in their cages and could intake water and food *ad libitum*. Antibiotics (penicillin G, 10,000–20,000 units) were given for 2–5 d following the operation.

Protocol.

Experiment 1 (nephrectomy group ; n=5 cats: effect of nephrectomy on renal mass). Under aseptic conditions with halothane anesthesia, the right kidney was approached retroperitoneally and nephrectomy was performed after ligation of the renal vessels and the ureter taking care not to injure the peritoneum and adrenal gland. The weight of the right kidney was measured. When the animals were sacrificed 3–5 d after the surgery, the left kidney was removed and weighed as described above.

Experiment 2 (nephrectomy and denervation group; n=7 cats: effect of nephrectomy and denervation on renal mass). The left kidney was exposed retroperitoneally and all branches of the renal nerves were separated from the renal plexus and surrounding connective tissue near the renal artery and vein using an operating microscope. All renal nerve branches were ligated with 7–0 silk sutures at two sites, and the branches were cut at the middle of the sites. The nephrectomy of the right kidney was done following the same method as the nephrectomy group. The weight of the right kidney was measured. When the animals were sacrificed 7 d after the surgery, the left kidney was removed and weighed as described above.

To examine whether the left kidney was completely denervated, we implanted a flow probe (lumen size 1.5–2 mm), connected to a pulsed Doppler flowmeter (Triton model 200-1,000), on the left renal artery close to the abdominal aorta in two anesthetized cats. We observed that renal blood flow of the denervated kidney was unchanged during stimulation of the splanchnic nerve, suggesting that renal denervation was complete.

Experiment 3 (denervation group; n=5 cats, effect of denervation on renal mass). Denervation of both kidneys was done following the same method as the nephrectomy and denervation group. The right and left kidneys were removed and weighed as described above on the 7th day after the surgery. The weight of the right and left kidneys were measured in intact cats

Table 1. Absolute values of whole body weight and right and left kidney weights in control, nephrectomy, denervation and nephrectomy, and denervation groups.

	Before intervention			After intervention		
	Body weight (g)	Right kidney weight (g)	Left kidney weight (g)	Body weight (g)	Right kidney weight (g)	Left kidney weight (g)
Control ($n=5$)	3,160 $\pm$ 147	22.8 $\pm$ 1.0	23.0 $\pm$ 1.0	—	—	—
Nephrectomy ($n=5$)	3,440 $\pm$ 234	22.7 $\pm$ 1.4	—	3,420 $\pm$ 235	—	30.8 $\pm$ 2.3
Dx+Nx ($n=5$)	3,540 $\pm$ 242	19.7 $\pm$ 1.4	—	3,500 $\pm$ 232	—	31.2 $\pm$ 2.7
Dx+Dx ($n=7$)	2,829 $\pm$ 254	—	—	2,757 $\pm$ 257	14.6 $\pm$ 1.3	15.2 $\pm$ 1.5

Values represent mean $\pm$ SE; Dx+Nx, left denervation and right nephrectomy; Dx+Dx, right and left denervation.

($n=5$) as the control group.

Data treatment and statistical analysis.

The percentages of right and left kidney weight per body weight (RKW, LKW) were subsequently calculated and analyzed. The effects of nephrectomy, nephrectomy and denervation, and denervation alone on RKW and LKW were analyzed using a two-way analysis of variance (ANOVA). When a significant F -value in the main effect was present, a Tukey's post hoc test was performed to determine a significant difference between mean values. The relative ratio between the right and left kidney weights was calculated and analyzed subsequently. The statistical significance of the changes in relative ratio obtained for the four groups was determined using Mann-Whitney's U -test. The effect of denervation alone on the RKW and LKW was also statistically examined using Mann-Whitney's U -test. The level of statistical significance was defined as $p<0.05$. The data are expressed as mean $\pm$ SE.

RESULTS

The body weight was unchanged by any intervention used in this study (nephrectomy, nephrectomy and denervation, and denervation alone group) as shown in Table 1.

The growth of the remaining kidney following nephrectomy

All absolute values of the weight of both kidneys and the whole body are summarized in Table 1. The changes in the percentage of the renal mass per body weight (LKW and RKW) are also shown in Fig. 1. In the control group, there was no difference in mass between the left and right kidneys (23.0 $\pm$ 1.0 g vs. 22.8 $\pm$ 1.0 g): LKW was 0.74 $\pm$ 0.06% and RKW was 0.74 $\pm$ 0.07%. In the nephrectomy group, the mass of the removed right kidney was 22.7 $\pm$ 1.4 g and the mass of the remaining left kidney was increased to

Fig. 1. The left (open column) and right (close column) kidney weights per body weight in the control, nephrectomy, and nephrectomy and denervation groups are shown. The left kidney's weight per body weight increased to 0.90–0.97% following nephrectomy with or without denervation. The data are indicated as means $\pm$ SE. * $p<0.05$, significant difference between left and right kidney weights per body weight.

30.8 $\pm$ 2.3 g 3–5 d after nephrectomy. LKW was increased to 0.90 $\pm$ 0.03% following nephrectomy, although RKW was 0.66 $\pm$ 0.01% (Fig. 1). The relative ratio of mass between the left and right kidney weights became 1.36 $\pm$ 0.04, greater than that of the mass in the intact kidneys (Fig. 2).

Effect of denervation on renal growth following nephrectomy

The mass of the remaining left kidney increased to

Fig. 2. The ratios of the left kidney's weight per body weight (LKW) and the right kidney's weight per body weight (RKW) are compared among the control, nephrectomy, and nephrectomy and denervation groups. The ratios of LKW and RKW in the nephrectomy group and nephrectomy and denervation group were significantly greater than that in the control group. The data are indicated as means $\pm$ SE. * $p < 0.05$, significant difference from control.

Fig. 3. The effect of denervation of both kidneys on the renal mass is shown. Both right (closed column) and left (open column) kidney weights per body weight decreased significantly to 0.54–0.56% in the denervation group compared to those in the control group. The data are indicated as means $\pm$ SE. * $p < 0.05$, significant difference from control.

31.2 $\pm$ 2.7 g following nephrectomy. LKW increased to 0.97 $\pm$ 0.15% following nephrectomy with denervation (Fig. 1). The relative ratio of mass between the left and right kidneys was 1.56 $\pm$ 0.12, which was greater than that in the control group but did not differ from that of the nephrectomy group (Fig. 2).

Effects of denervation alone on renal mass

The mass of the left and right kidneys following denervation were reduced compared to the renal mass in the control group to 15.2 $\pm$ 1.5 and 14.6 $\pm$ 1.3 g, respectively, although the total body weight of the denervated cats did not change significantly. LKW and RKW were 0.56 $\pm$ 0.05 and 0.54 $\pm$ 0.05% respectively, which were significantly smaller than those in the control group (Fig. 3).

DISCUSSION

We analyzed the effect of renal denervation on renal growth following nephrectomy using adult cats. The weight of the remaining kidney was increased by unilateral nephrectomy even after renal denervation was performed. On the other hand, we found that the renal mass was significantly decreased by renal denervation alone. Taking these results into account together, it is suggested that the renal nerves contribute to maintaining the renal mass and that the neural contribution to renal growth following nephrectomy may be covered by other compensatory factors.

We examined the effect of denervation of both kidneys on the renal mass, but not unilaterally, because it was possible that the renal denervation of one kidney might affect the opposite kidney due to the reno-renal reflex. Although we did not examine the effect of denervation on renal mass in the same cats, the percentages of left and right kidney weights per unit body weight (LKW and RKW) of the denervation group were significantly smaller than those in the control group. It is suggested that the renal nerves contribute to maintaining renal mass.

The renal nerves are the communication link between the central nervous system and the kidney. In response to normal physiological stimuli, changes in efferent renal sympathetic nerve activity importantly contribute to the homeostatic regulation of renal blood flow, glomerular filtration rate, renal tubular epithelial cell solute and water transport, and hormonal release [18]. Furthermore, our result suggests that the renal sympathetic discharge and/or a trophic substance released from the nerve contribute to homeostatic regulation of the renal mass in the baseline condition as previously considered [19].

It has been reported that chemical denervation of peripheral sympathetic nerve prevented compensatory growth in the adrenal cortical gland following unilateral adrenalectomy [20]. Moreover, our recent study showed that sympathetic nerve activity to the remaining kidney increased after unilateral nephrectomy [15, 17]. Therefore, it was possible that the renal nerves were also required for the compensatory growth of the remaining kidney following nephrectomy. To solve this question, the effect of renal denervation on renal compensatory growth following nephrectomy was examined using adult cats. We found that the renal mass was able to increase after unilateral nephrectomy with denervation in mature cats. This finding was in agreement with previous results obtained from young rats [11, 12]. Because these results showed the ineffectiveness of renal denervation on compensatory renal

growth following nephrectomy, it is likely that renal nerves may not have an essential role in producing renal hypertrophy after nephrectomy in the mature stage of animals as well as developing animals. However, it cannot be neglected that the renal nerve contribution to compensatory growth may be covered by other growth factors which appear simultaneously following nephrectomy.

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