Studies on Possible Mechanisms of Early Functional Compensatory Adaptation in the Remaining Kidney

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Two to 4 hours after unilateral renal exclusion in rats, urine flow rate from the remaining kidney had increased to twice the control level, whereas the filtration rate remained unchanged. After contralateral nephrectomy, NGFR was similar to that of controls, but fractional water reabsorption along proximal tubules decreased. Protein concentration in efferent arteriolar plasma, and hydrostatic pressure gradient between proximal tubules and peritubular capillaries were similar in experimental and control kidneys. Unilateral renal exclusion was followed by a rapid increase of blood pressure. Prevention of this rise depressed but did not abolish functional compensatory adaptation. The occurrence of compensatory adaptation was not affected by decreased renal perfusion pressure.

INTRODUCTION

The mechanisms by which water and electrolyte excretion from the remnant kidney is enhanced shortly after renal mass reduction remain unknown. Inhibition of net tubular reabsorption must play an essential role in this adaptation, since an increase of glomerular filtration rate (GFR) is not a prerequisite for the occurrence of compensatory changes [1,2]. We therefore investigated a possible role of intrarenal "physical factors" in the short-term adaptive changes after unilateral kidney exclusion. "Physical factors," such as peritubular protein concentration and the tubulo-capillary gradient of hydrostatic pressure are thought to modulate proximal tubular fluid and salt reabsorption [3].

Blood pressure (BP) has been shown to rise transiently immediately after renal mass reduction [2,4]. This small increase has not been considered as instrumental in inducing functional adaptation of the remaining kidney. Since we had confirmed, in preliminary experiments, that a transient rise of arterial blood pressure occurred consistently after functional exclusion of one kidney, we investigated in more detail the relationship between changes in arterial BP and the functional adaptation of the remaining kidney.

METHODS

Experiments were performed either on male Sprague-Dawley rats (Iffa-Credo, Les Oncins, France), or on female Wistar-type rats (Pathophysiologisches Institut, Bern, Switzerland), weighing between 160 and 240 g. Anesthesia was induced by IV injection of pentobarbital-Na (Nembutal\textsuperscript{R}: 30 mg/kg).

For micropuncture experiments, male Sprague-Dawley rats were used. Anesthetized animals were kept on a heated table, and were either nephrectomized unilater-
ally (right kidney) (experimental group: 12 rats), or sham-operated (control group: 10 rats). A flank incision was made, and the left kidney was placed in a lucite chamber. Mineral oil at 37°C covered the kidney throughout the experiment. End-proximal tubule punctures were performed with micropipets 10–12 μm OD; tubular fluid was collected after intratubular injection of an oil droplet colored with Sudan-black, and kept just distal to the puncture site during the collection, which lasted 3–5 minutes. The volume of the collectate was subsequently measured with calibrated micropipets. At the end of the collection, neoprene was injected through the puncture hole, and end-proximal localization was ascertained by subsequent cast dissection. The rats were infused with an isotonic saline solution at 0.05 ml/minute. Inulin was added to the infusion in amounts calculated to yield a plasma concentration of about 1 g/l.

After an equilibration period of 60 minutes, end-proximal punctures were performed. Final urine was simultaneously collected. Blood samples, obtained at 20–30 minute intervals from a femoral artery, were used for measuring hematocrit and inulin concentration. In 7 experimental and 6 control rats, superficial efferent arterioles were punctured with siliconized pipets. Arteriolar blood was centrifuged and plasma was assayed for protein concentration. Additionally, in 4 control and 6 experimental rats, hydrostatic pressure was measured in proximal tubules and peritubular capillaries with a servo-nulling micropressure apparatus (Dept. of Electronics, Yale Univ. School of Medicine, New Haven, Ct).

Female Wistar-type rats were used for experiments involving continuous blood-pressure recordings and alterations of renal perfusion pressure. Anesthetized animals were infused with isotonic saline containing inulin at a rate of 0.1 ml/min. The ureters of both kidneys were cannulated. An adjustable metallic clamp was placed around the aorta above the origin of the renal arteries, and a snare was placed around the vascular pedicle (in a few animals around the ureter) of the right kidney. After an equilibration period of 60 minutes, two timed (15–20 minutes) urine collections from both kidneys were obtained, and blood samples were taken at the mid-point of each collection period. The snare around the right vascular pedicle or ureter was then tightened, and after 20–30 minutes urine was collected from the left kidney for another two clearance periods. Femoral arterial BP was continuously registered with a recorder connected to a strain-gauge. In some animals the adjustable peri-aortic clamp was used to reduce renal perfusion pressure from the beginning of the experiment, in others to maintain femoral blood pressure at the level measured before unilateral kidney exclusion. Animals were studied in five different experimental situations with regard to femoral artery pressure:

Group 1 (7 rats): peri-aortic clamp left open throughout the experiment.

Group 2 (5 rats): peri-aortic clamp slightly tightened after exclusion of the right kidney, in order to prevent a rise in renal perfusion pressure.

Group 3 (6 rats): peri-aortic clamp tightened from the beginning of the experiment, in order to reduce renal perfusion pressure to 60–70 mmHg, but not adjusted after exclusion of one kidney.

Group 4 (4 rats): same as Group 3, but the peri-aortic clamp was further tightened after unilateral renal exclusion in order to prevent a rise of renal perfusion pressure.

Group 5 (4 rats): control group, in which an intestinal loop rather than the renal vascular pedicle was ligated. The peri-aortic clamp was left open throughout the experiment.

BP values were read on the record at the beginning of each experiment, 5 minutes
before tightening the snare, 30 seconds and 5 minutes after tightening the snare, and at the end of experiments.

Inulin concentration in plasma and urine was measured by the method of Vurek and Pegram [5]. Protein determinations in efferent arteriolar plasma were performed by the Lowry method as adapted to ultra-micro samples [6], using bovine serum albumin as standard.

Results are presented as means ± SEM. Statistical significance of differences was evaluated by the t test for unpaired or paired data, as appropriate.

RESULTS

During the second and third hour following unilateral nephrectomy, GFR of the remaining kidney did not significantly differ from that of the same kidney in sham-operated control rats (Table 1). At the same time, urine flow from the remaining kidney was twice as high as in the left kidneys of controls (Table 1). Proximal single-nephron filtration rate (NGFR) was similar in the two groups, but the fraction of filtered water reaching the end of the proximal convoluted tubules, as estimated by the inulin P/F ratio, was slightly but significantly larger in the remaining than in the control kidneys (Table 1). Calculation of absolute rates of fluid reabsorption along proximal convoluted tubules in individual rats did not show a significant difference between the two groups: 17.2 ± 0.9 nl/min in experimental versus 16.8 ± 0.7 nl/min in control kidneys. Protein concentration in efferent arterioles, and gradient of hydrostatic pressure between proximal tubules and peritubular capillaries were similar in experimental and control animals (Table 1). Hematocrit values in arterial blood at the beginning of the experiments were 43.5 ± 0.8 in unilateral nephrectomized rats and 42.7 ± 0.9 in controls, and 41.2 ± 0.9 and 39.3 ± 1.1, respectively, at the end of experiments. At no time did experimental and control values differ significantly.

Continuous recording of femoral arterial BP revealed a consistent pattern of

| TABLE 1 |
|-----------------|-----------------|------------------|
|                 | Left Kidney     |                 |
|                 | Right Nephrectomy | Control          |
| Kidney GFR (ml/min. KgBW) | 5.7 ± 0.2 (12) | 5.3 ± 0.2 (10) | NS               |
| Urine flow (µl/min. KgBW)     | 110.2 ± 9.0 (12) | 54.6 ± 4.0 (10) | *p < 0.001       |
| P/U inulin %               | 1.5 ± 0.1 (12)  | 0.9 ± 0.1 (10)  | *p < 0.01        |
| P/F inulin end-prox. %       | 51 ± 0.8 (29)   | 47 ± 1.3 (31)   | *p < 0.05        |
| Prox. NGFR (nl/min)        | 34.8 ± 1.9 (28) | 32.1 ± 1.1 (31) | NS               |
| Eff. arter. protein (g/100 ml) | 6.4 ± 0.2 (24) | 6.9 ± 0.2 (28) | NS               |
| Hydrostatic pressure gradient (prox. tub.–peritub. capill.) (mmHg) | 3.4 ± 0.3 (56) | 3.0 ± 0.4 (31) | NS               |
changes after exclusion of one kidney (Table 2). After an initial, short-lived decrease of blood pressure, maximal within 30 seconds, BP rose to reach a peak significantly above the values measured before renal exclusion (Table 2, group 1): 5 minutes after unilateral kidney exclusion, BP rose in all animals of Group 1 by +10 ± 2 mmHg. At the end of the experiment, average blood pressure fell back to pre-nephrectomy levels. Unilateral renal exclusion induced a large increase of urine, sodium and potassium excretion from the remaining kidney, while GFR remained constant (Table 3, Group 1). In contrast, blood pressure was not increased after ligation of an intestinal loop (Table 2, Group 5). When renal perfusion pressure was decreased to 60–70 mmHg at the beginning of each experiment (Table 2, Group 3), exclusion of the right kidney by ligation of the vascular pedicle induced a significant and even larger rise of femoral BP, 5 minutes after tightening the snare. Initial decrease of renal perfusion pressure reduced GFR, which then increased after exclusion of the contralateral kidney (Table 3, Group 3). Adaptation of water, sodium and potassium excretion were not suppressed during reduced renal perfusion pressure. In order to investigate the possible role of the initial transient peak of blood pressure in mediating the adaptive response of the remaining kidney, the post-nephrectomy blood pressure increase was prevented by appropriate tightening of the peri-aortic clamp in both conditions of initially normal or reduced perfusion pressure (Table 2, Groups 2 and 4). Whereas this maneuver abolished functional adaptation of water and sodium excretion as judged from the mean values, individual rats were not uniformly affected: 2 rats out of 5 in Group 2 showed an increase of urine and sodium excretion from the remaining kidney, which was larger by more than 50 percent of the level measured before contralateral renal exclusion. In Group 4, on the other hand, mean urine flow from remaining kidneys still increased despite the absence of a post-nephrectomy peak of BP. Finally, prevention of the post-nephrectomy increase of BP did not affect the compensatory adaptation of potassium excretion (Table 3, Groups 2 and 4).

DISCUSSION

A significant increase of urine flow and salt excretion in the first hours following contralateral nephrectomy of functional exclusion, occurring in the absence of a significant change of whole kidney GFR, in the present experiments, was accompanied by a slight but significant reduction of proximal fractional water reabsorption, whereas NGFR remained constant. Compensatory inhibition of proximal fluid and salt reabsorption of a larger magnitude than that in the present study has been measured in the dog [7] and the rat [8], and was accompanied by a fall of absolute proximal reabsorption. The proximal convoluted tubule, thus, appears to be an important site of mechanisms of compensatory adaptation. The intrarenal “physical

TABLE 2
Femoral artery blood pressure before and after unilateral renal exclusion or ligation of an intestinal loop. Statistical significance refers to values measured 5 minutes before ligation. *p < 0.05; **p < 0.025; ***p < 0.01 (paired t test).

| Group (see: Methods) | Initial | 5 min before Renal Excl. | 30 sec after Renal Excl. | 5 min after Renal Excl. | Final | After aortic unclamping |
|----------------------|---------|--------------------------|--------------------------|--------------------------|-------|-------------------------|
| **1**                | 126 ± 3 | 118 ± 2                  | 109 ± 3**                | 128 ± 3***               | 124 ± 3 NS | —                       |
| **2**                | 122 ± 3 | 120 ± 4                  | 92 ± 3***                | 105 ± 4**                | 108 ± 8 NS | —                       |
| **3**                | 127 ± 3 | 65 ± 2                   | —                        | 89 ± 7**                 | 79 ± 9 NS  | 139 ± 5***              |
| **4**                | 135 ± 5 | 66 ± 1                   | —                        | 64 ± 1 NS                | 63 ± 2 NS  | 139 ± 11***             |
| **5**                | 123 ± 2 | 117 ± 4                  | 104 ± 3*                 | 117 ± 7 NS               | 117 ± 4 NS | —                       |
### TABLE 3
Summary of renal functions of the left kidney before and after contralateral kidney exclusion or intestinal loop clamping. Differences were calculated in individual rats (values after ligation minus values before ligation). For a description of the groups, see "Methods." *p < 0.05; **p < 0.025; ***p < 0.01 (paired t test). Mean values are given ± SEM. When no indication is given, difference is statistically not significant.

| Group | GFR (ml. min⁻¹.kg⁻¹) | Urine flow (µl. min⁻¹.kg⁻¹) | Fract. excret. Na (% filtered) | Fract. excret. K (% filtered) |
|-------|------------------|-------------------------------|-------------------------------|-------------------------------|
|       | Before | After | Diff. | Before | After | Diff. | Before | After | Diff. | Before | After | Diff. |
| 1     | 5.4 ± 0.3 | 5.4 ± 0.4 | 0.0 | 33.3 ± 5.1 | 85.6 ± 12.9 | +52.3*** | 0.48 ± 0.18 | 2.04 ± 0.4 | +1.56*** | 19.2 ± 1.1 | 32.9 ± 2.1 | +14.7*** |
| 2     | 5.4 ± 0.3 | 5.2 ± 0.2 | -0.3 | 46.6 ± 9.4 | 50.8 ± 6.4 | +4.2 | 0.93 ± 0.29 | 1.0 ± 0.25 | +0.07 | 21.1 ± 3.2 | 29.7 ± 3.3 | +8.6*** |
| 3     | 3.3 ± 0.5 | 4.1 ± 0.6 | +0.8 | 25.9 ± 3.8 | 46.2 ± 3.6 | +20.3*** | 0.18 ± 0.13 | 0.43 ± 0.16 | +0.25** | 14.8 ± 2.4 | 38.4 ± 5.2 | +23.6*** |
| 4     | 3.0 ± 0.5 | 3.1 ± 0.4 | +0.1 | 15.6 ± 3.7 | 24.5 ± 5.6 | +8.9* | 0.07 ± 0.02 | 0.07 ± 0.02 | 0.00 | 10.9 ± 2.2 | 37.1 ± 6.7 | +26.2** |
| 5     | 4.6 ± 0.2 | 4.0 ± 0.4 | -0.6 | 35.0 ± 14.8 | 44.0 ± 21.5 | +9.0 | 0.50 ± 0.27 | 0.78 ± 0.41 | +0.28 | 15.9 ± 0.3 | 16.8 ± 1.5 | -0.9 |
factors,” which have been evaluated in the present experiments, however, do not appear to mediate the adaptive response.

Investigations on the mechanisms of volume expansion diuresis showed that, besides a decrease of salt and water reabsorption in proximal tubules, an inhibition of more distal sites of the nephron is required for the final natriuretic effect [9]. Though the behavior of nephron segments beyond the proximal convoluted tubule has not been extensively investigated in the course of the diuretic response to exclusion of the contralateral kidney, the present data, as well as earlier observations [10, 11], suggest that the extent of compensatory diuresis depends critically on changes in nephron sites downstream to the proximal convoluted tubules. Thus a compensatory diuretic response of the same magnitude may occur with a minor (present experiments) or a major [8] depression of proximal reabsorption.

Although the present data suggest the occurrence of consistent changes of BP after exclusion of one kidney, it remains questionable whether such changes are causally related to functional adaptation. The compensatory increase of sodium and fluid excretion appeared to be compromised when the BP rise following unilateral renal exclusion was prevented, but was not abolished in all animals. Additionally, the compensatory increase of potassium excretion was not impaired by keeping the renal perfusion pressure constant. Furthermore, the present study confirms the observations originally made by others [12], viz. that a decrease of renal perfusion pressure prior to kidney removal does not interfere with the adaptive changes of the remaining kidney. Thus, whereas significant and consistent changes of BP follow unilateral kidney exclusion, these changes do not appear to be the primary mediators of the functional adaptation of the remaining kidney.

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