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Compensatory Renal Growth: Interactions of Nephrectomy Serum and Urine Antisera Leading to a New Theory of Renal Growth Regulation

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Summary. Compensatory renal growth is mediated by a substance(s) found in the serum and in urine called renotropin. The interaction of nephrectomy serum and urine antisera upon compensatory renal growth was investigated by administering rat uninephrectomy serum and sham serum and urine to rabbits. The rabbit antisera was given intravenously to uninephrectomy and sham operated rats and kidney weight/body weight ratios were calculated. Antisera against sham serum and urine induced kidney growth as well as antisera against uninephrectomy serum and urine given to sham animals. These results suggest the presence of a circulating antigenic inhibitor to kidney growth and suggest that renotropin is made up of a inhibitory as well as a stimulatory substance.

Key words: Compensatory renal growth, Obligatory renal growth, Renotropin, Kidney growth regulation.

Introduction

Compensatory renal growth (CRG) is a recognized phenomenon that occurs when renal parenchyma is damaged or removed [8]. An uncharacterized substance(s) found in serum and urine which induces CRG has been named "renotropin".

Many clinical observations have aided the understanding of the physiology of CRG. The fetus does not exhibit CRG but CRG begins promptly after birth if one kidney is severely compromised [14, 19]. A kidney's ability to hypertrophy may decline with age [7, 13]. Transplanted kidneys undergo CRG suggesting a lack of direct neural control. Data from refluxing kidneys in children suggests that CRG is a reversible phenomenon [2].

These clinical observations have stimulated numerous laboratory investigations designed to elucidate the regulatory mechanisms responsible for CRG. Parabiotic animals have been used to demonstrate compensatory growth in unoperated kidneys. CRG seems to be a quantitative phenomenon depending on how many of the parabiotes' kidneys are removed [12, 22]. Bilateral nephrectomy of one parabiote results in CRG in its mate which is readily reversible in the mate after 12 h [3].

A three kidney animal (by transplantation) has made an excellent study model. By the addition or removal of functioning renal parenchyma hypertrophy and hypotrophy may be demonstrated [11, 15, 20]. The ability of CRG to be turned on and off remains unexplained but it does suggest the presence of a stimulatory as well an inhibitory substance(s).

Other investigators have used in vitro techniques including isolated organ perfusion [18] and tissue culture to demonstrate the effects of the serum and urine substance(s) on CRG.

Few facts are known about renotropin. It has a molecular range of 10,000 to 50,000 dalton. Renotropin crosses the peritoneal membrane poorly [4]. CRG is not dependent upon growth hormone [9], insulin [9], thyroid hormone [21], ACTH [17], or androgens [1].

This experiment is designed to test the interaction of nephrectomy serum and urine antisera upon CRG. Because the molecular weight range is suitable for antibody induction, study of this possibility would help in further efforts to characterize renotropin.

Materials and Methods

Six male Sprague Dawley rats weighing 230–280 g underwent a unilateral left nephrectomy through the flank under ketamine anesthesia. After 68 h the rats were placed in a metabolic cage and urine was collected for 4 h. The animals were then sacrificed and their serum obtained by centrifugation. One and one-half cc. of fresh uninephrectomy serum plus 0.5 cc of complete Freund's adjuvant was given subcutaneously to three 8 lb. New Zealand white rabbits. This process was repeated weekly for a total of four weeks. After five weeks blood was drawn from the rabbits by cardiac puncture and the serum was obtained and frozen at −20 °C.

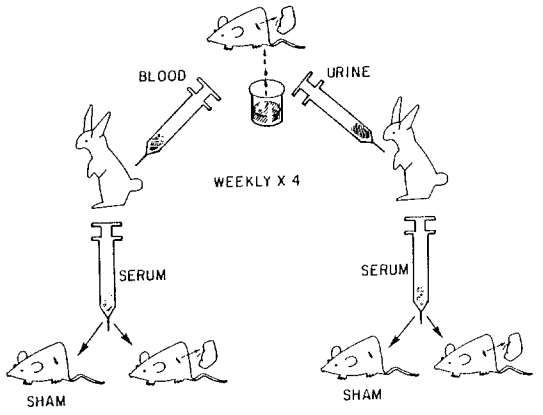

Fig. 1

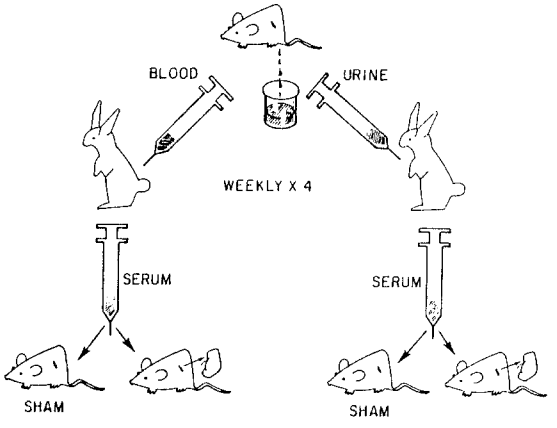

Fig. 2

A pilot trial of two nephrectomy groups and two sham groups were used to determine a crude dose-response curve of antisera given to stimulation or inhibition of renal growth. Groups of 10 male Sprague Dawley rats had a left unilateral nephrectomy through the flank or a left flank sham operation. Sham operations were performed by incising the flank musculature until the peritoneal cavity could be observed but under no circumstance was the kidney manipulated. Injections of 0.5 cc of the previously prepared rabbit anti-rat serum antisera were given intravenously (IV) through a tail vein without anesthesia once on day 0, or daily. The daily administration of antisera was found to yield the most discriminating results between the groups.

Groups of 10 male Sprague Dawley rats had a left unilateral nephrectomy through the flank or a left flank sham operation. After the surgery and for each of the four succeeding days, (5 injections total) 0.5 cc of rabbit anti-uninephrectomy rat serum antisera was given intravenously (without anesthesia) through the tail vein. Five days after surgery the rats were sacrificed, weighed, and the remaining kidneys harvested and weighed wet (Fig. 1). It has been shown that wet kidney weight is as accurate as dry kidney weight when renal hypertrophy is being measured [5]. In order to minimize individual animal variation a kidney weight/body weight ratio was calculated.

This protocol was followed using unilateral nephrectomy urine (Fig. 1) as well as sham serum and urine (Fig. 2). Statistical comparisions were made by using the Student's two tailed T test for unpaired samples.

Controls were determined by performing unilateral nephrectomy and sham operations on male Sprague Dawley rats with the sacrifice

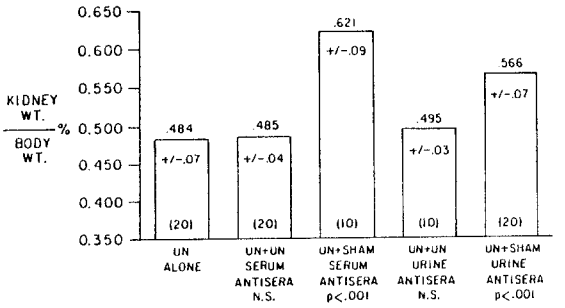

Fig. 3. Unilateral nephrectomy (*UN*). Results after 5 days. +/− = standard deviation; ( ) number of animals in each group; *p* values compared to UN alone; *N.S.*, not statistically significant

of 10 rats on postoperative days 3, 5, 8, 11 and 14. Kidney weight/body weight ratios were calculated.

A complete Freund's adjuvant control was performed by injecting 2 cc of complete Freund's adjuvant subcutaneously into New Zealand white rabbits weekly for 4 weeks. On the 5th week rabbit serum was obtained. Two groups of 10 rats each underwent either a unilateral nephrectomy or a sham operation and daily IV tail vein injections of 0.5 cc of rabbit anti-Freund's adjuvant antisera. After 5 days these animals were sacrificed and kidney weight/body weight ratios obtained.

Other 240–280 g male Sprague Dawley rats underwent unilateral nephrectomy and their serum was collected two days postoperatively. One-half cc of uninephrectomy serum was given IV to 10 male Sprague Dawley rats once, on day 0 and day 3, and daily. The animals in these groups were sacrificed after five days and kidney weight/body weight ratios were computed. Similar groups were given unilateral nephrectomy urine IV as well.

Commercially prepared rabbit anti-rat serum anstisera was administered IV to 6 male Sprague Dawley rats daily for 5 days. Afterwards these animals were sacrificed and kidney weight/body weight ratios were calculated.

All animals were handled identically and similar experimental and control groups were cared for simultaneously. All animals had free access to rat chow and water.

Selected kidneys were sectioned and stained with hematoxylin and eosin for further examination.

Results

The unilateral nephrectomy results are shown in Fig. 3. Figure 4 displays the sham operation results.

The expected contralateral kidney growth after unilateral nephrectomy is contrasted sharply against the results of the sham operated animals in Fig. 5.

The control results are compared in Fig. 6. It should be noted that the commercially prepared rabbit anti-rat serum antisera given IV daily gave similar results to the antisera prepared with this experimental protocol.

Pathological examination of the selected kidneys confirmed hypertrophy within the groups which had an increase in kidney weight/body weight ratios.

Animal weights varied for the first three days after surgery but by five days the weights stabilized and were not significantly different from preoperative weights. Control groups allowed to live longer than five days exhibited a

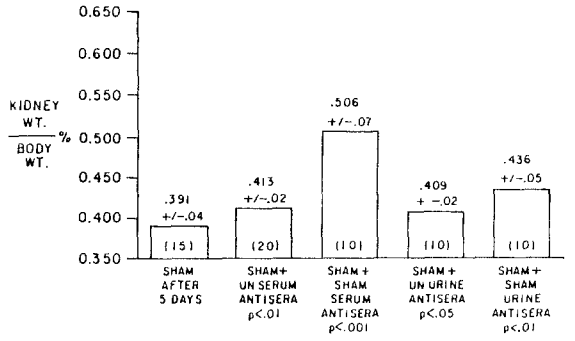

Fig. 4. Sham operation results after 5 days. *p* values compared to sham after 5 days; +/− = standard deviation; *UN*, unilateral nephrectomy; ( ) number of animals in each group

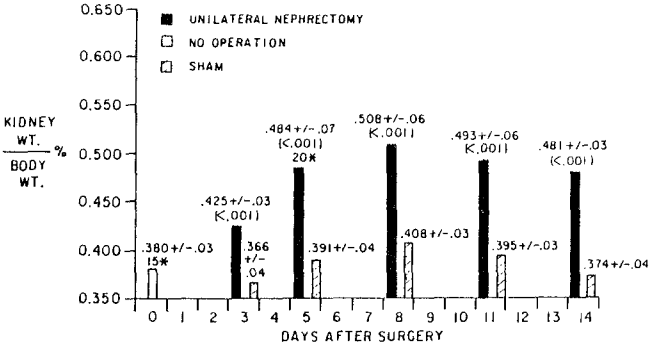

Fig. 5. Kidney growth after surgery. 10 animals per group except groups noted by *; +/− = standard deviation; ( ) *p* value by Student two-tailed t test for unpaired samples

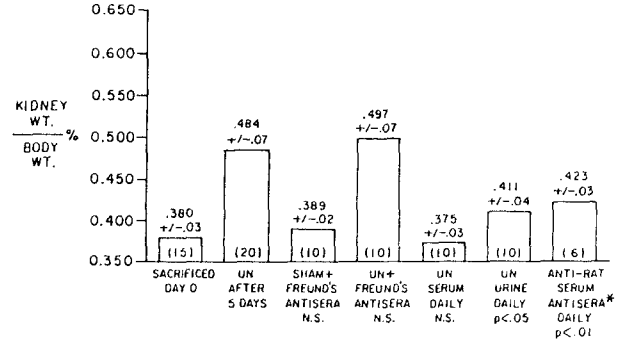

Fig. 6. Control results. *UN*, unilateral nephrectomy; +/− = standard deviation; ( ) number of animals in each group; *N.S.*, not statistically significant; all injections were given i.v.; * commercial antisera

sustained steady weight gain. In all groups wherein kidney weight/body weight ratios were increased, the actual body weight was not significantly different from preoperative body weight and the kidney weights were always significantly increased over controls.

Discussion

We had anticipated that renotropin would be an antigenic protein, and we expected that rabbit antisera to unilaterally nephrectomized rat serum would block CRG in nephrectomized animals. Our results showed that there was no such blocking effect. In fact, the antisera induced by sham operated animal's serum and urine stimulated significant renal growth in both sham operated and nephrectomized animals. This finding suggested that an antigen-antisera interaction interfered with the action of an inhibitor. Furthermore, the kidney growth effect of this blockade was additive to that of nephrectomy. Therefore, nephrectomy elicited a positive stimulus for growth independent of the inhibiting substance or its level in serum. Commerically prepared antisera to rat serum also induced growth implying that normal rats have a circulating inhibitor which was altered by an antigen-antisera interaction.

The fact that we identified no blocking effect in any group suggested that the nephrectomy-induced positive stimulus either did not circulate in the serum or it that was not affected by an antigen-antisera interaction. Additional evidence for this conclusion was that intravenous injection of serum from uninephrectomy animals failed to induce growth.

The concept of a two-factor system raises the possibility of two independent but related types of growth, one obligatory (developmental) and one compensatory. We must emphasize that a histologic or biochemical difference between the two types of growth has not been demonstrated, though the characteristics of CRG in the rat have been noted to be primarily proximal tubular epithelial hypertrophy with some hyperplasia. Variable interstitial proliferation has also been observed [10].

We propose a theory of renal growth control involving two tissue-specific factors. A circulating inhibitor probably renal in origin shuts off developmental or obligatory growth at an appropriate stage in postnatal life. Its continued presence is required, and inhibitor blockade by antisera results in resumed growth for an undetermined period of time. Obligatory growth is influenced also by nutrition, growth hormone, androgen, etc. The inhibitor is present in urine as well as in serum. Renal injury or removal may or may not modify the level of inhibitor. Renal damage, however, engenders a positive stimulus which causes compensatory growth independent of the presence or absence of inhibitor. Whether the stimulant circulates in serum or is excreted in urine remains unclear. Growth elicited in prior experiments by infusion of large amounts of uninephrectomy serum or urine may result from dilution of the inhibitor [6, 16, 18]. In our rats given small amounts of nephrectomy serum daily, no growth was seen; urine treated animals showed renal growth which was statistically significant.

Separate receptors may exist in renal tissue for each factor, or they may compete for the same binding site. Variations in numbers of receptors available could explain age differences in CRG response. Higher binding affinity or

the nature of the receptor itself could explain the ability of the stimulant to override the inhibitor. Simple decrease in circulating inhibitor levels would not account for the phenomenon because blockade by antigen-antisera interaction produces additional growth. Sufficient antisera was used in all cases to overwhelm all circulating inhibitor.

While many aspects of this theory remain to be substantiated by further studies, the existence of a circulating inhibitor in normal, sham operated and neprehctomized animals is suggested by these studies. This helps to explain the previous finding of renal hypotrophy when an adult kidney is transplanted into a child. Eventually as the child grows the kidney undergoes hypertrophy as seen in the adults. This inhibitor may be of great interest to investigators of renal cell cancer since this seems to be uncontrolled growth of proximal tubular cells suggesting failure of this inhibitor regulating mechanism. Further experiments will attempt to specify the nature of antisera induced growth, and to isolate the stimulatory factor, in the hope that one might someday be able to detect renal damage earlier via a renotropin assay and augment or maximize CRG in the clinical setting.

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