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A Denervated Nonfiltering Kidney Preparation in the Rat: A Model for Study of Renin Release (41622)

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Abstract. To examine the role of the renal vascular receptor in the control of renin secretion in the rat, a denervated, nonfiltering kidney model (DNFK) was developed. The left kidney was subjected to a 2-hr period of total renal ischemia followed by ureteral ligation and section. Denervation was accomplished by stripping all visible nerves and painting the renal vessels with 5% phenol. Forty-eight hours later lissamine green dye was injected iv and failed to appear in either the cortical or medullary tubules, indicating that glomerular filtration had ceased. Histological study of these kidneys revealed diffuse tubular necrosis with extensive intratubular cast formation. Norepinephrine content of the DNFK was reduced 91% compared to the contralateral normal kidney ($P < 0.001$). In another group of anesthetized rats with a single DNFK, 15 min of suprarenal aortic constriction (SAC) increased plasma renin activity (PRA) from 3.4 ± 0.6 to 11.5 ± 1.6 ng AI/ml/hr; in a time control series, PRA was unchanged. To exclude the influence of adrenal catecholamines in this response, bilateral adrenalectomy was performed in a separate group of animals with a DNFK. In this series, SAC also markedly increased PRA. The present data indicate that in the rat the macula densa, the renal nerves, and adrenal catecholamines were not essential for the hyperreninemia induced by a reduction in renal perfusion pressure.

In 1970–71, Blaine *et al.* (1, 2) developed a denervated, nonfiltering kidney preparation in the dog for the investigation of renin release mechanisms. This model has been very useful for the study of the renal vascular receptor or so-called renal baroreceptor which seems to function autonomously for the control of renin secretion. However, the characteristics of this receptor have not been defined in other species. Other investigators (3, 4) have studied the response to alterations in renal perfusion pressure in the isolated perfused rat kidney, but the macula densa input for the control of renin release was not eliminated.

The above considerations prompted the present experiments, in which the renal vascular receptor in the rat has been isolated from the influence of the macula densa, the renal nerves, and circulating adrenal catecholamines by a method similar to that of Blaine *et al.* (2). Under these conditions, the functional capacity of the renal vascular receptor in the denervated nonfiltering kidney (DNFK) was tested by an acute reduction in renal perfusion pressure. Our results indicate that the renal vascular receptor in the rat is responsive to a decrease in perfusion pressure and appears to function autonomously for the control of renin release. The development of this new model in the rat should help to provide a better understanding of the mechanisms that regulate renin secretion in this animal species, particularly in the study of baroreceptor mediated renin release.

Methods. *Preparation of the denervated, nonfiltering kidney.* Seven male Sprague–Dawley rats weighing between 250–350 g. were used. They were fed a commercial chow diet and tap water was available *ad libitum.* Under pentobarbital anesthesia (50 mg/kg, ip), the left kidney was exposed via a flank incision. The renal vessels were isolated and a serrefine microclamp (Sargil-Clip DBGM/DBP, S.P. 1.0–1.5 *N*, Aesculap Instruments) was applied for 2 hr to the renal artery near its origin from the aorta. Following this period of total ischemia, the clamp was removed and the ureter was ligated and sectioned. Denervation was accomplished by carefully stripping all visible renal nerves and thoroughly painting the renal vessels with 5% phenol. The kidney was repositioned and the incision closed. Forty-eight hours later the animals were anesthetized with pentobarbital and an indwelling polyethylene catheter (PE-50) was positioned in the left jugular vein. Both kidneys were exposed via a midline ventral incision. Lissamine green dye was injected into the jugular vein and the sur-

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face of both kidneys was observed through a dissecting microscope for the passage of dye into the tubules. The kidneys were removed and longitudinally sectioned for the observation of the presence of dye in the cortical and medullary tubular areas. The kidneys were placed immediately in 10% Formalin for fixation and prepared for histological studies. In a separate series of identical experiments ($n = 6$), after observation of the renal tubules for lissamine green, both kidneys were immediately frozen until assayed for norepinephrine content.

Effects of suprarenal aortic constriction in anesthetized rats with a single denervated nonfiltering kidney ($n = 8$). Forty-eight hours after the preparation of a DNFK as described above, the rats were anesthetized with pentobarbital (50 mg/kg, ip). They were placed on a surgical tray over a heating pad to maintain rectal temperature between 37–39°C. A tracheotomy was performed to facilitate ventilation and removal of secretions. Indwelling heparinized polyethylene catheters (PE-50) were placed in the left jugular vein, the right carotid artery, and in the inferior abdominal aorta via the right iliac artery. Through an abdominal midline incision the right kidney was removed and a loop of umbilical tape (snare) placed around the aorta above the left renal artery. Thirty minues were allowed for recovery from these preparatory procedures. Supra- and infrarenal blood pressures were measured constantly with Statham P23Db pressure transducers and tracings were made on to a Hewlett Packard 7702B recorder. Following the equilibration period, 1 cc of arterial blood was withdrawn for measurement of PRA. All blood samples were replaced with equal amounts of fresh donor blood. The aorta was then constricted to decrease renal perfusion pressure to a level between 40 and 60 mm Hg for 15 min. At the end of the period, arterial blood samples for PRA, plasma electrolytes, and hematocrit were collected. Following the last blood collection, the aortic constriction was released and lissamine green dye injected for the observation of the renal tubules as previously described. Studies in a time control series ($n = 8$) were made in a group of animals prepared with an identical methodology and following the same experimental design as described above with the exception that an inferior aortic abdominal catheter was not positioned and aortic constriction was not performed.

Effect of suprarenal aortic constriction in anesthetized, bilaterally adrenalectomized rats with a single denervated nonfiltering kidney ($n = 9$). To exclude the influence of adrenal catecholamines in the response of PRA observed after aortic constriction, a group of rats was studied in an identical manner as in the first series except that acute bilateral adrenalectomy was performed by surgically removing both adrenal glands immediately before the equilibration period.

Effects of suprarenal aortic constriction in anesthetized rats with a single intact filtering kidney ($n = 8$). In this group of experiments, the response of PRA to suprarenal aortic constriction was studied in rats with a single intact filtering kidney. The experimental design was identical to the first series, except that lissamine green dye was not infused at the end of the experiment. In addition, observations in a time control series ($n = 8$) were made in a group of animals with a filtering kidney following the same protocol with the exception that an inferior aortic abdominal catheter was not positioned and aortic constriction was not performed.

Analytical methods. Plasma electrolytes were measured by flame photometry. Hematocrits (Hct) were determined by the microcapillary tube method. Plasma renin activity and tissue norepinephrine content were determined by radioimmunoassay (5) and by high-pressure liquid chromatography (HPLC) with electrochemical detection (6), respectively. Histological examination was performed by fixing the kidneys in 10% buffered Formalin overnight, embedding them in paraffin, and obtaining sections of 7 micrometers. Alternate sections were stained with hematoxylin and eosin (H and E) and periodic acid–Schiff (PAS). All sections were examined microscopically by a pathologist (AL) without knowledge of the treatment given to the animals.

The experimental results are expressed as means ± SEM. Statistically significant changes are defined as $P < 0.05$ as determined by Student's t test and with unpaired t test for analysis between groups.

Results. *Gross, functional, and histological findings in the denervated nonfiltering rat kidney ($n = 7$).* Gross examination of the DNFK

revealed minimal hydronephrotic changes when compared to the contralateral normal kidney. After lissamine green dye was injected, the passage of the dye through the surface vessels of the kidney was observed. In the DNFK, in contrast to the contralateral normal kidney, the dye failed to appear either in the cortical or medullary renal tubules. These observations provided functional evidence for the absence of glomerular filtration in the preparation. This criterion was met by all of the kidneys made nonfiltering and denervated in this study.

The histological studies of DNFK revealed 2+ to 3+ cortical necrosis on the basis of a grading system ranging from a trace of necrosis (5–10% of surface area of the section) to 3+ necrosis (greater than 50% of surface area). Tubular necrosis was evidenced by eosinophilic homogenization of tubular epithelial cells and desquamation of epithelial cells; tubular cast formation was extensive (Fig. 1). Several of the kidneys revealed papillary necrosis; focal infarction was observed in one kidney. Medial necrosis of occasional small arteries was seen in some of the most severely affected kidneys. Histological analysis of the contralateral control kidneys revealed no pathologic abnormalities.

Tissue catecholamines in denervated nonfiltering kidneys (n = 6). Tissue norepinephrine (NE) content was assayed by HPLC in the DNFK and compared to that in the contralateral intact kidney. In the intact kidney the average norepinephrine content for the series was of 211 ± 27 pg of NE/mg of kidney wet weight. In the contralateral denervated nonfiltering kidney the mean tissue norepinephrine content was of 17.8 ± 2.1 pg of NE/mg of kidney wet weight. Comparison between the two values reveals a decrement of 91% ($P < 0.001$). This percentage decrement of norepinephrine content by our technique of denervation is similar to that reported by others (7).

Effects of suprarenal aortic constriction in anesthetized rats with a single DNFK (n = 8). For the group, mean arterial pressure (MAP) during the control period was 118 ± 4 and 118 ± 5 mm Hg above and below the renal arteries respectively (Fig. 2). During aortic constriction, MAP above the snare rose to 146 ± 6 mm Hg ($P < 0.001$) while the perfusion pressure below the constriction was decreased to 48 ± 2 mm Hg ($P < 0.001$). Heart rate decreased from a control level of 413 ± 10 to 393 ± 8 beats/min during aortic constriction ($P < 0.01$). PRA was elevated from a control value of 3.43 ± 0.60 to 11.51 ± 1.61 ng AI/ml/hr following the decrement in renal perfusion pressure ($P < 0.001$). Plasma electrolytes revealed a plasma sodium level of 144.2 ± 1.1 mEq/liter and a potassium concentration of 4.78 ± 0.34 meq/liter; hematocrit was 43% ± 0.55.

In the time control series, MAP was unchanged from a value of 121 ± 4 to 125 ± 3 mm Hg during the control and experimental periods, respectively ($P > 0.05$) (Fig. 2). Likewise, heart rate was unchanged from 403 ± 12 to 403 ± 13 beats/min during the same periods ($P > 0.05$). PRA was unchanged with levels of 4.13 ± 0.81 and 3.86 ± 0.83 ng AI/ml/hr ($P > 0.05$) during the control and experimental periods. At the end of the experiment, plasma sodium concentration was 143.7 ± 1.2 meq/liter, plasma potassium concentration was 4.81 ± 0.37 meq/liter, and hematocrit was 44% ± 0.32.

Effects of suprarenal aortic constriction in anesthetized, bilaterally adrenalectomized rats with a single DNFK (n = 9). This experiment was performed to exclude the influence of adrenal catecholamines in the renin response to suprarenal aortic constriction in the DNFK. For the group, the MAP during the control period was 105 ± 3 and 104 ± 2 mm Hg above and below the left renal artery, respectively (Fig. 3). During aortic constriction, MAP above the snare increased to 142 ± 3 mm Hg ($P < 0.001$) while the MAP below the constriction decreased to 48 ± 1 mm Hg ($P < 0.001$). Heart rate also decreased from 441 ± 7 during control to 411 ± 7 beats/min during constriction ($P < 0.001$). As in the previous series, PRA was significantly increased approximately three-fold from a control value of 5.7 ± 0.8 to a level of 14.5 ± 1.8 ng AI/ml/hr during the decrement in renal perfusion pressure ($P < 0.001$). At this time, plasma sodium concentration was 144.1 ± 1.3 meq/liter, plasma potassium concentration 4.92 ± 0.10 meq/liter, and hematocrit 44% ± 0.30.

Effects of suprarenal aortic constriction in anesthetized rats with a single intact filtering kidney (n = 8). For the group, control MAP was 123 ± 3 and 125 ± 4 mm Hg above and below the renal artery, respectively (Fig. 4).

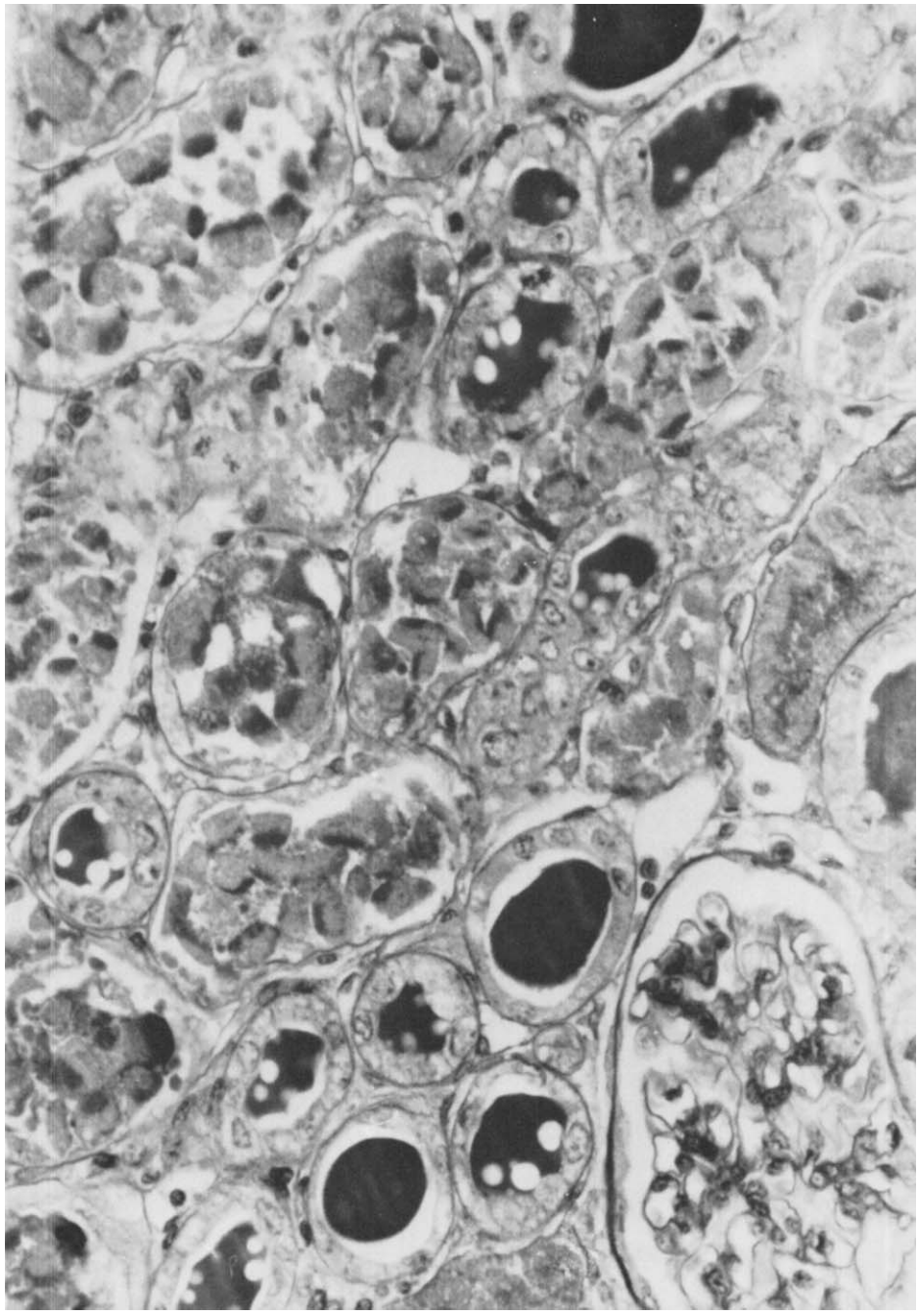

FIG. 1. Photomicrograph of a rat kidney which had been subjected to 2 hr of renal ischemia, ureteral ligation, and denervation 48 hr previously. Note the tubular necrosis and extensive tubular cast formation while the glomerular tufts appear normal.

During constriction, MAP above the snare rose to 156 ± 3 mm Hg ($P < 0.001$) while MAP below it decreased to 51 ± 2 mm Hg ($P < 0.001$). During clamping, heart rate decreased from a control of 418 ± 13 to 405 ± 14 beats/min ($P < 0.05$). The baseline PRA

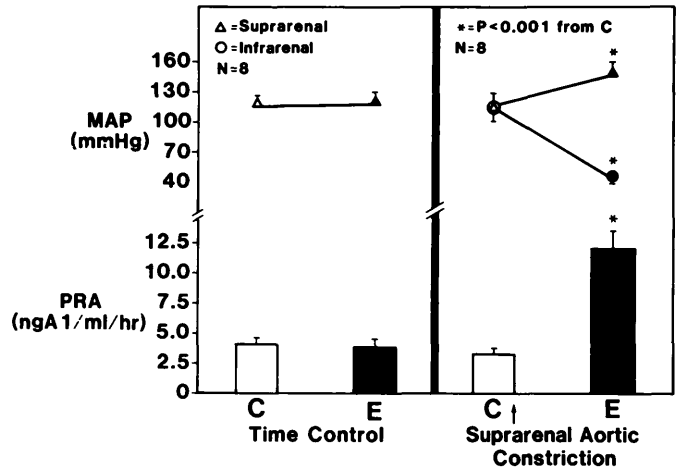

FIG. 2. Effects of suprarenal aortic construction in rats with a single denervated nonfiltering kidney (right panel) and a time control series (left panel). Values are expressed as means ± SEM. Abbreviations MAP and PRA are explained in the text. In addition; C = control and E = experimental period after 15 min of aortic constriction.

was 12.82 ± 2.89 ng AI/ml/hr and increased to 25.80 ± 2.32 ng AI/ml/hr during constriction ($P < 0.001$). Plasma sodium and potassium concentrations were 142.3 ± 0.45 and 4.33 ± 0.14 meq/liter, respectively, while hematocrit was 45% ± 0.65.

The results of the time control series with a single intact filtering kidney are also depicted in Fig. 4. Mean arterial pressure during the control and experimental periods was unchanged from a value of 125 ± 2 to 124 ± 3 mm Hg ($P > 0.05$). Heart rate was unaltered from a control of 418 ± 13 to a value of 409 ± 14 beats/min during the experimental period. Also, PRA was not significantly changed from a control value of 11.58 ± 1.62 to a level of 13.20 ± 1.36 ng AI/ml/hr during the experimental period ($P > 0.05$). Plasma sodium and potassium concentrations were 143.9 ± 0.30 and 4.05 ± 0.13 meq/liter, respectively, while hematocrit was 45% ± 0.27.

Discussion. More than two decades ago Tobian *et al.* (8) introduced the concept of an intrarenal "baroreceptor" in the control of

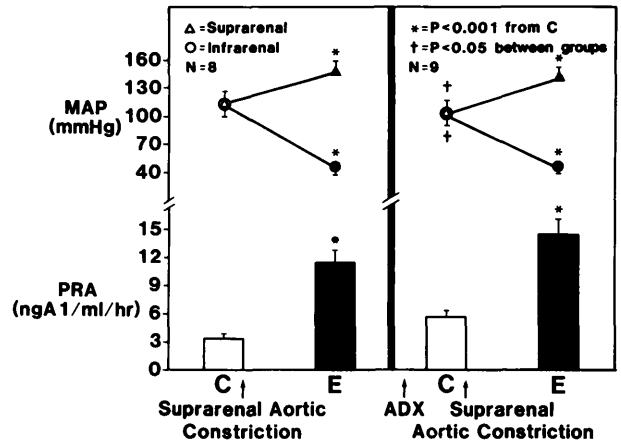

FIG. 3. Comparison of the effects of suprarenal aortic constriction in rats with a single denervated nonfiltering kidney with and without adrenal glands (left and right panels respectively); left panel is from Fig. 2. Period of constriction: 15 min. Values are expressed as means ± SEM. Abbreviations as in Fig. 2; in addition, ADX = adrenalectomy.

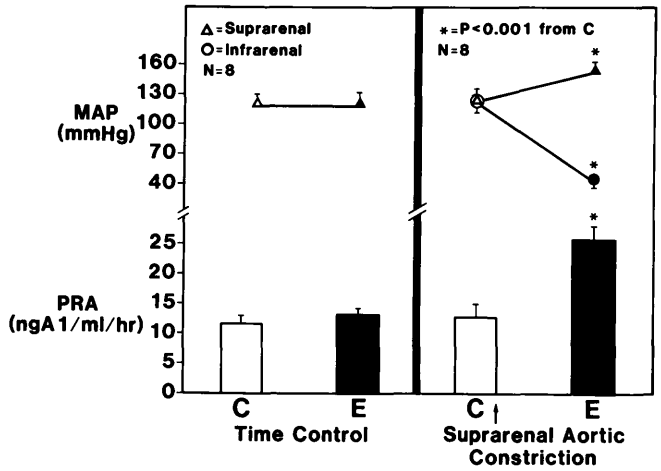

FIG. 4. Effects of suprarenal aortic constriction in rats with a single intact filtering kidney (right panel) and a time control series (left panel). Period of constriction: 15 min. Values are expressed as means ± SEM. Abbreviations as in Fig. 2.

renin secretion. By studying the response to changes in renal perfusion pressure in an isolated rat kidney preparation, these workers noted that juxtaglomerular (JG) cell granulation was consistently decreased in association with an increment in renal perfusion pressure. In 1962, Tobian suggested that the JG cells act as receptors that respond inversely to the degree of stretch of the renal afferent arteriole for the control of renin release (9). Skinner and associates (10) performed graded suprarenal aortic constriction in dogs and noted that changes in renin secretion were not necessarily dependent on either a decrease in renal blood flow or pulse pressure.

These first studies of the renal vascular receptor did not take into account the changes in the rate of glomeruler filtration that variations in renal perfusion pressure produce. For instance, the studies of Skinner *et al.* (10) did not consider that a decrement in renal perfusion pressure could have resulted in a fall of the filtered load of sodium chloride which the macula densa senses for the release of renin. Indeed, experiments by Vander and Miller (11) demonstrated that renin release secondary to aortic constriction in the dog was blunted or prevented by the simultaneous administration of diuretics which increased sodium chloride load or delivery to the macula densa. Thus, the difficulties in interpreting the various experimental manipulations that could influence either or both receptors for renin release became apparent.

To circumvent this problem, Blaine *et al.* (1) developed a nonfiltering kidney model in the dog, in which the influence of the macula densa was eliminated. With the aid of this preparation, they further explored and confirmed the "baroreceptor" hypothesis of Tobian. In these studies, these investigators found that suprarenal aortic constriction or hemorrhage elicited a striking increase in PRA in the absence of a functional macula densa. However, it was still possible that the baroreceptor-mediated renin response to these maneuvers could have been influenced by the renal nerves or circulating catecholamines (12, 13). With this realization, Blaine *et al.* (2) repeated their experiments in the nonfiltering kidney model in the dog in which the renal vascular receptor was further isolated from the renal nerves by renal denervation and from circulating adrenal catecholamines by bilateral adrenalectomy. In this preparation, both hemorrhage and suprarenal aortic constriction again produced striking increments in renin secretion. Collectively, the studies by Blaine *et al.* (1, 2) demonstrated both an important role and the functional autonomy of the renal vascular receptor in the control of renin release in the dog. During the last decade this dog model has proved to be quite useful in the study of renin release mechanisms.

The present study was undertaken to develop a denervated nonfiltering kidney model in the rat and to evaluate the renal vascular

receptor mechanism. It seemed important to characterize the renal vascular receptor in this species which is being used increasingly for study of renin secretion. The importance of creating such a model was recognized as early as 1946, when Selye created an "endocrine kidney" by a combination of a reduction in renal perfusion pressure and ureteral ligation (14). In this preparation he demonstrated tubular cast formation and loss of the tubular epithelial brush border, but unfortunately no functional evidence of the absence of glomerular filtration was provided. However, he reported that the endocrine function of the kidney probably remained intact, since "pressor substance" (probably renin) produced by this kidney was consistently detected.

In the present experiments, the renal vascular receptor was isolated by denervating a nonfiltering kidney and by adrenalectomy. Functional evidence that glomerular filtration had ceased was provided by the lack of appearance of lissamine green dye (injected iv) in the renal tubules. Histological evidence revealed marked tubular necrosis with extensive cast formation; under these circumstances, tubular flow with delivery of solute to the macula densa in a normal manner was not possible. Renal denervation reduced renal norepinephrine content 91%, and adrenal catecholamines were eliminated by bilateral adrenalectomy.

Physiological studies of the denervated, nonfiltering kidney model were made to evaluate its functional capacity and to provide evidence of its usefulness. Suprarenal aortic constriction produced a three-fold increase in PRA, whereas PRA was unchanged in a time control series. To eliminate the influence of adrenal catecholamines, bilateral adrenalectomy was performed in rats with a denervated, nonfiltering kidney and the response to suprarenal aortic constriction studied again. The response in PRA was essentially the same as in the presence of adrenal glands. The only noticeable difference was a lower control arterial pressure in the adrenalectomy group from loss of circulating catecholamines and a slightly higher control PRA secondary to the reduced renal perfusion pressure.

The response to suprarenal aortic constriction was also studied in a series of normal rats with intact kidneys for comparison with the data in the DNFK. The control values for PRA were three to four times higher in intact than in DNFK. The value of 12.5 ng AI/ml/hr for uninephrectomized but otherwise intact rats is in agreement with data from other laboratories for anesthetized, surgically stressed rats (15, 16) in which PRA is slightly elevated. The low control level for PRA in the DNFK could represent the loss of support from the renin stimulatory mechanisms deleted in the DNFK, namely, the macula densa, the renal nerves, and adrenal catecholamines. It is also possible that the JG mass was less in the DNFK due to the renal ischemia but there was no histological evidence of this. It should also be pointed out that the percentage and absolute increments in PRA following aortic constriction were not appreciably different in the normal and the DNFK.

In summary, a DNFK model has been developed in the rat in which the intrarenal vascular receptor was progressively isolated from the influence of (i) the macula densa by making the kidney nonfiltering, (ii) the renal nerves by surgical and chemical denervation, and (iii) the adrenal catecholamines by bilateral adrenalectomy. The experimental data indicate that the renal vascular receptor in the rat can function autonomously for the control of renin release and that a decrement in renal perfusion pressure is a potent stimulus for renin secretion via this receptor. Finally, this model should be a valuable tool in renin secretion experiments, particularly in the study of the renal vascular receptor.

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