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Direct Demonstration of Macula Densa–Mediated Renin Secretion

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An in vitro method has been used to examine whether secretion of renin from the juxtaglomerular apparatus is affected by changes in the sodium chloride concentration of the tubular fluid at the macula densa. Single juxtaglomerular apparatuses were microdissected from rabbits and the tubule segment containing the macula densa was perfused, while simultaneously the entire juxtaglomerular apparatus was superfused, and the fluid was collected for renin measurement. In this preparation, in which influences from renal nerves and local hemodynamic effects are eliminated, a decrease in the tubular sodium chloride concentration at the macula densa results in a prompt stimulation of the renin release rate.

RENIN IS AN ASPARTYL PEPTIDASE that is synthesized, stored, and released from granular cells in the renal vasculature (*1*). It catalyzes the cleavage of the decapeptide angiotensin I (AI) from angiotensinogen; AI is in turn transformed by converting enzyme to the physiologically active form, angiotensin II (AII). The active form AII is both a potent vasoconstrictor, with a regulatory role in control of renal and systemic vascular resistance, and a regulator of salt balance by promoting aldosterone release. Increased activity of the renin-angiotensin system may cause certain forms of hypertension. In keeping with its complex homeostatic roles, the regulation of renin secretion is under control of a number of factors. Release is stimulated by decreases in arterial pressure and increases in activity of the renal nerves (*2*); it is also probably influenced by the local concentrations of a variety of substances including prostaglandins, catecholamines, AII, and adenosine (*2*).

The renin-containing granular cells are located primarily at the glomerular vascular pole in the juxtaglomerular apparatus (JGA). This cell complex consists of three cell types—the granular cells, which contain renin, modified interstitial cells, and a specialized plaque of tubular epithelial cells called the macula densa. The macula densa is located in the wall of the thick ascending limb of Henle at the point where the tubule returns to its parent glomerulus. Sodium chloride concentration in tubular fluid at the macula densa varies widely with the physiological state of the animal and the flow rate in the tubule. In salt-depleted states, where tubular flow rate is slowed, NaCl concentration falls to low values (20 to 40 m*M*); it rises toward isotonic values in volume-expanded states where tubular flow rate is increased (*3–5*). This unique structural arrangement led, in 1944, to the proposition that renin release might be under control of tubular function at this site in the nephron (*6*).

It has, however, proven difficult to establish whether tubular fluid composition at the macula densa in fact influences renin release. The evidence usually cited to support macula densa–mediated renin release is indirect and inferential (*7*). Even the direction of change in renin secretion rate after changes in the tubular fluid composition is controversial. Two contradictory theories have emerged: one group of investigators propose that an increase in NaCl concentration at the macula densa (*8–11*) is a local signal for renin secretion, whereas others have maintained that renin release responds to a fall in NaCl concentration (*4, 12–15*).

A number of methods have been used to attempt to resolve this issue. Experimental preparations such as the "nonfiltering kid-

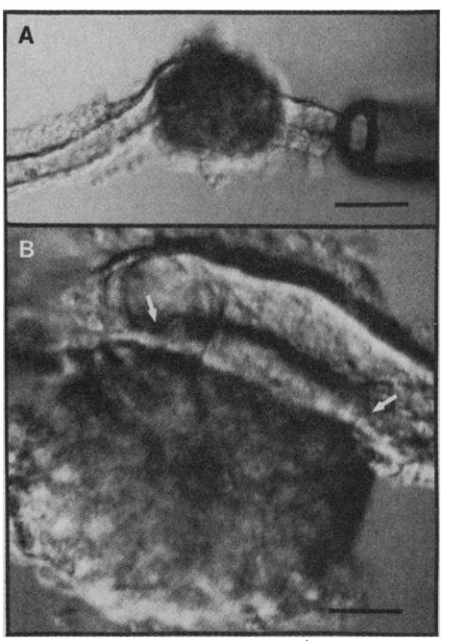

Fig. 1. Single JGA microperfusion. An individual JGA, consisting of portions of the thick ascending limb of Henle, macula densa, and early distal tubule with adherent glomerulus and hilar structures, was dissected from New Zealand white rabbits (body weight, 1.0 to 1.75 kg) and transferred to a thermoregulated chamber mounted on an inverted microscope. (**A**) The tubule was cannulated and perfused with a modified isolated perfused tubule apparatus (*24, 25*). The distal end was not cannulated. The tubular perfusion rate was maintained by a hydrostatic pressure gradient at about 10 nl per minute. Scale bar, 100 μm. (**B**) After perfusion had been established the macula densa could be visualized (arrows). Scale bar, 25 μm. An outer pipette was advanced to cover the perfused JGA. Flow was maintained at a rate of 600 nl/min through the outer superfusion pipette. After an equilibration period of 15 minutes, fluid in the bath chamber was replaced with warmed mineral oil. The droplets of superfusate that formed at the tip of the outer pipette were collected at 10-minute intervals for renin assay. Renin released from the JGAs was measured by radioimmunoassay of generated AI with the antibody-trapping technique (*20*). The sample was added to 20 μl of a mixture of purified AI antibody and purified renin substrate (1200 ng of AI per milliliter) and incubated for 24 hours at 37°C with subsequent radioimmunoassay of generated AI. After the experiments, the renin was extracted from the JGAs by freezing and thawing four times. Renin is expressed in terms of Goldblatt units compared to standards from the Institute for Medical Research (M.R.C., Mill Hill, London). The detection limit of the assay was found to be 1 nGU contained in 5 μl, which is equivalent to 160 fg of AI generated per hour of incubation.

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ney" model have been devised to compare response of renin secretion with and without an intact macula densa signal (*16*), but the results can be interpreted several ways. Standard renal micropuncture techniques have shown that changes in dietary NaCl intake lead to changes in the delivery of NaCl to the distal tubule and to inverse changes in plasma renin concentration (*4*), and it has become well-established that a diminished intravascular volume is generally associated with stimulation of renin secretion (*2, 14*). Nonetheless, volume depletion leads to increased sympathetic nervous activity and decreased arterial pressure, both independent stimuli for renin release.

In response to these problems, investigators have designed experiments in which the composition of the tubular fluid at the macula densa was manipulated in situ by micropuncture techniques. These studies have yielded contradictory results. In two large series of experiments the renin activity in single microdissected JGAs was increased after the macula densa segment had been perfused in situ with high-NaCl solutions (*9, 10*). In these studies renin secretion rate was not measured; whether the increase in renin content was accompanied by a parallel increase in secretory rate is not known. More recent studies have attempted to assess the effect of changes in the delivery of NaCl to the macula densa on renin secretion rather than renin content. In one study tubular blockade was found to result in a fall in the local renin concentration of venous blood (*11*), thus suggesting that the renin release rate is directly correlated with the flow rate at the macula densa and supporting the hypothesis that increased NaCl at the macula densa stimulates renin secretion. Another recent study came to the opposite conclusion. The renin concentration measured in blood from the efferent arteriole or in fluid from the proximal tubule was inversely correlated to changes in the flow rate in the loop of Henle (*15*). There are a number of reasons why the micropuncture studies may have yielded contradictory results. Quantitative collection of the total renin secreted from nephrons perfused in situ is not possible, and a substantial and possibly variable amount of renin may escape detection by local sampling techniques. Changes in macula densa fluid composition also activate local vasoconstriction (*17*), which may alter the baroreceptor signal to renin secretion. We therefore concluded that progress on this issue required an in vitro system in which renin secretion could be measured directly and in which the confounding influences of baroreceptors and renal nerves could be eliminated.

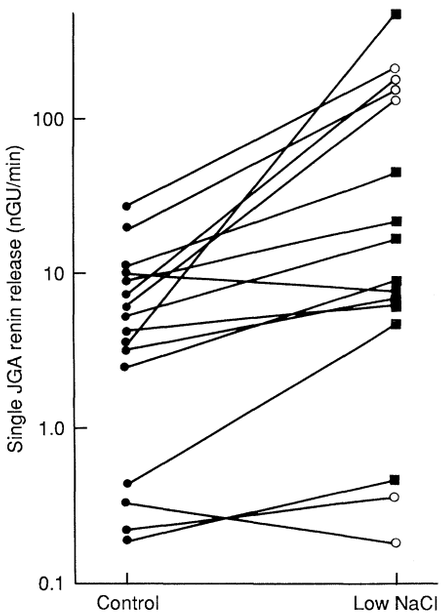

Fig. 2. Effect of lowering NaCl concentration on single JGA renin release. In 16 single JGAs the effect of lowering the NaCl concentration of the perfusate on the release rate of renin was tested. Shown are individual values for the control period (●) and the low-NaCl period, in nano–Goldblatt units per minute. Lines connect values measured from the same JGA. In the experimental period two solutions were tested, a hypo-osmolar solution (■) (n = 10) and an iso-osmolar solution (○) (n = 6).

Several methodological developments permitted a more direct approach to this question. Techniques for in vitro study of microdissected perfused renal tubules have become well established (*18*). The JGA can be dissected from the rabbit kidney and the macula densa segment of the tubule can be perfused (*19*). Renin assay methods now have sufficient sensitivity to measure the renin secreted by a single JGA with short collection intervals (*20, 21*). We used a modification of the isolated perfused tubule technique that allowed simultaneous perfusion of the tubular segment containing the macula densa and superfusion of the entire cell complex (Fig. 1). The superfusate was collected continuously to measure the secreted renin.

The basal renin secretion rate from 22 JGAs perfused and superfused with this new method was found to be 7.6 ± 2.7 (mean ± SEM) nano–Goldblatt units (nGU) per minute, with a range of approximately 0.1 to 30 nGU per minute. After 120 minutes of combined superfusion and perfusion, each JGA was removed from the bath, and the renin content was measured. The mean renin content found after the experiments was 0.34 ± 0.18 mGU per JGA. The fractional release rate was 1.17 ± 0.34% per hour. These values are similar to results from superfused rat and incubated rabbit afferent arterioles (*21, 22*).

In 16 JGAs, we examined the effect on renin release of changing the NaCl concentration of the fluid that perfused the macula densa. Two solutions were tested. In ten JGAs the perfusate was a hypo-osmolar solution with NaCl concentration reduced by 95 mM from the control solution. In six other JGAs the effect of the same reduction in NaCl concentration was tested, but constant osmolality was maintained by addition of mannitol. Results from all studies are summarized in Fig. 2. Lowering the NaCl concentration by 95 mM stimulated renin release in 14 of 16 perfused JGAs with the mean values rising from 7.7 ± 2.1 to 77.6 ± 30.8 nGU per minute ($P < 0.02$, paired t test). The response in the two groups was similar.

We also determined the time course of the response in the six JGAs perfused with the iso-osmotic low-NaCl solution and six JGAs perfused with control solution (Fig. 3). In the control JGAs a mock perfusate change was made to test whether the mechanical disturbance associated with perfusate change would stimulate renin release. Al-

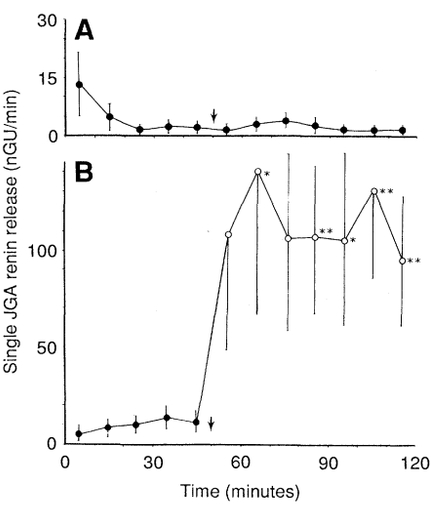

Fig. 3. The time course of renin release from single perfused JGAs. (**A**) The time course of renin secretion from six JGAs that were perfused with control solution throughout the experiments. After 50 minutes a mock perfusate change was made (arrow). (**B**) The time course of renin secretion from six JGAs perfused with control solution for the initial 50 minutes (●), after which the perfusate was changed to a low-NaCl, iso-osmotic solution (○). Values given are arithmetic means with bars showing SEM. Statistical analysis was performed on log-transformed data with the Systat statistical program. Log transformation was verified to produce homogeneity of variance by the Bartlett test (*26*). In the upper panel mean renin release rate did not differ between control and experimental periods, whereas in the lower panel a significant increase occurred in the experimental period relative to control ($P < 0.01$, paired t test). Asterisks indicate the P values for comparison of individual time periods with corresponding periods in the time control series (*$P < 0.05$, **$P < 0.02$).

though small rises in renin secretion rate were noted in the periods after perfusate change in two of the six preparations, these changes did not achieve significance and were only transient. Previous studies with isolated superfused glomeruli have shown that iso-osmotic replacement of NaCl with sucrose will suppress renin secretion (*23*). The stimulation of renin secretion that we observe when the macula densa was perfused with the iso-osmotic low-NaCl solution is therefore the opposite of that predicted if this solution were presented as the superfusion fluid. It indicates that the response is specific to exposure in the compartment containing the macula densa.

Our experiments show that renin secretion is affected by the composition of the tubular fluid at the macula densa. Changes in the NaCl concentration of the tubular fluid elicited an immediate change in the rate of renin release. This effect was demonstrable in an in vitro preparation where influences from renal nerves and local hemodynamic effects can be excluded. These results directly demonstrate that tubular fluid composition at the macula densa influences renin release and that a fall, rather than a rise, in NaCl concentration is the local tubular signal for stimulation of renin secretion.

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Molecular Analysis of a Constitutional X-Autosome Translocation in a Female with Muscular Dystrophy

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The gene responsible for Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) maps to the X chromosome short arm, band Xp21. In a few females with DMD or BMD, the Xp21 region is disrupted by an X-autosome translocation. Accumulating evidence suggests that the exchange has physically disrupted the DMD/BMD locus to cause the disease. One affected female with a t(X;21)(p21;p12) translocation was studied in detail. The exchange points from both translocation chromosomes were cloned, restriction-mapped, and sequenced. The translocation is reciprocal, but not conservative. A small amount of DNA is missing from the translocated chromosomes; 71 to 72 base pairs from the X chromosome and 16 to 23 base pairs from the 28*S* ribosomal gene on chromosome 21.

DUCHENNE MUSCULAR DYSTROPHY (DMD) and the less severe Becker muscular dystrophy (BMD) (*1*) are X-linked neuromuscular diseases that are allelic (*2*). The biochemical defect is unknown, but the gene has been localized to band p21 on the short arm of the X chromosome. The localization was established by linkage analysis (*3*), by the finding of cytologically detectable deletions of the Xp21 region in males with DMD and other phenotypes (*4*), and by the finding of X-autosome translocations involving band Xp21 in a group of 20 females with DMD or BMD (*5*). In these females, the position of the autosomal exchange point is variable, whereas the position of the X chromosome exchange point is consistently in band Xp21, which suggested that the translocations may disrupt the DMD/BMD locus. The disease is expressed in these translocation carrier females because of the preferential inactivation of the normal X chromosome (*5*). In one of the females (*6*), the autosomal breakpoint of the translocation occurred in a block of tandemly repeated ribosomal RNA genes (rDNA) on the short arm of chromosome 21 (*7*). The isolation of a human-specific rDNA probe located near the translocation breakpoint allowed the identification and cloning of a novel restriction fragment, XJ1, from the translocation junction

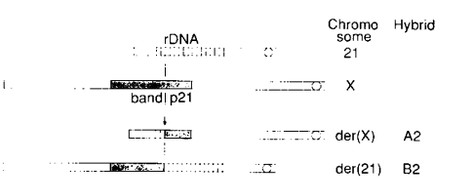

Fig. 1. Schematic diagram of the short arms of the chromosomes involved in the reciprocal translocation. The translocation-derived chromosomes have been segregated in somatic cell hybrids (*7, 8*) as the only human chromosomes on a mouse A9 background. Hybrid A2 has been fully described (*7*) and hybrid B2 is a subclone of the hybrid line C2-T10 reported earlier to contain the der(21) chromosome (*7*).

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