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The Juxtaglomerular Apparatus in the Normal Rat Kidney*

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Summary. Fifty juxtaglomerular apparatuses (JGA) from 6 normal rats of two different strains (BD9 and Sprague-Dawley) were studied with serial section technique after vascular perfusion with 1% Glutaraldehyde and embedding in Plexiglass.

The macula densa basal area and the contact area between macula densa and the Goormaghtigh cell field were significantly greater in the BD9 rats than in the Sprague-Dawley rats. The contact area between the efferent arteriole and the Goormaghtigh cell field was greater in the Sprague-Dawley than in the BD9 rats. The contact area between the afferent arteriole and the Goormaghtigh cell field was equal in both strains of rat.

Significant correlation was found in both strains between the size of the macula densa basal area and its contact area with the Goormaghtigh cells. Similarly there was a significant correlation of the area of contact between the Goormaghtigh cells and the macula densa on the one side and with the afferent arteriole on the other. No correlation was found with the efferent arteriole.

Direct contact between macula densa and the arterioles was not present in all juxtaglomerular apparatuses. It occurred in the BD9 rats in 92% on the afferent and in 52% on the efferent side. In the Sprague-Dawley rats direct contact between macula densa and the arterioles occurred in 59% with the afferent and 72% with the efferent arteriole.

Epithelioid cells were found in the preglomerular segment of the afferent arterioles. They replaced the smooth muscle cells in the whole thickness of the media.

The results point to the Goormaghtigh cells as the morphological link between the tubular and vascular parts of the juxtaglomerular apparatus.

Key words: Juxtaglomerular apparatus – Macula densa – Goormaghtigh cells – Contact areas of juxtaglomerular structures.

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Introduction

The normal juxtaglomerular apparatus (JGA) in rats has previously been investigated intensively by Barajas and Latta (1963); Barajas (1970) and Faarup (1965). They used serial section technique, but reported different results. Barajas found a greater amount of direct contact between the distal tubule and the efferent than the afferent arteriole. Faarup, however, found an equal contact length between macula densa (MD) and the efferent and afferent arteriole. Previously reported results from normal human kidneys showed that the contact between MD and the afferent arteriole occurred more often and was more extensive than with the efferent arteriole (Christensen et al., 1975). We therefore decided to investigate the JGA in the normal rat, with fixation by vascular perfusion.

Material and Methods

Two normal rats from our laboratory strain (Berlin Druckrey, Strain 9; BD9) and four normal Sprague-Dawley rats (SIFF, Oslo, Norway) were chosen for this study. They were all male and fed on a standard laboratory diet (Vestlandske Felleskjöp, Bergen, Norway), containing 0.5% NaCl. They had free access to tap water. The BD9 rats had been kept by brother-sister inbreeding since 1950 and are genetically very homogeneous (Druckrey et al., 1962). The Sprague-Dawley rats are from a genetically nonhomogeneous but previously inbred strain.

The animals, weighing between 250–300 g, were anaesthetized between 10 and 11 a.m. with 50 mg Nembutal-Na/kg (Abbott Laboratories) and the abdomen opened along the linea alba.

The aorta was freed from retroperitoneal tissue and a 1.2 mm injection needle was inserted in the abdominal part of the aorta approximately 1.5 cm cranial to the bifurcation (Maunsbach, 1966; Bohman, 1973). Then perfusion was started with 1% glutaraldehyde in 0.1 m Cacodylate buffer with 2% dextran 40. The whole fixative had an osmolarity of 300 mOsm, the perfusion pressure was kept constant at 120 mm Hg and perfusion lasted for 6 to 8 min. We thus followed the procedure described by Maunsbach (1966 a, b), Bohman and Maunsbach (1970) and Bohman (1973) exactly. In particular we watched the colour and the firmness of the kidneys. The fixed kidneys were cut into slices about 2 mm thick and left in the fixative at least overnight. Small cubes with a side length of about 2 mm were cut and washed in buffer overnight before being embedded in plexiglass. There was no selection of any particular cortical zone.

1.5 µm thick serial sections were cut on a Reichert Ultramicrotome Type OM U3 with glass knives. The sections were stained with Giemsa. 25 JGA from each rat strain were studied.

Each JGA was photographed with a Zeiss Photomicroscope with ×40 objective at a final magnification of 570:1. Great care was taken that the entire JGA was present in the series, that the arterioles could be unequivocally identified and that the JGA were not located marginally in the sections. All sections of each JGA were photographed serially and the copies taped together. As each section had been orientated in the same way before the micrographs were taken, it was easy to follow the different structures from one picture to the next. Measurements were taken on the micrographs of the direct contact between the different structures of the JGA, i.e. between:

Macula densa and Goormaghtigh cells (Goormaghtigh, 1932).

Macula densa and afferent arteriole.

Macula densa and efferent arteriole.

Goormaghtigh cells and afferent arteriole.

Goormaghtigh cells and efferent arteriole.

Further the length of the macula densa basement membrane was measured. From these data we calculated the areas of contact and the MD basal area taking into account the thickness and the number of sections.

The data were evaluated statistically with Spearman rank correlation test. Level of significance: $P=0.05$.

Results

The MD cells appeared in the perfused kidneys as taller cells with a somewhat lighter cytoplasm than in the neighbouring tubular epithelium, from which they were easily distinguished (Fig. 1).

The Goormaghtigh cells (Go. cells) lie between the two hilar arterioles, the distal tubule with MD and the glomerulus with the mesangial cells. The whole space between these structures is entirely filled out with Go. cells regardless of how far apart the arterioles lie. In some glomeruli with double efferent arterioles, the Go. cells were in contact with both efferent arterioles. This means that in the normal rat the Go. cells are always in direct contact with all the other components of the JGA. They are, furthermore, in direct contact with the mesangial cells into which the continue (Fig. 1). There is no morphologic criterion for separating the normal Go. cell from the mesangial cell in semithin sections. We, therefore, arbitrarily set the border between the Go. cells and the mesangium at the glomerular hilus where the parietal sheet of Bowman's capsule proceeds into the visceral.

The MD basal area was significant larger in the BD9 rats (1271 μ^2) than in the Sprague-Dawley rats (843 μ^2). The contact area between the MD and the Go. cell field was also significantly larger in the BD9 rats (741 μ^2) than in the Sprague-Dawley rats (517 μ^2, Table 1).

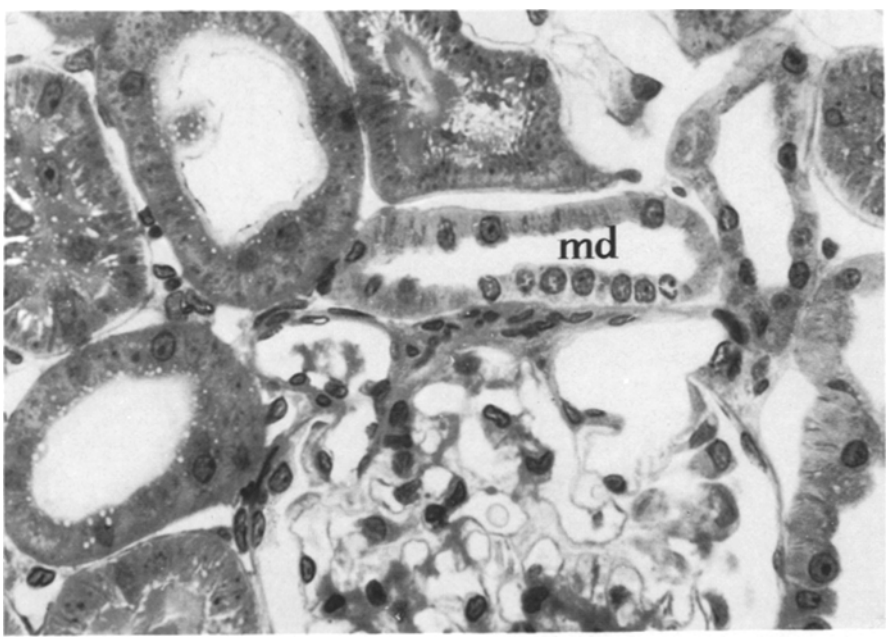

Fig. 1. Distal tubule with macula densa (*md*). Below the tubule, the afferent arteriole is on the right, the efferent arteriole on the left. The Goormaghtigh cell field is between the arterioles and in contact with them both and with the macula densa. Notice how the Goormaghtigh cells continue into the mesangium along the efferent arteriole. There is no contact between the macula densa and the efferent arteriole. Semithin section (1.5 μ), Giemsa stain, 570:1

Table 1. Basal area of the macula densa and areas of contact between the various structures of the juxtaglomerular apparatus

	BD9	Sprague-Dawley
	$\bar{x}$ SD (SE)	$\bar{x}$ SD (SE)
Macula densa basal area	1271 ± 300 (60)	834 ± 228 (46) S
Areas of contact between:		
a) Goormaghtigh cell field and macula densa	741 ± 235 (47)	517 ± 202 (40) S
afferent arteriole	302 ± 136 (27)	263 ± 134 (27) N.S.
efferent arteriole	177 ± 78 (16)	260 ± 144 (29) S.
b) macula densa and afferent arteriole	189 ± 135 (27)	69 ± 79 (16) S.
efferent arteriole	53 ± 85 (17)	71 ± 101 (20) N.S.

All numbers in μ^2. Mean ($\bar{x}$), standard deviation (SD), standard error pf the mean (SE), S = significant. N.S. = not significant. Level of significance $P = 0.05$

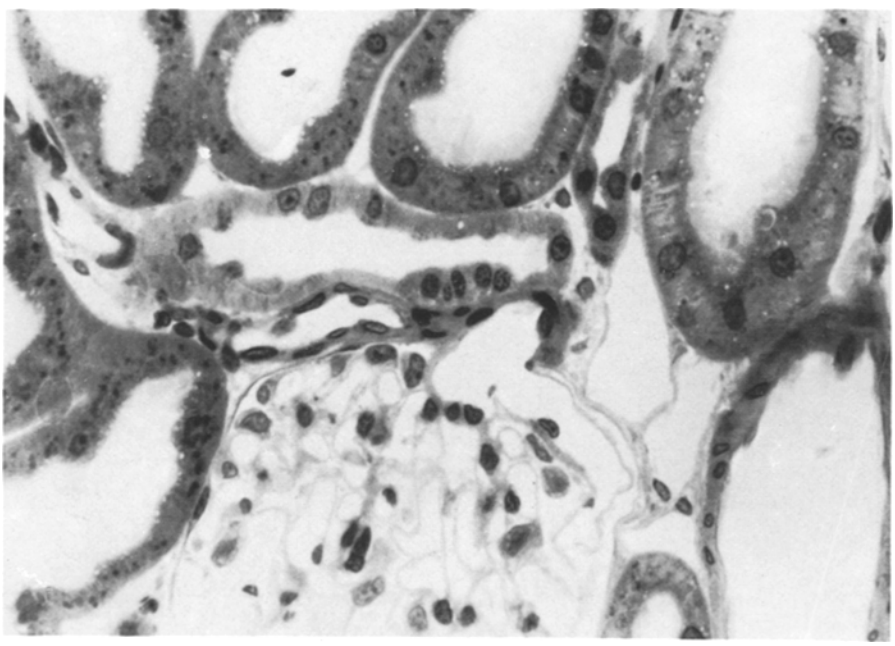

Fig. 2. Juxtaglomerular apparatus with macula densa, afferent arteriole (right), efferent arteriole (left) and the Goormaghtigh cell field between them. The efferent arteriole runs parallel to the distal tubule quite a long distance but no macula densa is present. Semithin section (1.5 μ), Giemsa stain, 570:1

The contact area between the afferent arterioles and the Go. cell field was of equal size in both rat strains. However, between the efferent arteriole and the Go. cell field the contact area was significantly larger in the Sprague-Dawley rats (260 μ^2) than in the BD9 rats (177 μ^2, Table 1).

Direct contact between the MD and the hilar arterioles did not occur in all JGA. The frequency of contact between MD and the afferent arteriole was considerably greater in the BD9 rats (92%) than in the Sprague-Dawley rats (59%). With the efferent arteriole, the contact area was somewhat greater and occurred more often in the Sprague-Dawley rats (72%) than in the BD9 rats (52%). This difference, however, was not statistically significant (Table 1).

The MD basal area was often found to be greater than the total contact area with the other JGA structures. On average 22% of the MD basal area extended beyond its contact area with the other JGA structures (23% in the BD9 and 21% in the Sprague-Dawley rats). It then bordered on capillaries, tubules and interstitial tissue.

On serial sections it became clear that the MD was correlated with the Go. cell field, the two structures resembling each other in extent. MD was seldom in more extensive direct contact with the arteriole, however, the efferent arteriole regularly ran parallel to the distal tubule with a variable contact with the latter. In these sites no MD was present (Fig. 2). The MD and its contact

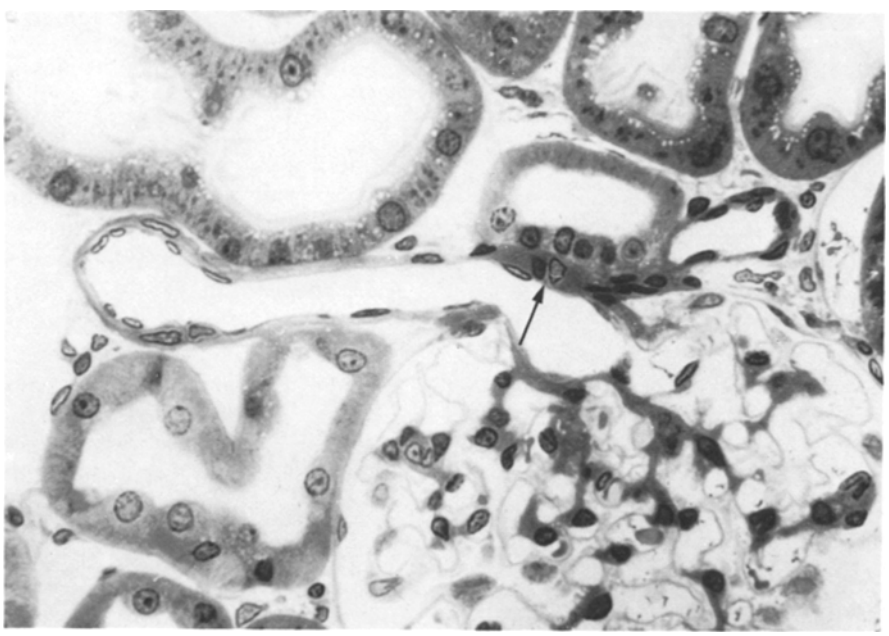

Fig. 3. Juxtaglomerular apparatus. From the right: efferent arteriole and Goormaghtigh cell field. Above: distal tubule with macula densa. On the left the afferent arteriole originating from the interlobular artery. Close to the glomerulus two epithelioid cells (arrow) in intimate contact with macula densa and the Goormaghtigh cells. Semithin section (1.5 μ), Giemsa stain, 570:1

Table 2. Statistical analysis of the contact areas

	BD9		Sprague-Dawley	
1. Macula densa basal area correlated to: the area of contact between Goormaghtigh cell field and macula densa	$r=0.5164$ $p=0.0041$	S	$r=0.7755$ $p<0.00001$	S
the area of contact between Goormaghtigh cell field and				
a) afferent arteriole	$r=0.3349$ $p=0.0509$	S	$r=0.4008$ $p=0.0236$	S
b) efferent arteriole	N.S.		N.S.	
2. Contact area between macula densa and Goormaghtigh cell field correlated with: the area of contact between Goormaghtigh cell field and				
a) afferent arteriole	$r=0.6060$ $p=0.0007$	S	$r=0.4759$ $p=0.0081$	S
b) efferent arteriole	N.S.		N.S.	

N.S. = not significant, S = significant. Spearman rank correlation test. Level of significance $p=0.05$; r = regression coefficient

area with the Go. cell field were statistically larger in the BD9 rats than in the Sprague-Dawley rats (Table 1):

Granular epithelioid cells were found only in the immediate preglomerular segment of the afferent arteriole in the BD9 rats, often, but not always, in contact with MD or the Go. cells (Fig. 3). We also found them in the opposite wall of the afferent arteriole. They invariably occupied the whole media. Sometimes the last segment of the afferent arteriole consisted entirely of epithelioid cells, separated from the blood stream by the endothelium only (Fig. 3). In the Sprague-Dawley rats epithelioid cells were also found at a greater distance from the JGA and in one interlobular artery.

Statistical analysis of the data obtained revealed a highly significant correlation in both rat strains between the MD basal area and its contact area with the Go. cell field. Further, the MD basal area correlated with the contact area between the Go. cell field and the afferent arteriole. Significant correlation of the contact areas was also found between the Go. cell field and the MD on the one hand and the afferent arteriole on the other. There was no significant correlation with the efferent arteriole and any other juxtaglomerular structure (Table 2).

Discussion

Our results suggest that the Go. cells, also called agranular or lacis cells, are an important morphologic structure linking MD to the vascular part of the

JGA. Further, they bridge the gap between MD and the glomerular mesangium. The importance of the Go. cells is underlined by the highly significant correlation between the size of the MD basal area and its contact area with the Go. cell field ($P<0.00001$ in the Sprague-Dawley rats and $P=0.0041$ in the BD9 rats, Table 2), and by the fact that these cells represent the only juxtaglomerular structure which is constantly in direct contact with both the tubular and vascular components of the JGA. Faarup (1965) also pointed out the constant contact between the Go. cells and the MD. He found only one JGA out of 25 in which the efferent arteriole possibly had no contact with the Go. cells. The amount of contact between the arterioles and the MD was found to be inconstant as previously described by other authors (Barajas and Latta, 1963; Faarup, 1965 and Barajas, 1971). Our results are in accordance with those of Faarup (1965) in respect of the direct contact between MD and the afferent arteriole as he found it to occur in 64% and with the efferent arteriole in 48% out of 25 examined JGA. The area of contact was larger with the afferent than with the efferent arteriole in the BD9 rats but of equal size in the Sprague-Dawley rats. These findings are at variance with those of Barajas (1970) who found a larger and constant amount of contact between the distal tubule and efferent arteriole. Our findings correspond to those reported by Faarup (1965) who found an almost equal amount of contact between MD and both hilar arterioles as far as the Sprague-Dawley rats are concerned. Faarup (1965), however, only estimated the length of contact and not the area. Further he reported having used albino rats without specifying the breed.

Barajas (1970) used vascular fixation with 1% glutaraldehyde as we did, but unfortunately did not differentiate between the distal tubule as a whole and MD as a specialized part of it. His areas of contact therefore represents MD plus the distal tubule beyond the MD. Moreover Barajas investigated only 4 JGA which makes direct comparison with our results difficult (Barajas, 1970).

The MD is a very specialized part of the distal tubule, easily recognized in kidney tissue fixed by vascular perfusion and well defined with the light and electron microscope (Thoenes, 1961; Bucher and Reale, 1962; Hatt, 1967). Because of this we only considered direct contact between MD and the hilar arterioles, not contact with the whole distal tubule to be of interest in reviewing JGA function.

In the BD9 rats we failed to confirm the results of Faarup (1965) and Barajas (1971) who found granular epithelioid cells in the rat kidney in the efferent arteriole, in the whole length of the afferent arteriole and in the interlobular artery. However, in the Sprague-Dawley rats, the same rats as Barajas and Latta (1963) used, the epithelioid cells were placed at a greater distance from the glomerular hilus and we found some epithelioid cells in one interlobular artery. There was also more frequent contact between the efferent arteriole and MD than in the BD9 rats.

It may be of interest with regard to the stretch receptor theory (Tobian, 1960) that the granular epithelioid cells replaced the smooth muscle cells in the entire thickness of the media of the afferent arterioles wherever they were found. The present findings, however, strongly support the MD-theory originally

introduced by Goormaghtigh (1932) and later supported experimentally by Thurau and Schnermann (1965) and Schnermann et al. (1970).

Compared to previous results in human kidneys (Christensen, 1974; Christensen et al., 1975) the findings in the rat disclosed the very same structural relationships and statistically significant correlation between the various structures in both species. With these morphological studies it seems therefore, that direct contact between MD and the hilar arterioles is not important in the function of the JGA.

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