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Gap Junctional Coupling between the JGA and the Glomerular Tuft

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Summary. The juxtaglomerular apparatus (JGA) in the rabbit kidney was examined by transmission electron microscopy and by freeze fracturing. It was found, that the Goormaghtigh cells of the JGA are extensively coupled with the mesangial cells within the glomerular tuft by gap junctions. A broad band of gap junctions starting within the Goormaghtigh cells, traversing the transitional area at the root of the glomerular tuft and continuing along the mesangial cells has been revealed by freeze fracturing. No gap junctional connections to the macula densa cells have been found. In accordance with data from literature it may be stated that all smooth muscle derived cell groups at the vascular pole of the glomerulus (smooth muscle cells of the vas afferens and efferens, granular cells, Goormaghtigh cells, mesangial cells) are extensively coupled by gap junctions with each other. It is supposed that this cell system may act as a synchronized functional unit.

Key words: Kidney (rabbit) – Juxtaglomerular apparatus – Mesangial cells – Gap junctions – Electron microscopy, freeze fracturing.

Introduction

It has been shown that at the glomerular vascular pole the ordinary smooth muscle cells of vas afferens and vas efferens, the granular cells and the lacis cells (Goormaghtigh cells) are connected among themselves as well as with each other by gap junctions (Pricam et al., 1974; Boll et al., 1975; Forssmann and Taugner, 1977). This is in accordance with the fact that these cell types all contain leiomyofilaments and are thought to be derived from smooth muscle cells (Barajas, 1970; Bucher and Kaissling, 1973). Moreover, also the mesangial cells within the glomerular tuft are connected among themselves by gap junctions (Pricam et al., 1974). The findings of the present investigation demonstrate that the mesangial cells – at the root of the glomerular tuft – are extensively coupled with the Goormaghtigh cells by gap

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junctions. These data confirm the recent suggestion (Forssmann and Taugner, 1977) that all the cells at the glomerular vascular pole coupled by gap junctions may act as a synchronized functional unit; the mesangial cells now have to be regarded as part of this system.

Materials and Methods

The kidneys of six male New Zealand rabbits were investigated by conventional transmission electron microscopy as well as by freeze-fracturing. Total body perfusion fixation was carried out from the abdominal aorta with a cacodylate-buffered 3% glutaraldehyde solution. Small pieces of the kidney cortex were washed in isoosmolar cacodylate buffer and then postfixed in cacodylate-buffered osmium tetroxide. For en bloc staining the tissue was immersed in maleate-buffered uranyl acetate. Dehydration and embedding was carried out according to Luft (1961). Sections were cut on a MT IIB Sorvall microtome. They were stained in conventional manner by uranyl acetate and lead citrate and investigated at a Philips 301 electron microscope equipped with a goniometer stage.

Freeze-fracture replicas were obtained on a Leybold-Heraeus EPA 100 freeze-fracture unit as described by Boll et al. (1975). In the figures the shadow castings are indicated by an arrow head; the shadows are white. The nomenclature according to Branton et al. (1975) has been used.

Results

The overall morphology of the JGA in the rabbit kidney does not appear to be fundamentally different from what has been described in other mammals (for review see Bucher and Kaissling, 1973). The Goormaghtigh cells (lacis cells) are located in the corner between afferent and efferent arterioles and the macula densa (Fig. 1). They are piled up to a pyramidal- or coneshaped cell complex the basis of which touches the macula densa, the sides face the afferent and efferent arterioles, whereas the top joins towards the root of the glomerular tuft. Here, at the top, the Goormaghtigh cells pass over into the mesangial cells of the glomerulus (Fig. 1). The flattened Goormaghtigh cells are predominantly oriented parallel to the basis of the cell pyramid, whereas the mesangial cell strands diverge from their origin at the top of pyramid into the glomerular tuft, where they establish the axes of the glomerular lobuli. Thus, the lacis cells gradually pass over into the mesangial cells.

Both cell types are embedded into a well developed intercellular matrix which in its electron density resembles the basal lamina material and which, in the case of Goormaghtigh cells, has been compared to a "lacis", i.e. network (Oberling and Hatt, 1960). By this matrix individual Goormaghtigh cells and mesangial cells are separated from one another. However, specific sites have regularly been found where the cells get into contact with each other.

In conventional electron microscopy the type of this cell-to-cell contact is only occasionally apparent. Tilting the sections on a goniometer stage the observed contact plaques are mostly identified as gap junctions which are equally structured within the Goormaghtigh cells (Fig. 2a, b) and among the mesangial cells (Fig. 2c). In addition to this type of junction, some of the cell-to-cell contacts have also been identified as modified desmosomes, a fact that would reveal also a mechanical connection of these cells; the investigation of this problem is in progress.

Also with the freeze-fracture technique gap junctions were repeatedly observed

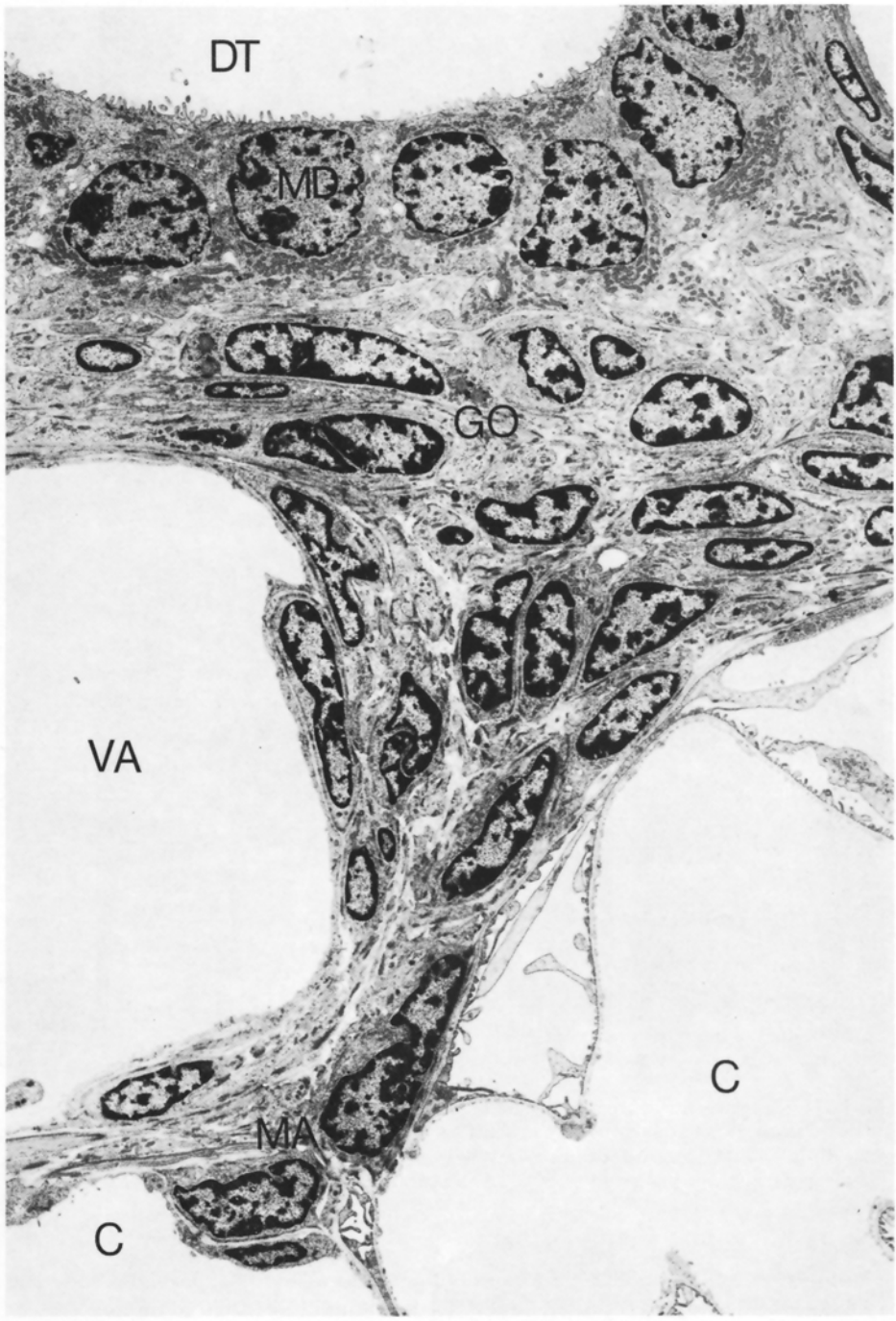

Fig. 1. EM-section through the JGA, exposing macula densa cells (*MD*) at the top, the Goormaghtigh cells beneath which pass over (at the bottom) into mesangial cells (*MA*). *DT* Distal tubular lumen; *VA* Vas afferens; *C* Glomerular capillary (× 3600)

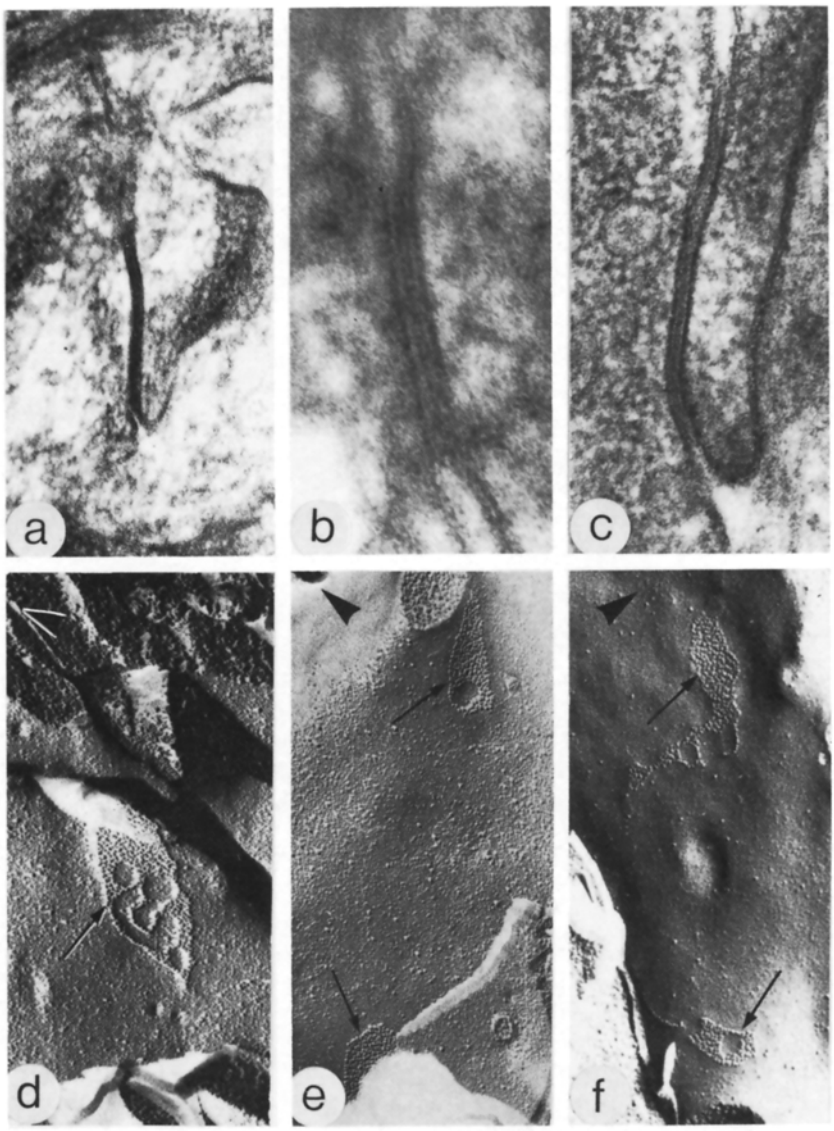

Fig. 2. a–c. Gap junctions (conventional thin EM-sections, tilted) between Goormaghtigh cells (a and b; ×90,000 and ×295,000) and between mesangial cells (c; ×145,000); **d–f** Details of Figure 3 (areas are marked), gap junctions (freeze fracture replicas) between two Goormaghtigh cells (d; ×40,000), between two cells of the transitional area (e; ×40,000) and between two mesangial cells (f; ×45,000)

among Goormaghtigh cells, in the glomerular stalk and among mesangial cells. The outstanding frequency of such junctions becomes fully obvious in the freeze-fracture replica demonstrated in Fig. 3. Here the freeze-fracture plane through the JGA exposes the wall of the afferent arteriole, the Goormaghtigh cells, the mesangial cells, and the transitional area between these cells at the stalk of the

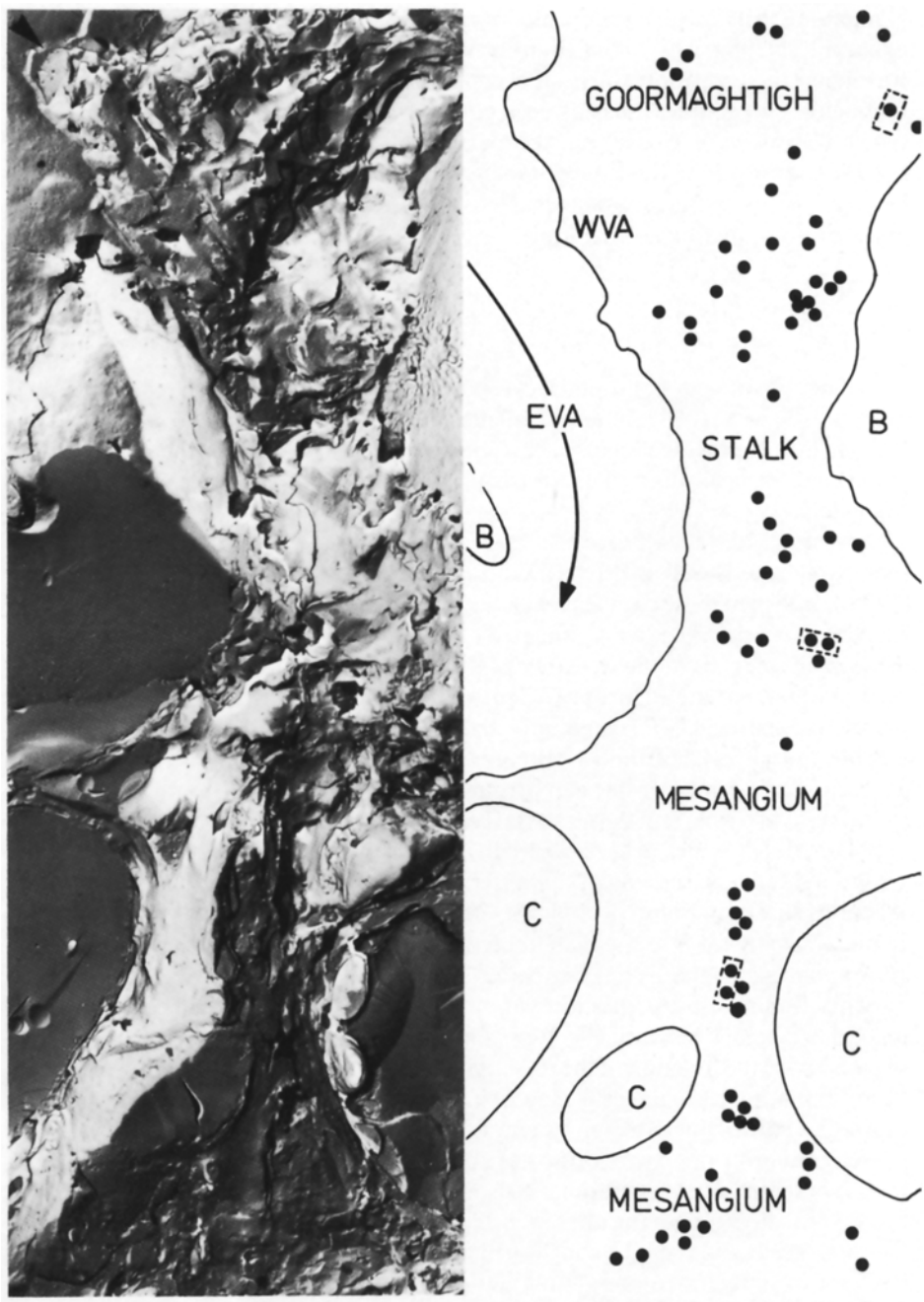

Fig. 3. Freeze fracture replica through the JGA (×2500). As indicated in the corresponding sketch (right) the fracture exposes Goormaghtigh cells, the wall of the vas afferens, the glomerular tuft root and mesangial cells within the glomerular tuft. *B* Bowman capsule; *C* glomerular capillaries; *EVA* endothelium vas afferens; *WVA* wall of vas afferens; the arrow indicates the entrance of the vas afferens into the glomerulus. The dots mark the position of the gap junctions; the surrounded areas correspond to the details given in Figure 2d–f

glomerular tuft. In this region a continuous band of gap junctions has become apparent starting within the smooth muscle cells and the Goormaghtigh cells, traversing the transitional area and continuing along the mesangial cells. A total number of 74 gap junctions has been counted within this band. The gap junctions typically consist of closely packed penetrating particles on the P-face and the corresponding pits on the E-face of the junctional cell membrane. The particles may be arranged in various macular patterns (Fig. 2d–f) which often contain rounded areas of non-junctional membrane.

Discussion

Gap junctions (nexus) connect the cytoplasm of adjacent cells, hydrophilic channels allowing the passage of ions and small molecules from cell to cell without significant leakage into the extracellular space. Cells connected by gap junctions may act as a sort of metabolically as well as electrically coupled functional system (McNutt and Weinstein, 1973).

Pricam et al. (1974) were the first who demonstrated gap junctions between mesangial cells as well as between Goormaghtigh cells in the rat kidney. Boll et al. (1975) confirmed these data and extended them, showing that gap junctions are also existent between individual granulated cells as well as between granulated cells and smooth muscle cells of the vas afferens. In addition to the above-mentioned data in rat Forssmann and Taugner (1977) proved the existence of gap junctions between granulated cells and Goormaghtigh cells as well as between Goormaghtigh cells and smooth muscle cells of the vas afferens and efferens in *Tupaia belangeri.*

This paper demonstrates gap junctions in the field of the Goormaghtigh cells, the afferent arteriole and the mesangial cells in the rabbit kidney. Most remarkably, gap junctions were also observed in the glomerular stalk region where Goormaghtigh and mesangial cells intermingle with each other. Therefore, the gap junctions shown in Figure 3 obviously connect Goormaghtigh and mesangial cells. In one single freeze-fracture plane a broad band of 74 gap junctions connecting the JGA and mesangial cells has been revealed.

This finding raises questions about the functional significance of the gap junction coupling between the JGA and the mesangial cell complex. As little is known about the function of the mesangial cells, the answer can only be speculative. In our opinion mesangial cells may well be regarded as part of the receptor system or/and as part of the effector system of the JGA:

According to the baroreceptor hypothesis (Tobian et al., 1959) regarding the release of renin out of the granular cells, variations in blood pressure are expected to become effective within the afferent arteriole. The gap junctional coupling between mesangial cells and the JGA suggests that, additionally, altered tensile forces might be effective at the root of the glomerular capillaries, and thus, at the mesangial cells.

On the other hand the gap junctional chain through the glomerular stalk might couple the mesangial cells to the JGA for effective mechanisms; a constriction of the afferent and/or efferent arterioles – as it is supposed to happen as part of the tubulo-glomerular feed-back mechanism (Thurau and Mason, 1974; Schnermann et al., 1976) – might spread down via the gap junctional route to the mesangial cells

causing them to contract and thereby to retract the glomerular tuft to its root at the vascular pole. Recent investigations (Hornych and Richet, 1977) demonstrate the ability of glomerular capillary loops to constrict; the mesangial cells are the only candidates to be responsible for a constriction.

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