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Ultrastructural endothelial cells peculiarities in glomerular capillaries in experimental subrenal aortic stenosis

S. Dyankova, G. Gyurov, S. Nikolov

Department of Anatomy, Histology and Embryology, University of Medicine, Varna

The endothelial cells ultrastructure has been studied in the glomerular capillaries in critical (above 70%) stenosed aortic lumen by means of operative metal clip installation below *a. renalis* in 10 immature dogs for a period of 10, 30 and 60 days. The changes observed concerned the intracellular organelles, fenestras, as well as the plasmalema: the syntheticosecretory structures were activated, the fenestras were reduced and the lumenal plasmalema formed branched labyrinth-like processi. The influence of the haemodynamic factors are discussed together with the ischaemia occurring in the cortex as most probable causes for the changes established in the endothelial cells in the glomerular capillaries.

Key words: endothelium, glomerular capillaries, aorta, stenosis.

The normal functioning of the glomerular capillaries (GC) is determined both by the condition of their structural components and the local haemodynamic conditions. Certain stenosis and obstructive diseases of the abdominal aorta most commonly localized below *a. renalis* exert adverse haemodynamic influences upon the kidneys. A redistribution of the blood flow occurs in them from the cortex toward the medulla conditioned by a neuroreflectory and humoural mechanism, known as "Trueta's shunt". The reduced blood flow in the cortex results in ischaemia and hypoxia of the structures. The cortical glomeruli are affected mainly. The changes resulting from influences impairing cortical haemodynamics have been extensively studied [1, 3, 8, 9, 10].

In the context of the altered conditions the nature of the ultrastuctural changes in the endothelial cells (EC) of the GC in critical experimental stenosis of the abdominal aorta at a subrenal level for a definite period of time is particularly interesting because the experiment reproduced efficiently the above mentioned pathological states. The EC, in their turn, play an important part in the local microcirculation regulation and, therefore, all changes within them are significant for the renal diseases pathogenesis.

Material and Methods

The abdominal aorta of 10 one-month-old non-pedigree dogs was stenosed operatively by means of metal clips below *a. renalis*. As a result the lumen was reduced by approximately 70%. The experiment lasted 10, 30 and 60 days, respectively. Material from the renal parenchyma cortex was obtained for ultrastructural investigations. The fixing was performed with 3% glutaraldechide in a phosphorus buffer (pH 7.4 for a period of 2 hours at 4°C and with 1% OsO_4 in the same buffer for a period of 2 hours at 4°C. The material was embedded in Durcupan ACM (Fluka-Switzerland/ and was investigated under a TEM Zeiss EM 109 Turbo/Zeiss-Germany).

Results

The results from the investigation in conditions of glomerular hypoxia following experimentally induced subrenal 70% - stenosis of the abdominal aorta indicate that observable qualitative ultrastructural changes take place in the EC of the GC. They concern the intracellular structures mainly, fenestras, certain plasmalema characteristics, as well as the cells shape, including their behaviour towards the glomerular basement membrane. Some of the indicators are related to the experiment duration.

In 10-day stenosis we established that the EC of the GC of the vascular circumference are less prominently fenestrated in certain regions and at places they do not form fenestras at all. They had a well-developed granulated endoplasmic reticulum

Fig. 1. 10-day stenosis. Cytoplasmic processi prominent in the lumen (Magnification 30 000 times)

with partially dilated cisterns, Golgi apparatus, Weibel Palade bodies, coated vesicles, microtubules. In some of the EC we found local paling of the cellular matrix with spiralised membranes due most likely to myelin-like bodies formation. At places the basal plasmalema of the EC was raised and separated from the glomerular basement membrane. In the space formed as a result, amorphous material was accumulated of middle electronic density. Prominent cytoplasmic processi of various sizes were commonly formed along the EC lumenal surface. They could be detected predominantly in the contact areas and made round formations, which were most probably paralumenal canals (Fig. 1).

In 30-day stenosis the GC lumen was significantly reduced: the nucleus-containing parts of the EC were prominent in it. Fenestras lacked along great parts of the capillary circumference. EC most frequently had a strongly developed granulated endoplasmic reticulum with dilated cisterns, Golgi apparatus, as well as a great number of coated vesicles. In this experimental period the myelin-like bodies were a more common finding (Fig. 2). The basal plasmalema was raised and separated from the basement glomerular membrane more commonly and to a greater extent. Along the lumenal surface the cytoplasmic processi were greater in number and longer, frequently forming canals and/or tunnels for the blood flow.

In 60-day stenosis we established a complete lack of fenestras in the GC. Around and within the extranuclear EC areas the granulated endoplasmic reticulum was very well developed together with numerous dilated cisterns. The Golgi apparatus was less developed as compared to the other experiment durations. There were single coated vesicles and vacuoles with an electronpale matrix. Local rarities were

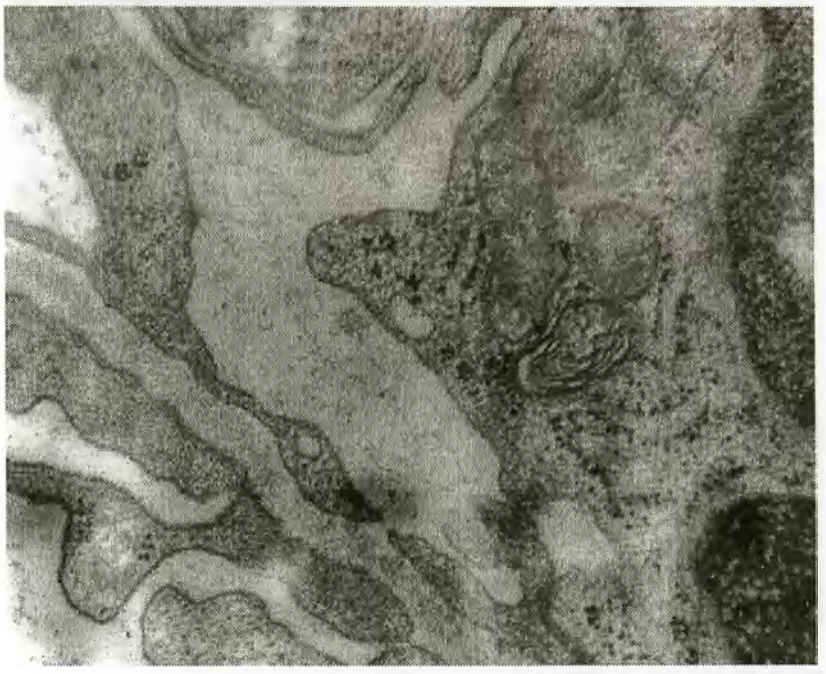

Fig. 2. 30-day stenosis. Myelin-like structures (Magnification 30 000 times)

detected in the cytoplasm together with secondary lysosomes and possible microclasmatosis. In this period we also observed regions of raised basal EC plasmalema separated from the basement membrane, as well as accumulation of heterogeneous basal-like membrane material of middle density (Fig. 3). The strongly activated EC lumenal surface formed thin, long and branched cytoplasmic processi, penetrating deeply into the GC lumen (Fig. 4). They became linked and formed a paralumenal labyrinth of grooves and canals.

Discussion

The strategic anatomical position of the EC, e.g. between the circulating blood vascular smooth muscle, juxtaglomerular and mesangial cells, determines their role as a highly active endocrine organ and has a crucial impact on functional regulation in GC and on kidney disease pathogenesis [5]. It is known that EC take an active part in processes controlling coagulation, inflammation, immune response, as well as contribute to the vasomotor tone regulation by cytokine secretion [4]. Being a component of the blood-urine barrier the EC of the GC are also considered to be a valve controlling accessibility to the filter [8]. At the same time, however, they are directly influenced by haemodynamic factors. The changes occurring in these factors affect the EC morphology and physiology.

In the framework of our experiment the alteration in the blood flow intensity, the pressure on the vascular wall above the stenosis and the resulting ischaemia in

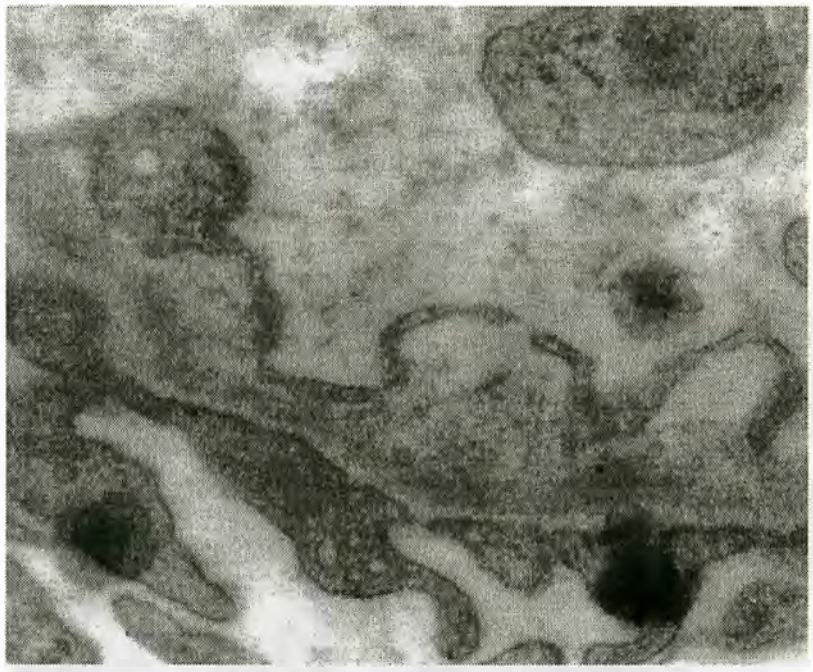

Fig. 3. 60-day stenosis. EC raising and separation from glomerular basement membrane (Magnification 30 000 times)

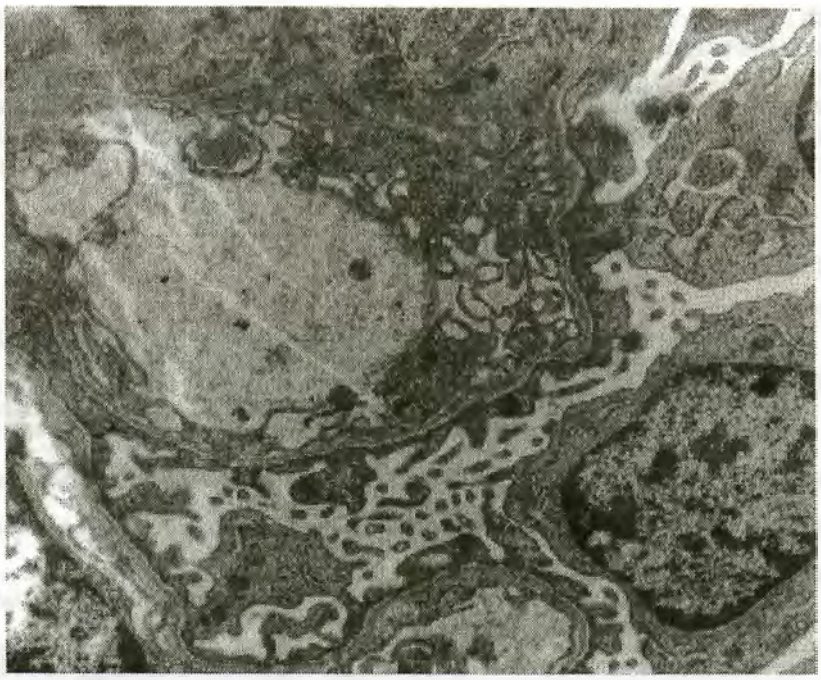

Fig. 4. 60-day stenosis. Almost complete lack of fenestras in certain GC. Strongly activated lumenal surface with numerous processi, forming labyrinths (Magnification 7000 times)

the renal cortex obviously provoke the EC reactivity, this inducing the described changes. The various experiment durations enabled us to monitor their development dynamically. We established the following rule: by increasing influence duration the EC of the GC fenestration is reduced drastically. This fact corresponds to the reduction in the fenestras size and number, reported by Solez et al. [6] in acute early postischaemic renal failure following transplantation, as well as by Мельман, Шутка [10] in acute ischaemia resulting from left kidney vessel ligature. By increasing the experiment duration in the EC, hyperplasia is observed of structures responsible for cellular products synthesis and export. Certain destructive changes can also be detected such as segregation of pale cytoplasmic areas and formation of myelin-like structures. Protrusions are increased in number along the lumenal surface, forming intricate intralumenal labyrinths for the blood flow apparently leading to definite reological changes. The nucleus-containg EC parts prominent in the lumen could also act in the same direction. Similar changes have been reported by Мельман, Шутка [10] in acute renal ischaemia following renal vessels ligature and by Yokota and Ogawa [8] in experimental congestion in clamped *v. renalis.*

The raising and separation of the basal EC plasmalema from the glomerular basement membrane with the accumulation of amorphous material produced by the hyperplastic endoplasmatic reticulum and the Golgi complex can also be related to the haemodynamic stress in our experimental model. Such changes have been observed in nephritic proteinuria after transplantation and following vascular disorders

[2] which makes us consider that this type of morphological reaction has no specific relation to the cause.

The unspecific and relatively in our experiment and confirmed by other studies, are comparatively independent of the qualitative and quantitative characteristics of the stimuli affecting renal haemodynamics. They indicate the EC ability to react most economically and adapt most flexibly to the new functional requirements.

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