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Review article

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INVOLVEMENT OF P2 RECEPTORS IN REGULATION OF GLOMERULAR PERMEABILITY TO ALBUMIN BY EXTRACELLULAR NUCLEOTIDES OF INTRA-/EXTRA-GLOMERULAR ORIGINS

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Plasma filtration through glomerular filtration barrier (GFB) is a key process to maintain fluid and electrolyte homeostasis. GFB consisting of endothelial cells, podocytes and basement membrane restricts passage of albumin but is permeable for smaller plasma molecules. Various biological agents, such as extracellular nucleotides influence activity of cells, which in turn affects permeability of GFB. Nucleotides are released from cells outside and within the glomeruli that activate the purinoceptors - P2Rs classified into ATP-gated non-selective ion channels, P2X receptors (P2XRs), and G-protein-coupled metabotropic P2Y receptors (P2YRs). P2Rs are expressed on cellular components of GFB. P2Rs activation triggers intracellular calcium concentration and calcium-dependent metabolism with subsequent affect on glomerular permeability to albumin. Purinergic-dependent glomerular cell activation also affects the biophysical properties of acelluar glomerular basement membrane (GMB). Finally, P2Rs stimulation may lead to increased proteins excretion in urine. The involvement of P2Rs in increased GFB permeability to albumin may be expected under pathophysiological conditions characterized by increased albumin excretion in urine.

Key words: *albumin, permeability, glomeruli, purinoceptors, P2 receptors, vascular endothelial growth factor, reactive oxygen species*

INTRODUCTION

Human kidney contains approximately one million of functional glomeruli, which are specialized capillary tufts responsible for plasma filtration. During plasma filtration glomerular capillary wall functions as a size-selective, multicellular glomerular filtration barrier (GFB) that restricts passage of albumin (66-kD, 585-amino acid, negatively charged globular protein), but remains permeable to smaller plasma molecules. The glomerular sieving coefficient (Θ) of albumin in rats evaluated by a two-photon microscope technique indicates that 1 albumin molecule per $1 – 4 \times 10^3$ albumin molecules entering the renal glomerulus passes GFB (1, 2), that represents 2 – 3 g of albumin in 24 h at normal glomerular filtration rate (GFR) (~100 ml/min). Finally, only 30 mg of this albumin occurs in the urine, denoting the post-glomerular metabolism of albumin. Accordingly, after glomerular filtration albumin is mostly reabsorbed by proximal tubular cells (PTC) through receptor-mediated clathrin-dependent endocytosis and fluid-phase endocytosis. Albumin is targeted for degradation in lysosomes-degradation pathway, or transcytosis *via* basolateral membrane into extracellular fluid and next to the blood - reclamation pathway (3). Albumin filtered in the glomeruli is considered to be the major source of urinary albumin. Thus, the changes in GFB result in increased level of proteins in the urine, a hallmark of diverse kidney diseases and a major risk factor for renal and cardiovascular diseases.

GLOMERULAR BARRIER COMPLEX

The GFB is organized into three interdependent layers: 1) glomerular endothelial cells (GEC) with fenestrations covered by glycocalyx, a meshwork of glycosaminoglycans; 2) glomerular basement membrane (GBM), a thin layer of extracellular matrix composed of extracellular matrix proteins; and 3) slit diaphragm, an adhesion junction interconnecting the neighbouring foot processes derived from different podocytes bodies (POD) (4). The glomerular filtrate passes through the endothelial fenestrae, crosses the GMB and through the slit diaphragm thereby reaching the Bowman's space. GFB scheme and extracellular route of glomerular filtrate is presented in *Fig. 1*. *In vitro* each layer of GFB is characterized by individual sieving coefficient i.e. Θ_{GEC}, Θ_{GMB}, Θ_{POD}, however, final glomerular sieving coefficient (Θ_{GLO}) is not a result of simple multiplication or the sum of these values but seems to be a result of layer interaction forming integrative glomerular barrier complex (5, 6). The Θ_{GLO} is not a static or constant parameter. Infusion of atrial natriuretic peptide (ANP) in rats induces cyclic alternation in a filtrate-to-plasma concentration ratio of Ficoll Θ_{Ficoll} (7). Θ_{GLO} also depends on GFR. It has been shown that Θ_{Ficoll} decreases with increase in GFR (8). Accordingly, visualization of podocyte ultrastructure revealed oscillation of glomerular contraction (9). Moreover, multiphoton fluorescence imaging showed regular oscillations of fluorescence intensity levels recorded in Bowman's urine space (2). These observations

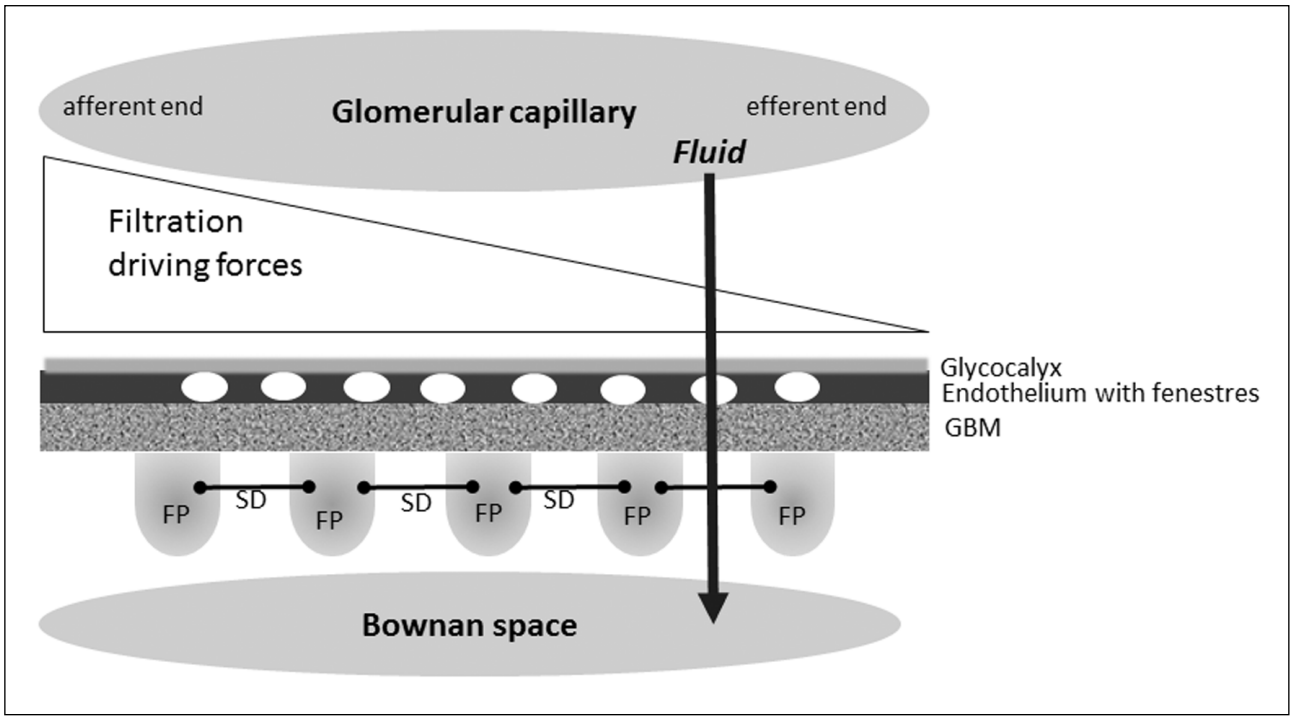

Fig. 1. Scheme of glomerular filtration barrier. SD, slit diaphragm; FP, foot processes; GBM, glomerular basement membrane.

may support the 'glomerular pump' hypothesis (10). Post-GFB fluid passes into subpodocyte space (SPS) that covers at least 60% of the total filtration surface and through subpodocyte space exit pore (SEP) is connected with another urinary space - interpodocyte space (11). SPS is restrictive to fluid flow and its characteristics depend on the dimensions of SEP. Podocytes cytoskeleton may press tightly on SEP and as a consequence, increase the hydrostatic pressure and decrease the filtration through the SPS. Hydrostatic pressure in SPS may be affected by podocytes dynamics and cause a 3-fold excess in the hydrostatic pressure in Bowman's urinary space (12) which in turn may result in pressure-induced changes in GBM permeability. Increasing transmembrane pressure is accompanied by decreasing effective pore size likely due to compression of the gel leading to increase in density of GBM fibre proteins network, such as laminin-521 and type IV collagen (13). Furthermore, SPS shows ability to retain macromolecules. The hyaluronan removal from endothelial glycocalyx is accompanied by albumin accumulation close to podocytes foot processes, especially in SPS (14). The glomerular permeability may be indirectly modulated by mesangial cells located between the glomerular capillaries and possessing contractile properties, thereby assisting in the maintenance of glomerular organization and control of glomerular blood flow. These cells produce and release biological factors, such as nitric oxide, affecting function of endothelial cells and podocytes and in turn properties of GFB (15). Many pharmacological agents affect renal function and GFB properties. Cyclosporine A, an immunosuppressant affecting calcineurin activity and NFAT-dependent transcription, is associated with drug-induced nephrotoxicity in log-term therapy (16) but an NFAT-independent mechanism for calcineurin i.e stabilization of synaptopodin protein in podocytes, has been identified and this one is responsible for antiproteinuric effect of cyclosporine A (17). Furthermore, the peroxisome-proliferator-activated receptors gamma (PPAR-γ) ligands have protective effect against cyclosporine A-induced nephrotoxicity (18).

GLOMERULAR PURINOCEPTORS

The cells of GFB, podocytes, GEC, as well as mesangial cells express receptors for extracellular nucleotides - P2 purinoceptors, P2Rs (19-25). These receptors can be classified into ATP-gated non-selective ion channels P2X receptors (P2XRs) and into G-protein-coupled metabotropic P2Y receptors (P2YRs). At present, seven P2XRs (P2X1-7Rs) and eight P2YRs (P2Y1,2,4,6,11-14Rs) distinct mammalian receptors have been cloned and characterized. The localization of ionotropic and metabotropic mRNAs and proteins of P2Rs on GFB cells is reported in *Table 1*. However, the detection of a transcript encoding a receptor by PCR does not mean that the receptor is actually expressed in a functional form. P2XRs respond only to ATP by elevating the intracellular calcium concentration either by direct Ca^{2+} permeation, activation of voltage-gated Ca^{2+} channels, or Ca^{2+} release from sarcoplasmic reticulum *via* ryanodine receptors (RyRs) and inositol 1,4,5-triphosphate (IP_3Rs) (26, 27). Additional prolonged ATP stimulation of P2X7R leads to the formation of a larger reversible pores, which allows for the uptake of organic ions with a mass up to 900 Da or size up to 1.4 nm (28). P2YRs recognize ATP and several other nucleotides, including ADP, UTP, UDP, UDP-glucose, and signal through G_q, G_i, or G_s proteins producing changes in concentration of intracellular cAMP or Ca^{2+} (29).

P2Rs expressed on the glomerular cells can be activated by nucleotides released external from the glomerulus and reach the kidney with plasma. Unstimulated plasma ATP concentration is approximately 28 ± 16 nmol/l but may reach values greater than 1000 nmol/l (30), while its concentration in Bowman's space is about 32 ± 7 nmol/l (31). Moreover, glomerular cells may release ATP. The basal experimental rate of ATP release from isolated glomeruli is estimated to be 0.3 pmol/mim/1000 glomeruli and mechanical perturbation by medium displacement method increased ATP release rate by about 40% (32).

Table 1. P2XRs and P2YRs localization in glomerular filtration barrier cells.

GFB cells	Receptor	mRNA /protein	Species	Experimental model	Reference
Podocytes	P2Y1	Protein	Mouse	Immortalized cell culture	(21)
			Rat	Freshly isolated glomeruli	(22)
		mRNA	Mouse	Immortalized cell culture	(17)
	P2Y2,6,7 P2X7	mRNA	Mouse		
	P2Y2	Protein	Rat	Rat renal slides	(19)
	P2Y1,2,4,6,11 P2X4,5,6,7	mRNA	Human	Visceral glomerular epithelial cells	(18)
Endotheliocytes	P2Y1,2,4 P2X1,4,7	mRNA	Mouse	Cell culture	(20)
	P2Y2	Protein	Bovine	Cell culture	(16)

Nonstimulated mesangial cells release ATP as well (33). Furthermore, ATP may be released in response to pharmacological agents. Nebivolol and carvedilol, third-generation α-adrenoreceptor antagonists, induce mechanosensitive channels-dependent ATP release from GEC (34). Additionally, hypoglycaemic agent, metformin, increases ATP concentration by mechanism based on inhibition of ecto-ATPase activity expressed on podocytes (35). Once released, ATP may act in autocrine or paracrine manner using connexin hemichannels (36). Connexin isoforms are localized in the intraglomerular mesangium, GEC and podocyte, which may facilitate the intraglomerular purinergic signaling (23, 37). The recent multiphoton imaging experiments have provided evidence that glomerular retention of neutrophils occurs in normal kidneys (38), thus these cells might be a potential source of intraglomerular ATP. The effect of P2Rs on GFB permeability is summarized in *Fig. 2*. Conversely, dephosphorylation of the nucleotides by ectonucleotidases reduces bioavailability of endogenous P2 receptor agonists (39). While this review is focused on P2 receptors, it is important to note that the P1 receptors, which are preferentially activated by adenosine, are also reported in glomeruli cell and play significant role in purinergic control of glomeruli function (40).

PURINERGIC-DEPENDENT REGULATION OF GLOMERULAR HEMODYNAMICS

Glomerular capillary hydrostatic pressure is the driving force for filtration but a net ultrafiltration driving Starling type forces for filtration is about 10 mm Hg in human kidney. The glomerular capillary network is interposed between two resistance beds - afferent and efferent arteriole. The afferent arteriolar resistance (AAR) determines the fraction of pressure that is transmitted to the glomerular capillary network, whereas the efferent arteriolar resistance determines the outgoing pressure. AAR is automatically adjusted within seconds of changes in arterial perfusion pressure through the combined influences of two distinct mechanisms - myogenic control and tubuloglomerular feedback (TGF). Under physiological conditions, glomerular hemodynamics are stable due to the renal autoregulation working through extracellular ATP, P2Rs and gap junctions. Activation of P2X1R with subsequent activation of voltage-dependent L-type calcium channels and 20-hydroxyeicosatetraeonic acid (20-HETE) is an essential mechanism for pressure-mediated afferent arteriolar vasoconstriction (41). Moreover, renal autoregulatory capacity is reduced in hypertensive animal models and is associated with reduced P2X1Rs reactivity (42).

TGF mechanism is a negative-feedback system that stabilizes renal blood flow (RBF) and GFR and operates within the juxtaglomerular apparatus (43). The specialized cells of cortical thick ascending limb of Henle's loop called macula densa cells sense the changes in luminal Na^+/Cl^- concentration *via* activity of Na^+-K^+-$2Cl^-$ cotransporter. The affected luminal uptake of ions initiates adjustment of glomerular arteriolar and mesangial vasomotor tone that results in inverse changes of GFR. This signaling pathway involves ATP release across the macula densa basolateral membrane through 'maxi' anion channels. Under some conditions, activation of certain potent vasoactive systems can exceed the normal autoregulatory mechanisms and abrogate the expected maintenance of RBF and GFR. Increased albumin excretion may occur due to increased RBF and/or increased glomerular capillary hydrostatic pressure. The increased renal interstitial ATP is observed due to an increase in renal perfusion pressure (44) and angiotensin II (Ang II)-induced hypertension with upregulation of P2X1R in the cortical tissue (45). Moreover, P2Y6R heterodimerizes with receptors for Ang II (AT1R) and prevents Ang II-induced desensitization, and in consequence promotes the development of Ang II-induced hypertension (46). Importantly these animals are characterized by increased AAR, glomerular capillary pressure and albumin excretion in urine (47). Furthermore, Ang II *via* AT1R induces rapid release of ATP in freshly isolated blood-free kidneys and this effect is enhanced in hypertensive rats (48).

PURINERGIC-DEPENDENT REGULATION OF GLOMERULAR PERMEABILITY TO PROTEIN

A short-term activation of P2Rs expressed on GEC or long-term activation of these receptors expressed on mesangial cells induces the nitric oxide (NO) production through endothelial or inducible isoforms of nitric oxide synthase (eNOS, iNOS), respectively. NO increases glomerular permeability for albumin through mechanism involving tyrosine phosphorylation (49). Our preliminary experiments suggest that activation of P2Rs leads to increase in permeability of isolated glomeruli and podocytes to albumin and this process is dependent on NOS and cytoplasmic guanylyl cyclase (50). This effect may be modified. The statins by increasing levels of eNOS and NO production and affecting hemodynamics (51) may have probably impact on effects of P2-receptors stimulation. However, NO may also have positive

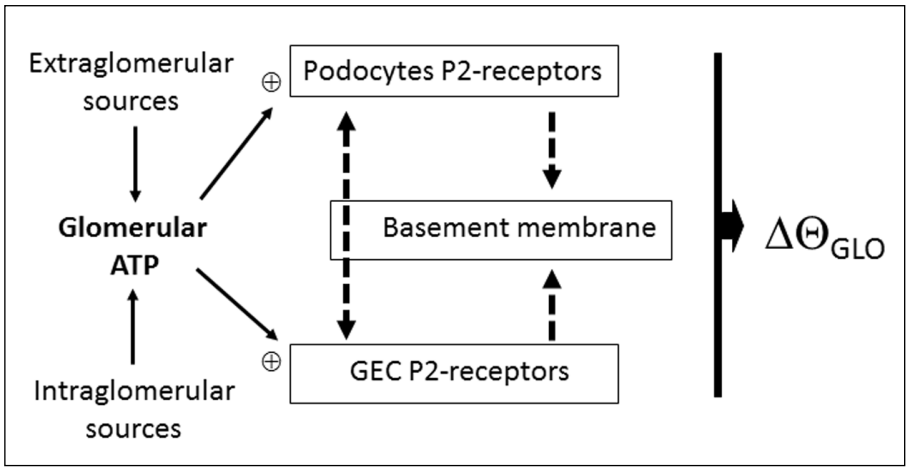

Fig. 2. The proposal of P2-receptors influence on glomerular permeability to albumin. GEC, glomerular endothelial cells; Θ_{GLO}, glomerular sieving coefficient; GBM, glomerular basement membrane.

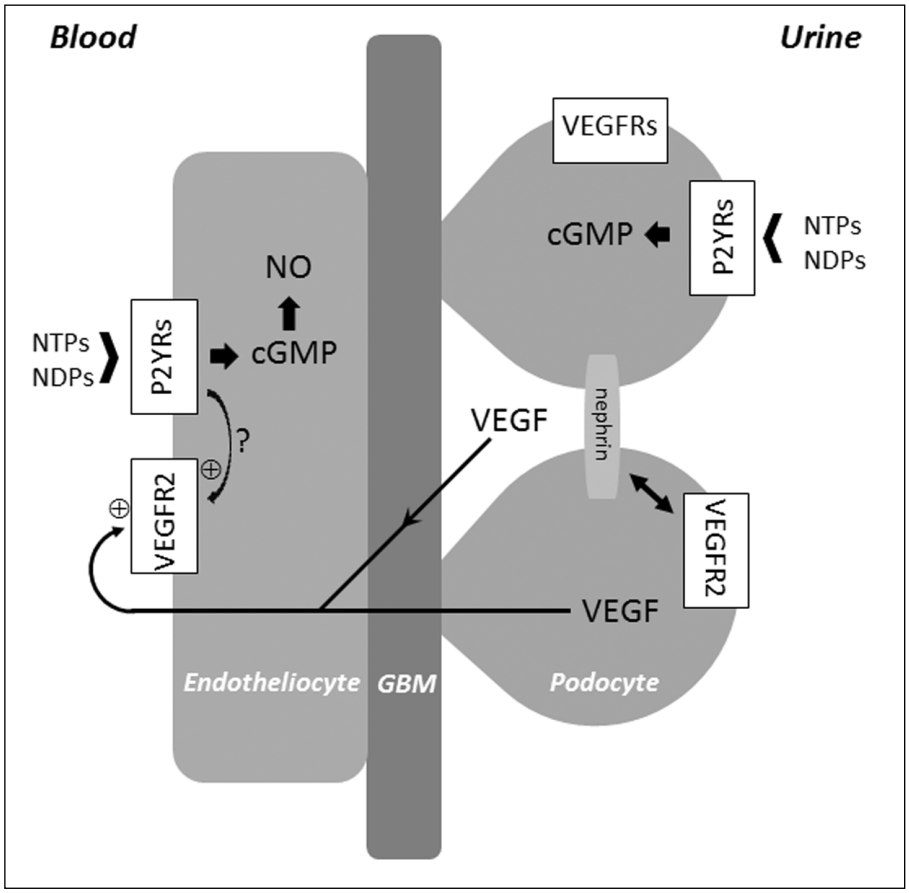

Fig. 3. Interactions between podocytes and endothelial cells in regulation of glomerular filtration barrier permeability, role of P2Rs and VEGF. cGMP, cyclic guanosine monophosphate; NTPs, nucleotide triphosphates; NDPs, nucleotide diphosphates; NO, nitric oxide; VEGF, vascular endothelial growth factor; VEGFR-2, VEGF receptor 2.

influence on GFB permeability to protein by antagonizing the superoxide (O_2^-) and this can explain the exacerbated proteinuria after NOS inhibition using L-NMNA (52). Superoxide and other reactive oxygen species (ROS) may be generated after P2Rs activation (53), but there are also reports demonstrating reduced ROS generation due to P2Rs activation (24). Moreover, ROS may also mediate action of biological agents on glomerular permeability (54), disrupt the GEC glycocalyx composed of proteoglycans, glycosaminoglycans (GAG) and adsorbed plasma proteins without affecting the GAG biosynthetic pathway (55). GEC is a fenestrated layer where glycocalyx covers fenestral and interfenestral domains. Structure and properties of GEC are also regulated by hemopexin, heme-scavenging protein inhibited by ADP. Hemopexin reduces glycocalyx, induces nephrin-dependent reorganization of the actin cytoskeleton in cultured podocytes and increases albumin diffusion across GEC (56). The decreased hemopexin and increased ATP concentration in plasma is observed in preeclampsia, syndrome of pregnancy characterized by proteinuria and morphological changes in GEC including cell swelling with loss of fenestrae and occlusion of the capillary lumens. It has been shown that infusion of ATP induces albuminuria in pregnant rats (57), thus suggesting that ATP and its signaling *via* P2Rs may be harmful, particularly during pregnancy. Activation of P2Rs induces the changes of intracellular calcium concentration affecting the cytoskeleton protein dynamics, which is critical for elaboration and maintenance of podocyte foot processes (58). Rearrangement of the actin cytoskeleton, reflected by changes in F-actin pattern

from a random to more stellate with reduced peripheral F-actin staining, is key in foot process effacement, disruption of the slit diaphragm, and albuminuria development (59). Activation P2YRs in podocytes expressing podocin evokes calcium current that appears to flow through canonical transient receptor potential-6 (TRPC6) and require ROS generation affecting rearrangement of cytoskeleton and disrupting the linkage between podocytes and GBM with subsequent detachment of the podocytes from GBM (60). The expression of TRPC6 mRNA and protein in podocytes is increased by vascular endothelial growth factor, VEGF (61). This molecule is constitutively expressed in podocytes whereas its receptors are predominately localized on the GEC (62). The kinetic model of VEGF synthesis suggests its diffusion from the podocytes to GEC through GBM against the flow of glomerular filtration (63). VEGF is essential for the integrity of GFB. VEGF increases the ultrafiltration coefficient in isolated intact rat glomeruli, which may contribute to the high physiological glomerular permeability to water through an action on GEC (64). This action may be reflected by increased fraction of albumin crossing GFB by liquid flow, nonetheless predominant fraction of albumin passing the GFB is due to diffusion. Furthermore, VEGF causes podocytes effacement and its level is higher in hypertensive patients in the presence of increased albumin excretion in urine (65). P2Rs affect VEGF signalling. Stimulation of P2X7Rs in monocytes promotes the release of VEGF from monocytes (66). Moreover, activation of P2YRs transactivates VEGF receptor 2 (VEGFR-2) in endothelial cells (67). Furthermore, VEGFR-2 directly interacts with the slit diaphragm protein nephrin and this complex involves Nck and actin in podocytes (68). In conclusion, P2Rs activation may affect amount of albumin crossing GFB powered by mechanism of VEGF-VEGFR interactions. The proposal of interaction between P2YRs and VEGF-VEGFR axis in cross-talk between podocytes and GEC is presented in *Fig. 3*.

The pathological conditions, characterized by increase GFB permeability to albumin and albuminuria, such as diabetes mellitus, hypertension and glomerulonephritis, are marked by enhanced level of P2X7Rs expression in podocytes (69, 70). These receptors play pro-apoptotic role and may be responsible for progressive reduction of podocyte numbers in glomerulus under pathophysiological conditions accompanied by increased albumin excretion in urine (71). Despite the low level of P2X7Rs expression under physiological conditions mice lacking P2X7Rs are characterized by lower level of proteinuria (72). Furthermore, P2X7Rs deficiency attenuates renal injury in experimental glomerulonephritis, and A-438079, an agonist of P2X7Rs induces dose-dependent reduction of proteinuria in nephrotoxic nephritis rats. Similarly, P2X7Rs deficiency reduces albumin excretion in urine in deoxycorticosterone acetate-salt hypertension mice and P2X7Rs antagonism with brilliant blue G or A-438079 markedly attenuated urinary albumin excretion (73, 74). Activation of P2X7Rs can lead to the generation of ROS affecting GFB permeability to albumin. Importantly, antioxidant supplementation of N-acetylcysteine in diabetic rats shows attenuated P2X7Rs expression and decreased albumin excretion in urine. Furthermore there is a correlation between P2X7Rs activity and proteinuria (75).

In summary, purinoceptors P2 may play important role in the regulation of the glomerular permeability to proteins by direct action on GEC and podocytes or indirect on GBM.

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