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REVIEW
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Cannabinoid Receptor 1 Inhibition in Chronic Kidney Disease: A New Therapeutic Toolbox

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Chronic kidney disease (CKD) concerns millions of individuals worldwide, with few therapeutic strategies available to date. Recent evidence suggests that the endocannabinoid system (ECS) could be a new therapeutic target to prevent CKD. ECS combines receptors, cannabinoid receptor type 1 (CB1R) and type 2 (CB2R), and ligands. The most prominent receptor within the kidney is CB1R, its endogenous local ligands being anandamide and 2-arachidonoylglycerol. Therefore, the present review focuses on the therapeutic potential of CB1R and not CB2R. In the normal kidney, CB1R is expressed in many cell types, especially in the vasculature where it contributes to the regulation of renal hemodynamics. CB1R could also participate to water and sodium balance and to blood pressure regulation but its precise role remains to decipher. CB1R promotes renal fibrosis in both metabolic and non-metabolic nephropathies. In metabolic syndrome, obesity and diabetes, CB1R inhibition not only improves metabolic parameters, but also exerts a direct role in preventing renal fibrosis. In non-metabolic nephropathies, its inhibition reduces the development of renal fibrosis. There is a growing interest of the industry to develop new CB1R antagonists without central nervous side-effects. Experimental data on renal fibrosis are encouraging and some molecules are currently under early-stage clinical phases (phases I and IIa studies). In the present review, we will first describe the role of the endocannabinoid receptors, especially CB1R, in renal physiology. We will next explore the role of endocannabinoid receptors in both metabolic and non-metabolic CKD and renal fibrosis. Finally, we will discuss the therapeutic potential of CB1R inhibition using the new pharmacological approaches. Overall, the new pharmacological blockers of CB1R could provide an additional therapeutic toolbox in the management of CKD and renal fibrosis from both metabolic and non-metabolic origin.

Keywords: cannabinoid, cannabinoid receptor type 1, chronic kidney disease, endocannabinoids, renal fibrosis

INTRODUCTION

Chronic kidney disease (CKD) is an important problem worldwide and remains a burden for public health (1). CKD corresponds to the replacement of renal functional tissue by extracellular matrix proteins that progressively and irreversibly alter renal function, i.e renal fibrogenesis. Although only 1% of people with CKD reach end-stage renal disease (ESRD), it remains the most expensive of chronic diseases and significantly reduces lifespan (1). To date, only the renin angiotensin system (RAS) blockade using either angiotensin converting enzyme inhibitors or angiotensin II receptor blockers has been shown to be effective in slowing the progression of CKD (2). Recently, new therapeutic classes have improved the management of diabetic nephropathy, which is the main cause of CKD and ESRD worldwide (3), particularly sodium glucose co-transporter 2 (SGLT2) inhibitors (4–8) and glucagon-like peptide-1 receptor (GLP-1R) agonists (9, 10). SGLT2 inhibitors can prevent CKD progression, ESRD and death from renal or cardiovascular causes and are currently approved for use in adults with type 2 diabetes by the U.S. Food and Drug Administration. Interestingly, SGLT2 inhibitors could be efficient regardless of the presence or absence of diabetes (4–8). There is therefore a critical need for new therapeutics in CKD especially in non-metabolic nephropathies. A growing body of evidence suggests that the endocannabinoid system (ECS) could be a new therapeutic target to prevent CKD (11–18). The ECS includes two receptors, the cannabinoid receptor type 1 (CB1R) and the cannabinoid receptor type 2 (CB2R), and about sixty endogenous ligands. The foremost exogenous ligand is Δ9-tetrahydrocannabinol (Δ9-THC). CB1R was the first endocannabinoid receptor to be identified within the brain (19). CB1R belongs to the 7-transmembrane regions receptor family and is a G-protein coupled receptor (19, 20). CB1R is best known to be involved in the regulation of addictive behavior, emotional behavior, analgesia, and memory (21, 22). CB1R is also involved in metabolic pathways in peripheral tissues (11, 23). A second G-protein coupled receptor, CB2R, was later discovered. CB2R is mainly expressed in the immune system and it contributes to its regulation (20). The best-known endogenous ligands are N-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG). Most endogenous ligands are ecosanoïds which derive from phospholipids of cellular membranes (24, 25). Whereas CB1R expression is low in normal kidneys (12, 26–28), we previously found that its expression increased in metabolic CKD but also in non-metabolic CKD such as IgA nephropathy, acute interstitial nephritis, and chronic allograft nephropathy (12, 28). Recent studies have demonstrated that CB1R is involved in CKD during diabetes and/or obesity (13, 15–18) by its role on metabolism but also through a direct action on podocytes and tubules. Activation of CB1R enhances oxidative stress, inflammation, and fibrosis within the kidney, whereas CB2R exerts opposite effects. However, CB2R agonists do not provide any additive effect to CB1R inhibition (11, 12). We will therefore mainly focus on CB1R inhibition as a novel target in CKD. The first human clinical trials exploring the therapeutic potential of the ECS were designed to investigate the effect of CB1R inhibition in obesity and metabolic syndrome. Rimonabant, a CB1R inverse agonist which belongs to the first generation of CB1R antagonists, was the first approved for clinical use (29–36). However, due to central nervous side effects, mainly depression, rimonabant was withdrawn from the market in 2007. Since then, compounds with minimal blood brain barrier (BBB) penetration, such as AM645 or JD5037, have been developed (12, 16, 18, 37–40). Recently, third generation CB1R antagonists, which are peripherally restricted dual-target CB1R antagonists, have been designed to improve therapeutic efficacy by targeting a second pathway involved in the pathological conditions (37, 41). More and more compounds are under development to both prevent BBB penetration and enhance peripheral CB1R inhibition efficacy, including β-Arrestin-2 biased peripheral CB1R antagonist (42) and CB1R blocking antibodies (NCT03261739; NCT03900325 clinical studies ongoing). In the present review, we will first describe the role of the endocannabinoid receptors, especially CB1R, in renal physiology. We will next explore the role of endocannabinoid receptors in both metabolic and non-metabolic CKD and renal fibrosis. Finally, we will discuss the therapeutic potential of CB1R inhibition using the newest pharmacological approaches.

Abbreviations: 2-AG, 2-arachidonoylglycerol; ACC, acetyl-CoA carboxylase; AEA, anandamide; AMPK, AMP-activated kinase; Ang II, angiotensin II; AT1, Ang II type 1 receptors; BBB, blood brain barrier; BP, blood pressure; CAD, chronic kidney allograft dysfunction; CB1R, cannabinoid receptor type 1; CB2R, cannabinoid receptor type 1; CCR2, CC chemokine receptor 2; CKD, chronic kidney disease; COX-2, cyclooxygenase type 2; DAGL, sn-1-specific diacylglycerol lipase; ECS, endocannabinoid system; ESRD, end-stage renal disease; FAAH, fatty acid amide hydrolase; GFR, glomerular filtration rate; GLUT2, glucose transporter 2; HK2, human proximal tubular cell line; HP, hemopressin; HTN, hypertension; IHC, immunohistochemical assay; LKB1, tumor suppressor liver kinase B1; MAGL, monoacylglycerol lipase; MCP-1, monocyte chemoattractant protein-1; NAPE, N-arachidonoyl phosphatidyl ethanolamine; NAPE-PLD, NAPE-specific phospholipase D; PIP2, 2-arachidonoyl-phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; RAS, renin angiotensin system; SGLT2, sodium glucose co-transporter 2; SHR, spontaneously hypertensive rat; RPTC, renal proximal tubular epithelial cells; RT-PCR, Reverse transcription and polymerase chain reaction; SR141716A, rimonabant; STZ, streptozotocin; TAL, thick ascending limb; THC, tetrahydrocannabinol; TGFβ, transforming growth factor β; TRPV1, transient receptor potential vanilloid type I channels; UUO, unilateral ureteral obstruction; WB, western blot; WIN, WIN55,212-2; ZDF, Zucker diabetic fatty.

THE ROLE OF THE ENDOCANNABINOID SYSTEM IN RENAL PHYSIOLOGY

The Endocannabinoid System Within the Kidney

Little is known about the exact role of the ECS within the kidney, data being rather scarce and controversial (11) (**Figure 1**). As previously stated, ECS combines receptors, CB1R and CB2R, and ligands, the most prominent within the kidney being AEA and 2-AG. We will first describe what is known within the kidney about CB1R and CB2R expression, the pathways involved in endocannabinoid synthesis and the relationship between cannabinoid receptors and their ligands. Secondly, we will

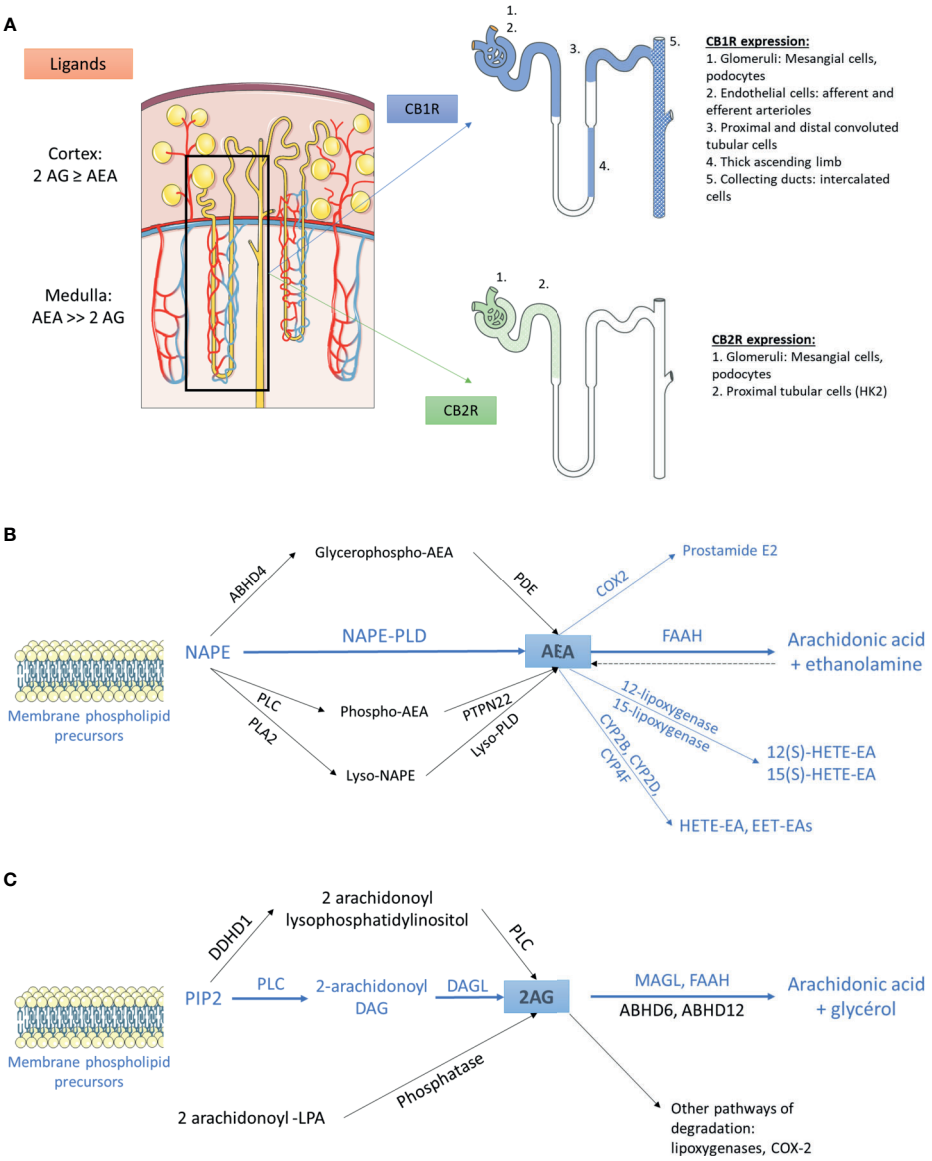

FIGURE 1 | The endocannabinoid system within the kidney **(A)** Distribution of main endogenous ligands and cannabinoid receptors in healthy kidneys. **(B)** Biosynthesis and degradation of anandamide. The pathways that were demonstrated within the kidney are in blue. The other pathways are in black. **(C)** Biosynthesis and degradation of 2-arachidonoyl-glycerol. The pathways that were demonstrated within the kidney are in blue. The other pathways are in black. 2AG, 2-arachidonoyl-glycerol; ABHD, α/β-Hydrolase domain containing; AEA, anandamide; CB1R, cannabinoid receptor type 1; CB2R, cannabinoid receptor type 2; COX2, cyclooxygenase 2; CYP, cytochrome P450; DAG, diacylglycerol; DAGL, *sn*-1-specific diacylglycerol lipase; DDHD1, phosphatidic acid-preferring phospholipase A1; EET-EA, epoxyeicosatrienoic acid-ethanolamine; FAAH, fatty acyl amide hydrolase; HETE-EA, hydroxyeicosatetraenoic acid-ethanolamine; LPA, lysophosphatidic acid; MAGL, monoacylglycerol lipase; NAPE, N-arachidonoyl phosphatidyl ethanolamine; PDE, phosphodiesterase; PIP2, 2-arachidonoyl-phosphatidylinositol 4,5-bisphosphate; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D.

detail the role of the ECS in renal physiology and will mainly focus on the aspects that could be involved in the therapeutic management of CKD.

The Cannabinoid Receptors Within the Kidney

The cannabinoid receptors expression within the kidney was studied using transcriptional and protein approaches. Reverse transcription and polymerase chain reaction (RT-PCR) for *Cnr1*, the gene encoding for CB1R, western blot (WB) and immunohistochemical assays (IHC) demonstrated that CB1R was present in human and rodents (12–14, 27, 43–48). Assessing the specificity of proteins expression by IHC and WB, especially G-protein coupled receptor, needs adequate positive and negative controls to prevent improper interpretation of results (49, 50). Therefore, in the present review, we paid particular attention to the negative and positive controls used to confirm

staining specificity (**Table 1**). Whereas CB1R expression was extremely low in healthy kidneys, we and others found than it was expressed in many cell types, especially in the vasculature (12, 26, 43). Its expression was also documented in other parts of the nephron including glomeruli (podocytes and mesangial cells) (13, 14, 43, 45) and tubular cells (27, 46, 48) (**Figure 1A**). Unlike CB1R, the expression of CB2R within the kidney remains controversial (52). Several groups failed to detect *Cnr2* mRNA, encoding for CB2R, and to demonstrate protein expression by WB and IHC (27, 46). Conversely, other groups found that CB2R may be expressed in normal renal cortex (43, 48, 53). In rats, *Cnr2* mRNA was found in mesangial cells (43). In human and mice, the pattern of CB2R staining by IHC was suggestive of podocyte labeling (53). Transcripts and proteins by WB were also found in human proximal tubular cell line (HK2), suggesting that CB2R could also be expressed in proximal tubules within the kidney (48) (**Figure 1A**). Taken together, these conflicting results suggest that CB2R is expressed at an extremely low level in normal kidneys. As previously stated, assessing the protein expression of G-protein by IHC is very tricky and antibodies specificity had to be carefully checked (49, 50). Transcriptional approach is helpful, especially when it targets a specific cell type. To date, data about the physiological role of CB2R within the kidney are lacking. Therefore, we will mainly focus on the role of CB1R.

The Endogenous Ligands of the Endocannabinoid System Within the Kidney

Whereas AEA was the first endogenous cannabinoid receptor ligand to be discovered (24), later evidence indicated that 2-AG was both the most abundant and the most efficacious endogenous natural ligand for the cannabinoid receptors in several tissues (54). Data about the main cannabinoid endogenous ligand within the kidney are contradictory: we and others found that 2-AG was the main endogenous ligand (12, 44), conversely to authors finding that AEA was more abundant than 2-AG (26, 43, 55, 56). This difference is possibly explained by the difference in the renal compartment analyzed in these studies: while kidney cortex exhibits similar or higher levels of AEA and 2-AG, AEA is the major endocannabinoid ligand of CB1R within the medulla.

AEA and 2-AG are eicosanoids that are synthesized on-demand from arachidonic acid containing phospholipids (57–59). The main source of AEA depends on the conversion of the membrane-bound N-arachidonoyl phosphatidyl ethanolamine (NAPE) (60–64). To date, the exact system responsible for NAPE's synthesis within the kidney remains unknown (56). The next step is the conversion of NAPE to AEA. Several pathways have been described so far (54, 56, 60, 65). A direct mechanism involves the conversion of NAPE to AEA by the NAPE-specific phospholipase D (NAPE-PLD) (66). In addition, using NAPE-PLD$^{-/-}$ mice, at least 3 groups demonstrated that multi-step NAPE-PDL-independent pathways were also able to convert NAPE to AEA, involving the formation of intermediate compounds which are thereafter converted to AEA (**Figure 1B**) (67–73). The NAPE-PDL could play a major role within the kidney as NAPE-PDL$^{-/-}$ mice exhibited significantly higher NAPE level in kidney than wild-type mice (70). The presence of NAPE-PDL within the kidney was assessed by RT-PCR in immortalized epithelial cells derived from pig kidney proximal tubule (LLC-PK1 cells) (74). After biosynthesis, AEA is rapidly catalyzed by the fatty acid amide hydrolase (FAAH), an integral membrane serine hydrolase, to form arachidonic acid and ethanolamine (75, 76). Former studies demonstrated that FAAH hydrolyzed various N-acylethanolamines with a higher reactivity toward AEA and that FAAH was ubiquitously present in various tissues, including the kidney (75, 77, 78). Biochemical analysis revealed a higher relative FAAH activity in renal cortex than medulla, which could explain the high content of AEA in renal medulla (79). In addition to FAAH, other enzymes could also be involved in the degradation of AEA, such as cyclooxygenase type 2 (COX-2), lipoxygenases and cytochrome P450s. All these enzymes are expressed in the kidney (56, 80–84).

As for the biosynthesis of AEA, multiple pathways are involved in the biosynthesis of 2-AG (64, 85, 86) (**Figure 1C**). 2-arachidonoyl-phosphatidylinositol 4,5-bisphosphate (PIP2) is the main precursor of 2-AG. PIP2 is hydrolyzed by phospholipase C to form 2-arachidonoyl-diacylglycerol, which is further deacylated

TABLE 1 | CB1R expression within healthy kidneys.

Structure	Species	Technical approaches	References
Whole kidney	Human	RT-PCR, IHC, WB	(27, 47, 51)
	Rats	RT-PCR, WB	(47, 48)
	Mice	RT-PCR, WB	(13, 44)
Endothelial cells	Human	IHC*	(12)
	Rats	RT-PCR, IHC#	(26, 43, 45)
Glomerular cells	Rats	RT-PCR, IHC (mesangial cells)	(43, 45)
	Mice	RT-PCR, IHC	(13, 14)
		RT-PCR, IHC**/IF (podocytes)	
Tubular cells	Human	IHC (proximal and distal convoluted tubule cells; intercalated cells in the collecting ducts)	(27)
		RT-PCR, WB (HK2)	(48)
	Rats	WB (TAL tubule cells)	(46)

Mice cnr1$^{-/-}$ tissue was used to assess the specificity of IHC staining (12).

***Specificity of the antibody binging was confirmed by disappearance of the staining when antibody was preabsorbed with a 10-fold excess of control peptide (26).*

#*Two-photon microscopy revealed a greater immunostaining for CB1R in afferent arterioles compared with efferent arterioles.*

CB1R, cannabinoid receptor type 1; HK2, human proximal tubular cell line; IF, immunofluorescence; IHC, immunohistochemistry; RT-PCR, reverse transcription/polymerase chain reaction; TAL, thick ascending limb; WB, western blot.

by *sn*-1-specific diacylglycerol lipase (DAGL) to yield 2-AG. 2-AG is then hydrolyzed to arachidonic acid and glycerol (64). This reaction can be catalyzed by several enzymes, including monoacylglycerol lipase (MAGL), FAAH, lipase α-β hydrolase (ABHD) 6 and ABHD12. The relative contribution of these enzymes differs among tissues and cells. Data about the pathways involved in biosynthesis and degradation of 2-AG in the kidney are scarce. To our knowledge, only Sampaio et al. demonstrated by RT-PCR that DAGL and MAGL were expressed in LLC-PK1 cells (74) (**Figure 1C**). Other endocannabinoids include N-acyl dopamine and 2-arachidonyl glyceryl ether, both of which binding strongly CB1R (87, 88). In addition to lipid compounds, some endogenous peptides could also bind to cannabinoid receptors. Recent studies found that hemopressin (HP), a peptide produced by the cleavage of the α1 chain of hemoglobin, behaved as an inverse agonist of CB1R (89, 90).

Relationship Between Ligands and the Cannabinoid Receptors

AEA and 2-AG bind both CB1R and CB2R, although with different affinities. Both ligands exhibit much higher affinity for CB1R than for CB2R: Ki of AEA were respectively 61-565 nM for CB1R *versus* 279-1940 nM for CB2R and Ki of 2-AG were respectively 58-472 nM for CB1R *versus* 145-1400 nM for CB2R (43, 54, 91). Numerous studies demonstrated that endogenous ligands also acted through non-cannabinoid receptors, the most studied being the transient receptor potential vanilloid type I channels (TRPV1) (92–100). TRPV1 belong to the transient receptor potential cation channel receptor family. Its activation was found to elicit renoprotection in rodent models of acute kidney injury (AKI) following ischemia/reperfusion (101–104). AEA could activate TRPV1, even though with a 20-fold lower affinity than CB1R (92, 95, 99). Experimental studies also demonstrated than AEA directly inhibited T-type calcium channels (100). Therefore, ECS is a complex system with numerous ligands lacking specificity and precisely decipher its role within the kidney remains challenging. However, as stated earlier, not only CB1R is the main cannabinoid receptor within the kidney, but also kidneys are enriched in AEA and in enzymes involved in its metabolism. As AEA had a better affinity for CB1R than for other receptors, many groups studying the ECS within the kidney used AEA infusion to explore the role of CB1R, although non-canonical effects of AEA may blur the results. Indeed, an effect of AEA through non-CB1R, such as TRPV1, could not be completely excluded. Many synthetic ligands were also developed to clarify the role of the ECS. These ligands behave as CB1R agonist or antagonist and exhibit various affinities for the cannabinoid receptors. Pharmacological properties of both endogenous and exogenous ligands that we cite in the present review, are summarized in **Table 2**. The most-studied pharmacological ligand is SR141716A (rimonabant), a CB1R inverse agonist which belongs to the first generation of CB1R antagonists. Rimonabant was briefly approved for clinical use but it was withdrawn from the market in 2007 due to severe central nervous side effects, mainly depression (29–36). Pharmacological studies demonstrated that rimonabant bound CB1R with much higher affinity than CB2R, Ki being respectively 1.98-12.3 nM for CB1R and 702-13200 nM for CB2R (108). However, like endogenous ligands, exogenous ligands also exert non-canonical effect and bound non-cannabinoid receptor as TRPV1 (93). However, directly targeting the endocannabinoid receptors seems the better approach to explore the therapeutic potential of the ECS since endogenous ligands are synthesized on-demand from arachidonic acid containing phospholipids through numerous pathways and bind both canonical than non-canonical receptors.

The Vascular Functions of the Endocannabinoid System Within the Kidney

In the normal kidney, CB1R expression is more prominent in the vasculature where it contributes to the regulation of renal

TABLE 2 | Affinity of ligands for CB1R and CB2R.

Ligands	Ki for CB1R (nM)	Ki for CB2R (nM)	References
Endogenous ligands			
2-arachidonolyglycerol	58.3-472	145-1400	(43, 54, 91)
Anandamide	61-543	279-1940	(43, 54, 91)
Exogenous ligands			
MRI-1867[#]	1.2-8.0	>1000	(105)
WIN55,212-2	1.89-123	0.28-16.2	(106, 107)
SR141716A (rimonabant)	1.98-12.3	702-13200	(108)
JD5037	2	>1000	(109, 110)
AM6545	3.3	624	(38, 111)
Δ9-THC	5.05-80.3	3.13-75.3	(107)
AM251	7.49	2290	(112, 113)
(S)-SLV 319 (ibipinabant)	7.8	7.9	(114)
AM281	12	4200	(115)
(R)-Methanandamide	17.9-28.3	815-868	(116)
SR144528	400	0.6	(117)
AM1241	580	7	(118)
JWH-133	677	3.4	(119)
AM630	5152	31.2	(120)

CB1R, cannabinoid receptor type 1; CB2R, cannabinoid receptor type 2; Ki, inhibition constant; THC, tetrahydrocannabinol.
[#]*MRI-1867 is a dual CB1R/inducible NOS antagonist.*

hemodynamic (12, 26, 43). Deutsch et al. demonstrated that renal endothelial cells express mRNA for CB1R, and selectively bind AEA (43). In rats, AEA injection induced afferent arterioles vasodilation through CB1R binding by increasing nitrogen monoxide (NO) production since this response was inhibited by SR141716A (43) (**Figure 2**). The arteriolar responses to AEA were also assessed *ex vivo* by the blood-perfused juxtamedullary nephron technique (26). Whereas high doses of AEA significantly increased the diameter of both afferent and efferent arterioles, lower doses were responsible for predominant efferent arteriolar dilation, inducing a fall in glomerular filtration rate (GFR). In this model, selective antagonism of CB1R by AM251 (**Table 2**) attenuated the afferent arteriolar response to AEA and inhibited the efferent arteriolar response to AEA. Conversely, AM281, a nonselective antagonist of cannabinoid receptors inhibited responses to AEA in both afferent and efferent arterioles. Taken together, these results suggest that efferent arterioles are more sensitive to AEA and that AEA causes greater efferent arteriolar dilation *via* CB1R, which is consistent with a decreased hydrostatic pressure in glomerular capillaries and therefore a decrease in GFR (26, 56). However, the best way to fully decipher the vascular role of CB1R within the kidney would be to study endothelial cells specific CB1R knockout rodents. To our knowledge, such studies are lacking nowadays. As both endogenous and pharmacological ligands may also act through non-cannabinoid receptors, afferent arteriolar dilatation by high doses of AEA could therefore be amplified by non-cannabinoid effect of AEA. In CKD, the increase of capillary pressure participates to nephron reduction. Thus, the decrease of capillary pressure possibly mediated by CB1R activation could be beneficial in CKD. Conversely, an acute drop of capillary pressure could be damaging in normal kidneys, similarly to what is observed during RAS inhibition. Therefore, a better understanding of the vascular effects of CB1R and of its inhibition is mandatory if CB1R inhibition become a therapeutic target in CKD.

The Role of the Endocannabinoid System in Tubular Cells

The ECS could also be involved in tubular functions, especially in the regulation of water and sodium balance (**Figure 2**). Thus, it could offer therapeutic perspectives in the management of hypertension (HTN) or conversely promote sodium retention. Former studies investigating the renal effects of marijuana shown that Δ9-THC, binding both CB1R and CB2R with similar affinity (**Table 2**), increased urine production (121, 122). This effect was first thought to be centrally mediated (123–126). Indeed, both AEA and 2-AG, released from the hypothalamus, act directly on the hypophysis to modulate glutaminergic and GABAergic pathways that induce hormone release such as vasopressin (125, 126). However, recent data shown that a peripheral modulation could also be involved (46, 79, 127–129). As mentioned above, several studies confirmed that tubular cells express the main components of ECS, including CB1R and possibly CB2R as well as the enzymes for biosynthesis and degradation of their ligands (74, 130). However, not only the physiological role of CB1R in tubular cells, but also the mechanism responsible for an aquaretic and/or diuretic effect of ECS, remain controversial. In rat kidneys, the effect of oral THC administration on urine and Na^+ output was comparable to the thiazide diuretic, hydrochlorothiazide (122). More recently, Ritter et al. reported a natriuretic effect of AEA (79, 130). They found that AEA infusion into the mouse renal medulla increased both sodium excretion and urine output (79, 130). The authors

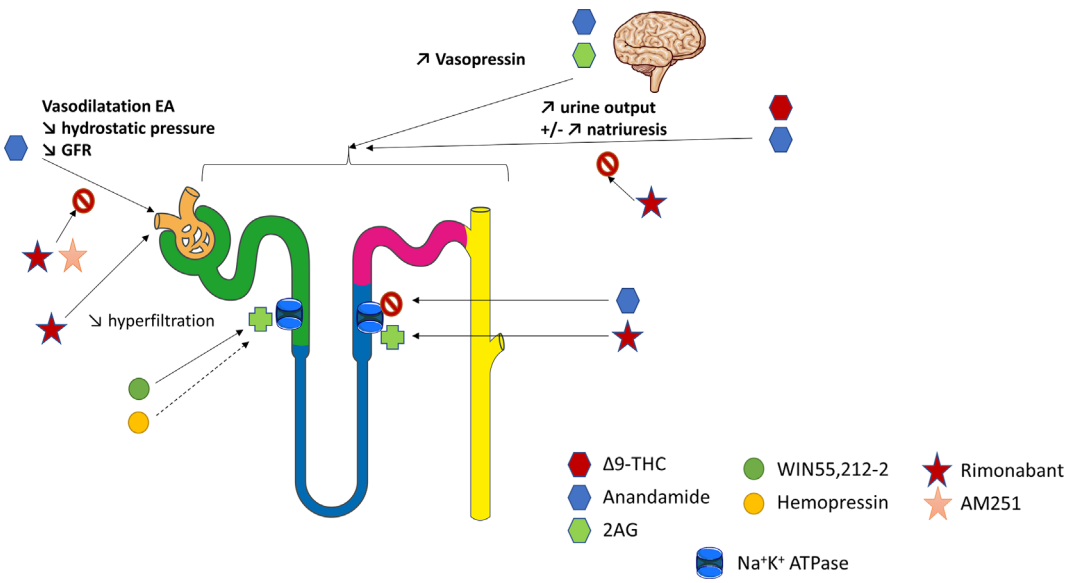

FIGURE 2 | The physiological role of CB1R within the kidney. Overview of the current knowledge of the role of CB1R in renal vasculature, tubular cells and blood pressure regulation. The figure has taken intp account both *in vitro* and *in vivo* experiments. 2AG, 2-arachidonoyl-glycerol; EA, efferent arterioles; GFR, glomerular filtration rate; THC, tetrahydrocannabinol.

initially reported that a non-canonical CB receptor-based mechanism could be involved (79). Indeed, celecoxib, a selective COX-2 inhibitor, blocked the effects of AEA. Therefore, the action of AEA could be mediated indirectly by its COX-2 metabolite, prostamide E2. Their hypothesis was reinforced by the fact that infusion of prostamide E2 mimicked the effects of AEA in stimulating natriuresis and diuresis in mice. More recently, Ritter et al. found that rimonabant also blocked the effects of AEA, suggesting a CB1R-dependent effect of intramedullary AEA on flow rate and natriuresis (130). Using *ex vivo* experiments on isolated mouse kidney thick ascending limb (TAL) tubule cells, they demonstrated an inhibitory effect of AEA on the Na^+/K^+-ATPase activity (130). Another team measured oxygen consumption in rat TAL suspensions to monitor the effects of AEA (46). AEA reduced oxygen consumption in a concentration-dependent manner. WIN55,212-2 (WIN), a non-selective lipid cannabinoid agonist binding both CB1R and CB2R with a better affinity for CB2R (**Table 2**), also reduced oxygen consumption whereas rimonabant blocked the effect of AEA. Conversely, both the CB2R-selective agonist JHW-133 and the CB2R antagonist AM630 were ineffective (**Table 2**). The authors concluded that AEA inhibited TAL Na transport-related oxygen consumption by activating CB1R and NO stimulation, which, in turn, could block apical transporters and therefore be associated with a natriuretic effect (**Figure 2**).

Conversly, other authors found that ECS was involved in sodium retention (26, 128). In rats, intramedullary infusion of methanadamide (**Table 2**), a stable analogue of AEA, increased diuresis without any effect on natriuresis (128). Similarly, intrarenal AEA injection did not alter urinary sodium excretion (26). These results were enhanced by an *in vitro* study on LLC-PK1 cells in which the authors have shown that ECS could decrease renal sodium reabsorption by directly stimulating Na^+/K^+-ATPase (74). WIN was responsible for an early and sustained increase of Na^+/K^+-ATPase activity whereas HP displayed a biphasic effect leading to an early significant increase in the Na^+/K^+-ATPase activity and acting as a potent inhibitor of the pump after 15 min. The CB1R antagonist AM251 (**Table 2**) was used to demonstrated that the fast effect of both WIN and HP on Na^+/K^+-ATPase activity was independent of CB1R whereas the slow one was dependent on CB1R. Different signaling pathways were stimulated by WIN and HP, respectively protein kinase C (PKC) and protein kinase A (PKA), resulting in different effects on the Na^+/K^+-ATPase (74). Sampaio et al. also studied the role of ECS in an acute model of experimental ischemia reperfusion, both *in vivo* in Wistar rats and *in vitro* using antimycin A treatment on LLC-PK1 epithelial cells (131). Antimycin A induced ATP depletion and was therefore used as an *in vitro* model of ischemia reperfusion. The authors demonstrated that the ECS and Na^+/K^+ ATPase were downregulated 24 hours after the ischemia reperfusion. *In vitro*, they found that the downregulation of Na^+/K^+ ATPase could be reversed by WIN in a CB1R dependent manner (131).

Thus, the role of ECS in tubular cells within the kidney remains largely unknown. Either CB1R may promote natriuresis and its therapeutic inhibition will give HTN and sodium retention (like endothelin-1 antagonists), or conversely CB1R inhibition could cause natriuresis and be beneficial in HTN treatment. Effects of AEA may depend on dose, route of injection, cells (TAL *versus* renal proximal tubular epithelial cells, RPTC). Different pathways and sodium channels are involved in sodium reabsorption throughout the various tubular segments within the nephron and AEA may act through different mechanisms. Once again, as not only endogenous ligands but also pharmacological compounds could exert CB1R-independent effects, the best way to fully decipher the tubular role of CB1R within the kidney would be to study tubular-specific CB1R knockout rodents. As explained earlier for CB1R and vascular function, a better understanding of CB1R role in water and sodium balance is paramount if CB1R become a new therapeutic tool in CKD.

The Role of the Endocannabinoid System on Blood Pressure Regulation

Both the vascular functions of the endocannabinoid system within the kidney and the role of the ECS in tubular cells highly suggest an involvement of the ECS on blood pressure (BP) regulation, which represents a major factor in the management of CKD progression (1) (**Figure 2**). Former studies demonstrated that chronic use of Δ9-THC not only increased urine production, but also decreased heart rate and BP, both in human and animals (132–135). The same effect was observed in rats after intravenous perfusion of Δ9-THC (133, 136). A triphasic response was described involving a vagal-mediated fall in blood pressure (phase I), followed by a brief pressor effect (phase II) and finally a prolonged hypotensive effect (phase III) (136–138). Multiple mechanisms seemed to be involved, both central, mainly through cardiovascular centers of brainstem and hypothalamus, and peripheral (58). Central mechanisms of BP regulation by the ECS were deciphered first. Several studies demonstrated that activation of CB1R was responsible for a decrease of sympathetic activity leading to neurologic-mediated hypotension (132, 133, 136, 139). AEA could modulate the baroreflex through activation of CB1R within the nucleus tractus solitarius, possibly by modulating effectiveness of GABA and/or glutamate neurotransmission (140). CB1R also participates to the pressor effect of angiotensin II (Ang II) infusion in the brain (58, 141).

Next, peripheral mechanisms of BP regulation by the ECS were also identified. Cardiac activation of CB1R exerted negative chronotropic and inotropic effects independently of the central nervous system, therefore promoting a decrease in BP (58, 142). In addition to direct cardiodepressant effects, studies highlighted a vasodilatory effect of the ECS ligands in aorta and coronary arteries, through both CB1R-dependent and independent pathways that could also participate to a decrease in BP (143–148). Interestingly, *Cnr1*$^{-/-}$ mice have the same baseline BP than their wild-type littermates (21, 149). Treatment of normotensive rats and mice with the CB1R antagonist SR141716A alone has also little effect on BP (133, 136), as well as inhibition of AEA transport (150). Taken together, these results suggest that, under baseline conditions, CB1R is not tonically active in vessels and do not participate to

baseline BP regulation (56). Conversely, effects of the ECS through CB1R modulation were observed in 3 experimental models of hypertension (HTN): the spontaneously hypertensive rat (SHR) model, the Dahl salt-sensitive and salt-resistant rat model and chronic Ang II infusion- induced hypertension in Sprague Dawley rats (151–153). Once again, BP of the normotensive control rats was not altered by either CB1R agonists (Δ9-THC, AEA) or CB1R antagonists (SR141716A, AM251) which were used in these studies (151–153). Bátkai et al. demonstrated the involvement of CB1R in BP regulation: the CB1R antagonists SR141716A and AM251 aggravated HTN in SHR whereas URB597, an inhibitor of FAAH, reduced BP in SHR and in the chronic Ang II infusion induced HTN (151). Conversely, in SHR, not only the CB2R antagonist SR144528 (**Table 2**) did not affect BP, but also the TRPV1 antagonist capsazepine did not alter BP decrease by AEA intra-venous administration (151). The mechanisms of BP reduction in these models were attributed to the effects of endocannabinoids on cardiac contractility and vascular resistance. Endocannabinoids tonically suppressed cardiac contractility in hypertension and could normalize blood pressure by enhancing the CB1R-mediated cardiopressor and vasodilator effects of endogenous AEA (151). Furthermore, an interaction between the RAS and the ECS was identified in rodent peripheral arterioles. Ang II stimulated vascular endocannabinoid formation, which attenuated its vasoconstrictor effect, suggesting that endocannabinoid release from the vascular wall and CB1R activation reduced the vasoconstrictor and hypertensive effects of Ang II (154, 155).

CB1R may also modulate the Ang II pressor effect by its action on sodium and water balance. As described earlier, CB1R may be involved in the regulation of salt and water balance (46, 79, 122, 127–130). Furthermore, Ang II type 1 receptors (AT1) are largely expressed within the kidney. A recent review highlighted that the juxtaglomerular and tubulo-interstitial areas were conserved expression sites for AT1 across species and could represent the main target sites for Ang II in adult human and rodent kidneys (156). In addition, as demonstrated using tissue-specific AT1a knockout mice (157), AT1 receptors within the kidney (and not in the heart) promote the pressor response to Ang II. Several studies found interactions between AT1 and the ECS in numerous organs and systems (58, 141, 154, 155). On the molecular basis, the signal integration between CB1R and AT1 could be due to AT1R–CB1R heteromerization (15, 158). AT1R–CB1R heteromerization was first demonstrated in Neuro2A cells, a neuroblastoma cell line that contains endogenous CB1R (158). In hepatic stellate cells from ethanol-administered rats in which CB1R is upregulated, Rozenfeld et al. found a significant upregulation of AT1R–CB1R heteromers and enhancement of angiotensin II-mediated signaling. Moreover, CB1R inhibition by rimonabant prevented angiotensin II-mediated mitogenic signaling and profibrogenic gene expression (158). Therefore, the interaction with CB1R could confer new signaling properties to AT1R and enhanced responsiveness to Ang II. Jourdan et al. found that losartan, an AT1R antagonist, attenuated diabetic nephropathy in rats by downregulating the expression of CB1R in podocytes, reinforcing this hypothesis (15). Thus, a role for the ECS and CB1R within the kidney in the physiopathology of HTN cannot be excluded and it deserves to be studied, especially because a large proportion of CKD patient, who could be treated by CB1R antagonists, may suffer from HTN.

THE ROLE OF CANNABINOID RECEPTORS IN CHRONIC KIDNEY DISEASES AND RENAL FIBROSIS

CB1R, and Not CB2R, Is the Main Actor of the Endocannabinoid System Promoting Renal Fibrosis

Fibrogenesis is a multifactorial process leading to excessive extracellular matrix deposition, regardless of the organ and cause. Several studies demonstrated that endocannabinoids acting *via* CB1R promote fibrosis and that CB1R blockage reduces fibrogenesis in many organs including the liver (159–161), the heart (162), the lungs (163, 164) and the skin (165). As for the kidneys, CB1R is also an active direct player in renal fibrogenesis. This was first suggested by a microarray analysis performed by our team (12). Using the unilateral ureteral obstruction (UUO) experimental model, a rapid and reproducible model of renal fibrosis in mice, we compared the gene expression profile of fibrotic kidneys with contralateral undamaged kidneys by microarrays analysis (12). As expected, we found many overexpressed genes well-known to be involved in renal fibrosis, such as *tgfb, mmps* and *timp.* Remarkably, *Cnr1* was among the ten most significantly upregulated genes during renal fibrosis (12). In addition, a growing body of evidence highlighted that CB1R could also be a new therapeutic target to prevent renal fibrosis in both metabolic and non-metabolic diseases, as we will discuss next in our review (11–18). Conversely, the role of CB2R during renal fibrosis remains largely unknown, studies being scarce and contradictory. Several studies elicited that CB2R blockage promoted fibrosis in the liver (166), the heart (167) and the skin (168), whereas CB2R agonists decreased fibrogenesis (166–168). Surprisingly, Zhou et al. found that CB2R overexpression could promote renal fibrosis (169, 170). We and others found opposite results with a protective effect of CB2R activation in various models of CKD (12, 53, 171). During UUO, we demonstrated that CB2R antagonist alone (SR144528) increased the development of renal fibrosis whereas CB2R agonist (JWH 133) alone blunted it (12). However, neither SR144528 nor JWH133 further reduced the development of fibrosis when compare with CB1R pharmacological blockade by rimonabant (12). Barutta et al. demonstrated that CB2R was downregulated in kidney biopsies from patients with advanced diabetic nephropathy (53). They also found that AM1241, a CB2R agonist (**Table 2**), ameliorated albuminuria, podocyte protein downregulation, and glomerular monocyte infiltration, without affecting early markers of fibrosis. In addition, AM1241 reduced CC chemokine receptor 2 (CCR2) expression in both renal cortex and cultured podocytes, suggesting that CB2R activation may interfere with the deleterious effects of monocyte chemoattractant protein-1 (MCP-1) signaling (53). In streptozotocin (STZ)-induced diabetic mice, the genetic

deletion of CB2R exacerbated the downregulation of podocin and nephrin, mesangial expansion, monocyte infiltration, and reduced renal function overexpression of extracellular matrix. The deletion of CB2R also enhanced the expression of fibrosis marker (171). Given the contradictory results of the various studies, the exact role of CB2R in renal fibrosis needs to be further explored. To date, there is no ongoing clinical study investigating the effect of CB2R on renal function. We will therefore focus on the therapeutic potential of CB1R inhibition in renal fibrosis.

CB1R Promotes Renal Fibrosis Associated With Metabolic Disorders

The role of CB1R in renal fibrosis was first documented during diabetes and metabolic syndrome (13, 15–18, 45) (**Figure 3**). We and others demonstrated that renal CB1R expression was increased in diabetic nephropathy, both in humans (12, 45) and rodents (14, 15, 17, 18). Numerous studies demonstrated that CB1R blockade improves multiple parameters involved in metabolic syndrome and diabetes, such as waist circumference, glycemia, glycated hemoglobin, HDL and LDL cholesterol and triglycerides (13, 29, 30, 45). Both global genetic CB1R inactivation and pharmacological blockade by first generation CB1R antagonists dramatically improves metabolic parameters (13, 14, 45) (**Figure 3**). Indeed, CB1R inhibition may protect the kidney from metabolic induced fibrosis and injury by improving systemic metabolic parameters. Thus, experimental studies in rodents demonstrated that rimonabant and AM251, belonging to the first generation CB1R antagonists with BBB crossing, protect the kidney from the development of albuminuria, CKD, renal fibrosis, glomerulosclerosis and renal inflammation in diabetes (13, 14, 45). However, recent studies also exhibited a role of peripheral CB1R in metabolic nephropathies. Udi et al. highlighted that CB1R regulated obesity-induced CKD by acting on RPTC (16). Indeed, specific deletion of CB1R in RPTC (RPTC-CB1R$^{-/-}$) did not prevent obesity in mice, but significantly reduced the obesity-induced lipid accumulation in the kidney as well as renal dysfunction, urinary albumin-to-creatinine ratio (ACR), inflammation, and renal fibrosis. Deciphering the pathways involved in this experimental model, the authors found that CB1R acted through the $G\alpha_{i/0}$-PKA axis. Therefore, CB1R blockade mediated the downstream activation of the tumor suppressor liver kinase B1 (LKB1), which modulates AMP-activated kinase (AMPK) activity by inducing its phosphorylation. AMPK inactivates acetyl-CoA carboxylase (ACC) by its phosphorylation, increasing fatty β-oxidation in renal proximal tubules. *In vitro*, CB1R blockade by JD5037 increased fatty acid β-oxidation in proximal tubular cells and protected the kidney from obesity-induced fibrosis.

Similarly, the peripheral role of CB1R during renal fibrosis in diabetic nephropathy was recently documented. The first data supporting a peripheral role of CB1R in diabetic nephropathy and an antifibrotic effect independent from the improvement of systemic metabolic parameters were reported by Janiak et al. (45). Rimonabant, the most-known first generation CB1R antagonists crossing BBB, significantly reduced body weight, blood glucose and improved lipid profile in rodents (14, 45). Comparing obese Zucker rats receiving either rimonabant or vehicle for 12 months to pair-fed but untreated group of obese rats, Janiak et al. demonstrated that rimonabant significantly reduced proteinuria and renal failure as well as tubulo-interstitial

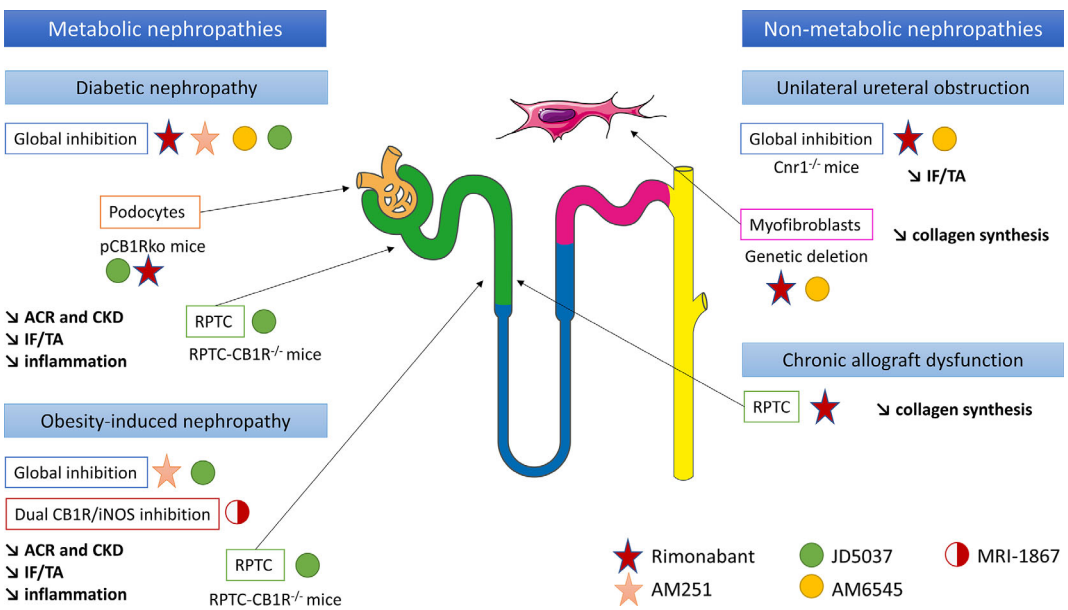

FIGURE 3 | CB1R inhibition could be a new therapeutic target in chronic kidney diseases and renal fibrosis, in both metabolic and non-metabolic nephropathies. Both *in vitro* and *in vivo* experiments have been taken into account. ACR, urinary albumin-to-creatinine ratio; CB1R, cannabinoid receptor type 1; CKD, chronic kidney disease; IF/TA, interstitial fibrosis and tubular atrophy; iNOS, inducible nitric oxide synthase; pCB1Rko, podocyte-specific deletion of CB1R; RPTC, renal proximal tubular cells.

fibrosis and glomerulosclerosis compared to per-fed animals (45). In addition, in cultured podocytes *in vitro*, high glucose stimulation increased CB1R expression whereas rimonabant abolished high-glucose-induced up-regulation of collagen and plasminogen activator inhibitor-1 synthesis, also suggesting that CB1R blockade protected against renal injury through both metabolic and antifibrotic effects in type 2 diabetic nephropathy (14). A study of Barutta et al. reinforced this hypothesis (13). Whereas CB1R blockade by AM251 in STZ-induced diabetic mice had no effect on body weight, blood glucose and BP, it significantly reduced ACR and prevented down-regulation of nephrin, podocin and zonula occludens 1 (ZO-1). Because histological lesions, including renal fibrosis, were very mild, a possible beneficial effect of CB1R blockade preventing overt lesions in diabetic nephropathy could not be demonstrated (13).

The same results were observed using JD5037 and AM6545, peripherally restricted CB1R antagonists that did not cross BBB. Whereas JD5037 and AM6545 were quite ineffective in controlling blood glucose and glycated hemoglobin levels (13, 15, 17, 18), they significantly improved renal parameters, especially renal function, ACR and renal inflammation (13–15, 17, 18, 39, 40, 45) in Zucker diabetic fatty (ZDF) rats (13, 15), STZ-induced diabetic mice and Akita diabetic mice (17). Several pathways seem to be involved. In ZDF rats, Barutta et al. found that JD5037 improved glomerular filtration and reversed albuminuria, activation of the RAS, oxidative/nitrative stress and podocyte loss (13). However, no evidence of chronicity (glomerulosclerosis, interstitial fibrosis or tubular atrophy) was observed at the point (90 days) at which the cohort was sampled for histopathological analysis, preventing any conclusion regarding a decrease in overt renal diabetic lesions (13). Thus, these results suggest that improvement mainly depends on renal hemodynamic in this model. Another work published by Jourdan et al. in ZDF rats reinforces this hypothesis (15). Indeed, not only the authors demonstrated that CB1R signaling in podocytes increased RAS activity, which was down-regulated by CB1R blockade, but also they found a direct link between AT1 activation and CB1R expression *in vivo* in ZDF rats (15). In podocytes *in vitro*, they demonstrated a cross talk between reactive oxygen species, angiotensin II and slit-diaphragm protein expression such as nephrin (15). In STZ-induced diabetic nephropathy, some authors extended the delay before analysis to allow time for renal lesions, especially renal fibrosis, to develop (18, 39, 40). After 14 weeks, Barutta et al. found that AM6545 reduced mesangial expression, upregulation of glomerular fibronectin and collagen deposition in both the mesangial and the interstitial area (39). AM6545 also significantly reduced down-regulation of nephrin and podocin (40) and prevented diabetic-induced downregulation of markers of anti-inflammatory M2 macrophages which play a key role in resolving inflammation and promoting repair (39, 40). To precisely assess the role of CB1R podocyte expression in diabetic nephropathy, Jourdan et al. reported a mouse model with podocyte-specific deletion of CB1R (pCB1Rko) STZ-induced diabetic nephropathy (18). Whereas hyperglycemia was similar in both pCB1Rko and their wild-type littermate, pCB1Rko mice exhibited less ACR, podocytes loss, tubular dysfunction and interstitial fibrosis. These parameters were mediated in part by podocyte-derived endocannabinoids acting *via* CB1R on proximal tubular cells (18). In addition, the role of CB1R expression in RPTCs in diabetic nephropathy was studied by Hinden et al. in STZ-induced diabetic mice using RPTC-specific deletion of CB1 (RPTC-CB1R$^{-/-}$) (17). RPTC-CB1R$^{-/-}$ mice developed only mild diabetes manifesting by modest hyperglycemia and were almost completely protected from the development of diabetic nephropathy compared to their littermate counterparts, with a better renal function and a decrease of ACR, kidney inflammation and tubule interstitial fibrosis. These effects were due to a downregulation of glucose transporter 2 (GLUT2) expression in RPTC. Indeed, GLUT2, localized in RPTCs, affects the basolateral efflux of glucose from the tubular cell back to the circulation (172, 173). Its expression is increased in humans with diabetes and in murine models of diabetes (174–177). Hyperglycemia increases tubular GLUT2 expression and shifts its localization from basolateral membrane to the apical/brush border membrane, contributing to increase glucose reabsorption (174, 178). CB1R blockade was related with the disruption of glucose-induced Ca^{2+}-dependent PKC -β1 activation which, in turn, modulated GLUT2 translocation and/or expression in RPTC, through the $G_{q/11}$ signaling. *In vitro*, CB1R stimulation mimics these effects whereas blockade of CB1R by JD5037 blunts these effects (18).

Thus, all these studies documented that the beneficial effect of CB1R blockade was not only due to its role on metabolic parameters, but also through a direct action on renal cells (podocytes and RPTCs) independently of metabolism. These studies also demonstrated that different cellular pathways are involved in renal fibrogenesis during metabolic nephropathies.

CB1R Activation Promotes Non-Metabolic Renal Fibrosis

So far, only our group studied the role of CB1R in an experimental model of non-metabolic renal fibrosis *in vivo* in mice (12) (**Figure 3**). Whereas CB1R expression was low in normal kidneys, it was increased both in metabolic (diabetic nephropathy) and non-metabolic (acute interstitial nephritis, IgA nephropathy) nephropathies (12). Furthermore, there was a significant negative correlation between CB1R expression and kidney function. In mice in the UUO model, both the pharmacological blockade by rimonabant or by AM6545 and the genetic disruption of CB1R profoundly reduced the development of renal fibrosis. This effect was mainly due to a direct paracrine/autocrine role of CB1R in myofibroblasts, which are the final effector cells in renal fibrogenesis. We also found that upon transforming growth factor β (TGFβ) stimulation, renal myofibroblasts expressed CB1R and secreted endocannabinoid ligands, whereas CB1R blockade reduced collagen synthesis (12). During chronic kidney allograft dysfunction (CAD) in humans, we found that CB1R was highly expressed in tubular cells (28). CAD remains the first cause of graft loss (179) and corresponds to the progressive and inevitable impairment of renal graft function. It is a multifactorial and complex process, in which a lot of immunological and non-immunological causes are involved (180–184). Recently,

antibody-mediated rejection as emerged as one of the major causes of CAD (184–186). During CAD, the functional renal tissue is replaced by extracellular matrix proteins, mainly collagens, leading to both interstitial fibrosis and tubular atrophy. Other histological damages associated glomerulosclerosis and vascular intimal hyperplasia (187). We highlighted that CB1R expression significantly increased in the first three months after kidney transplantation, and it remained stable thereafter. CB1R expression in preimplantation kidney graft biopsies was higher than CB1 expression we previously found in normal kidneys (23% ± 15% *versus* 6.5 ± 4.8%) and was not correlated with renal fibrosis at this particular time-point (12, 28). Therefore, we hypothesized that the high level of CB1R expression in preimplantation biopsies could be a consequence of cold ischemia-induced acute tubular necrosis and could be predictive for the development of further renal graft fibrosis. Next, we found a significant positive correlation between CB1R expression and renal graft fibrosis at 3 months post-transplantation (28) (**Figure 3**). Moreover, patients with stable renal fibrosis during the first-year post-transplantation tended to have lower increase in CB1R expression than patients in whom renal fibrosis increased. Thus, CB1R could promote the early steps of the development of CAD or at least be a marker of renal fibrosis. *In vitro*, we found that an anticalcineurin treatment by tacrolimus significantly induced mRNA and protein expression of CB1R, concomitantly to collagen up-regulation. Administration of rimonabant blunted collagen synthesis. The impact of cannabinoid system modulation during CAD as well as the cellular and molecular pathways involved, remains to be clarified.

Overall, we found evidence of a profibrotic role of CB1R in experimental mice models, human renal biopsies and *in vitro* studies. However, the respective contribution of tubules and myofibroblasts remains to decipher. In the UUO model, we found that whereas CB1 expression was drastically increased in the tubules, interstitium and glomeruli, CB1R blockade significantly reduced the increase of renal fibrosis through a direct paracrine/autocrine role of CB1R in myofibroblasts with no strong evidence for a direct role of CB1R expressed in tubules (12). During CAD, we found that CB1R was highly expressed in tubular cells and that CB1R blockade reduced collagen synthesis by tubular cells. Thus, identifying the respective role of CB1R in tubules and myofibroblasts during non-metabolic renal fibrosis remains to be established. The best way to fully answer the question would be to study cell-specific CB1R knockout rodents. The cellular pathways involved in non-metabolic renal fibrosis mediated by CB1R also remain to be elucidated.

CB1R INHIBITION: A NEW PROMISING THERAPEUTIC TARGET IN CHRONIC KIDNEY DISEASES

As presented earlier, experimental data highlighted the therapeutic potential of CB1R blockade in renal fibrosis and CKD, regardless of the cause (metabolic or not) (12, 13, 15–18, 45). Therefore, there is a growing interest for the industry to develop CB1R inhibitors with no BBB passage to improve tolerance compared with rimonabant (188).

The First Generation CB1R Antagonists: Rimonabant

Rimonabant was the only CB1R antagonist approved for clinical use in obesity. However, clinical trials with rimonabant mainly focused on metabolic syndrome and patients with CKD were excluded from these studies (29–36). The RIALTO study (NCT00458081) aimed to assess the effect of rimonabant on microalbuminuria in patients with metabolic syndrome. Assessment of glomerular filtration rate was a secondary objective of the study. Due to central nervous system adverse events, especially an important risk of severe depression, rimonabant was withdrawn in 2008, before the end of the RIALTO study. The study was also prematurely terminated. Therefore, to date, no clinical trial investigated the effect of CB1R inhibition on CKD course in diabetes and obesity.

The Second Generation CB1R Antagonists: Peripherally Restricted CB1R Antagonists

Multiple peripherally CB1R antagonists, without central nervous system side-effects, were developed these past 10 years (37). As discussed earlier in the present review, experimental studies demonstrated that some molecules, such as AM6545 or JD5037, could be useful to prevent renal fibrosis and therefore be good candidates to be part of the therapeutic tools against CKD (12, 16, 18, 37–39). AM6545 is a non-brain-penetrant neutral CB1R antagonist, based on the rimonabant template but with an alkynyl chain off the 4-aryl group. It retains high affinity and selectivity for CB1R with Ki of 3.3 nM for CB1R, which is similar to Ki of rimonabant (38). Importantly, AM6545 displays markedly reduced brain penetrance (38, 111). AM6545 significantly reduced diabetic-induced albuminuria in diabetic mice (39, 40) as well as collagen deposition in both the mesangial and the interstitial area (39). Using UUO, a non-metabolic experimental model of renal fibrosis, we demonstrated a 25 to 60% reduction of renal fibrosis in AM6545 compared to vehicle treated mice.

While the rimonabant template was the most common choice for developing peripherally restricted CB1R antagonists, the Jenrin group developed JD5037 from ibipinabant, another first generation CB1R antagonist (109, 189). It was found that modifications of the central N-methyl group of ibipinabant with polar pendants provided analogs that followed physicochemical guidelines for diminishing blood brain barrier penetration. JD5037 contained pendants of N-substituted valinamide and exhibited 15-fold greater affinity for CB1R (IC50 1.5 nM) than ibipinabant (109). Preclinical toxicity studies were performed with high doses of JD5037 in rats and dogs (respectively 150 mg/kg/d and 20-75 mg/kg/d) without observed adverse effects. Experimental studies in rodents demonstrated that JD5037 was effective in mitigating both diabetic nephropathy by blocking overactive CB1R in podocytes (15) and regulating renal GLUT2 in proximal tubular cells (18) and obesity-induced nephropathy by blocking overactive CB1R in proximal tubular cells (16). The CB1R blockade by JD5037 increases fatty acid β-oxidation in proximal tubular cells and protects the kidney from obesity-induced dysfunction and injury.

To date, data about the effect of JD5037 in non-metabolic renal fibrosis are lacking. Therefore, peripherally restricted CB1R antagonists may be good candidates for slowing CKD progression, especially during metabolic nephropathies, and experimental data are encouraging to move up forward clinical trials in the short term. JD5037 is now developed under the name of CRB-4001 (Corbus Pharma) and a Phase II study in patients with non-alcoholic steatohepatitis is underway. Recently, Liu et al. described a peripheral CB1R antagonist (MRI-1891) highly biased toward inhibiting CB1R-induced β-arrestin-2 recruitment over G-protein activation. In mice, MRI-1891 reduced food intake, body weight and obesity-induced muscle insulin resistance *via* β-arrestin-2 signaling (42).

The Third Generation CB1R Antagonists: Peripherally Restricted Dual-Target CB1R Antagonists

Recently, hybrid CB1R antagonists were designed to improve therapeutic efficacy for instance in liver fibrosis (105, 190) and in pulmonary fibrosis (164). Experimental studies demonstrated the usefulness of peripherally restricted hybrid CB1R/inducible nitric oxide synthase (iNOS) antagonists during CKD (37, 41). Indeed, both CB1R and iNOS are increased during CKD (51). iNOS overactivity was found to contribute to tubular dysfunction in obesity-induced CKD in mice (191). Interestingly, iNOS overactivity was also found to contribute to renal fibrosis in UUO, a non-metabolic experimental model of renal fibrosis (192). Udi et al. demonstrated that MRI-1867, a dual CB1R/iNOS antagonist, was more efficient than JD5037 or an iNOS inhibitor (1400W) alone to prevent obesity-induced kidney injury, inflammation, fibrosis and kidney dysfunction in mice (51). Similarly, data about the effect of MRI-1867 on non-metabolic renal fibrosis are lacking.

Another hybrid molecule could also be an activator for the secondary target, such as AMPK activator (37). Supplemental experimental data are mandatory to move forwards to therapeutics. No phase II trial is ongoing yet. Assessing safety and tolerability, especially central nervous system, would be the first stage of clinical development in human pathologies.

CB1R Blocking Antibodies

Blocking antibodies targeting CB1R is another interesting approach to prevent central nervous system side-effects induced by CB1R inhibition. The Bird Rock Bio, Inc group developed RYI-018 (nimacimab), a negative-allosteric modulating antibody targeting CB1R. Early-stage clinical development is ongoing. Clinical trials are assessing safety, tolerability and pharmacokinetics of RYI-018 in patients with non-alcoholic fatty liver disease (phase 1 study, NCT03261739) and diabetic gastroparesis (phase 2a study, NCT03900325). The Goldinch Bio, Inc group also developed GFB-024, another peripherally restricted CB1R inverse agonist monoclonal antibody, targeting patients at high risk of CKD due to severe insulin resistant patients. Phase 1 study in healthy volunteers is ongoing. To date, there is no published studies with RYI-018 nor GFB-024.

CONCLUSION

There is now a growing body of evidence for a prominent role of CB1R in a broad range of renal diseases. In metabolic syndrome, obesity and diabetes, CB1R inhibition not only improves metabolic parameters, but also exerted a direct role in the protection of renal function. Since diabetic nephropathy remains one of the main causes of CKD and ESRD and since a large proportion of CKD patients suffer from metabolic syndrome and obesity, the metabolic benefits of CB1R inhibition represents a major advantage in the therapeutic management of these patients. In addition, recent studies highlighted that CB1R also promotes renal fibrosis in non-metabolic nephropathies and that its inhibition reduced the development of renal fibrosis. Rimonabant, belonging to the first generation CB1R antagonists, was approved for clinical use but its development was stopped due to an important risk of severe depression, and it was definitively withdrawn in 2008. To date, second and third generation CB1R antagonists without central nervous side-effects are under development with encouraging experimental data on renal fibrosis prevention. Due to the weak expression of CB1R in healthy kidneys, few side effects of its peripheral blockage are expected, although, as detailed in our review, CB1R could impact GFR and sodium and water balance. There is a growing interest of the industry to develop new CB1R antagonists and some molecules are currently under early-stage clinical phases (phases I and IIa studies), paying a peculiar attention to safety and tolerability. These new pharmacological blockers of CB1R could therefore provide an additional therapeutic toolbox in the management of CKD and renal fibrosis from both metabolic and non-metabolic origin.

AUTHOR CONTRIBUTIONS

Both MD and HF authors contributed to the final version of the manuscript. HF supervised the project. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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