

REVIEW

T-Type Ca Channel Blockade as a Determinant of Kidney Protection

Koichi Hayashi, Koichiro Homma, Shu Wakino, Hirobumi Tokuyama,
Naoki Sugano, Takao Saruta and Hiroshi Itoh

Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan

(Received for publication on December 15, 2009)

(Accepted for publication on February 18, 2010)

Voltage-dependent Ca channels are classified into several subtypes based on the isoform of their $\alpha 1$ subunits. Traditional Ca channels blockers (CCBs), including nifedipine and amlodipine, act predominantly on L-type Ca channels, whereas novel CCBs such as efonidipine, benidipine and azelnidipine inhibit both L-type and T-type Ca channels. Furthermore, cilnidipine blocks L-type and N-type Ca channels. These CCBs exert divergent actions on renal microvessels. L-type CCBs preferentially dilate afferent arterioles, whereas both L-/T-type and L-/N-type CCBs potentially dilate afferent and efferent arterioles. The distinct actions of CCBs on the renal microcirculation are reflected by changes in glomerular capillary pressure and subsequent renal injury: L-type CCBs favor an increase in glomerular capillary pressure, whereas L-/T-type and L-/N-type CCBs alleviate glomerular hypertension. The renal protective action of L-/T-type CCBs is also mediated by non-hemodynamic mechanisms, i.e., inhibition of the inflammatory process and inhibition of Rho kinase and aldosterone secretion. Finally, a growing body of evidence indicates that T-type CCBs offer more beneficial action on proteinuria and renal survival rate than L-type CCBs in patients with chronic kidney disease (CKD). Similarly, in CKD patients treated with renin-angiotensin blockers, add-on therapy with N-type CCBs is more potent in reducing proteinuria than that with L-type CCBs, although no difference is found in the subgroup with diabetic nephropathy. Thus, the strategy for hypertension treatment with CCBs has entered a new era: treatment selection depends not only on blood pressure control but also on the subtypes of CCBs. (Keio J Med 59 (3) : 84–95, September 2010)

Keywords: renal microcirculation, voltage-dependent Ca channels, efonidipine, T-type calcium channels, chronic kidney disease

Introduction

Calcium channel blockers (CCBs), which target voltage-dependent Ca channels, are widely used in the field of hypertension therapy and rank second in the pharmaceutical market for hypertension treatment. Indeed, CCBs are believed to have less serious adverse effects than other antihypertensive drugs and are recognized as reliable drugs in terms of lowering blood pressure. A growing body of evidence, however, points to a problem associated with kidney function. Conventional CCBs, including nifedipine, diltiazem and amlodipine, elicit marked increases in the glomerular filtration rate and re-

nal blood flow^{1–3} by dilating afferent arterioles preferentially,^{4–8} whereby glomerular hypertension and subsequent renal injury are expected to ensue unless systemic blood pressure is sufficiently controlled.^{9–12}

Voltage-dependent Ca channels are widely distributed throughout the body and play a critical role in the maintenance of vascular tone. Ca channels are classified into several subtypes, including L-type, T-type, N-type, P/Q-type and R-type Ca channels based on their electrophysiological properties;¹³ among these subtypes, the L-type Ca channel has been most extensively investigated for elucidation of its function. The blockade of L-type Ca channels dilates the systemic vasculature and substan-

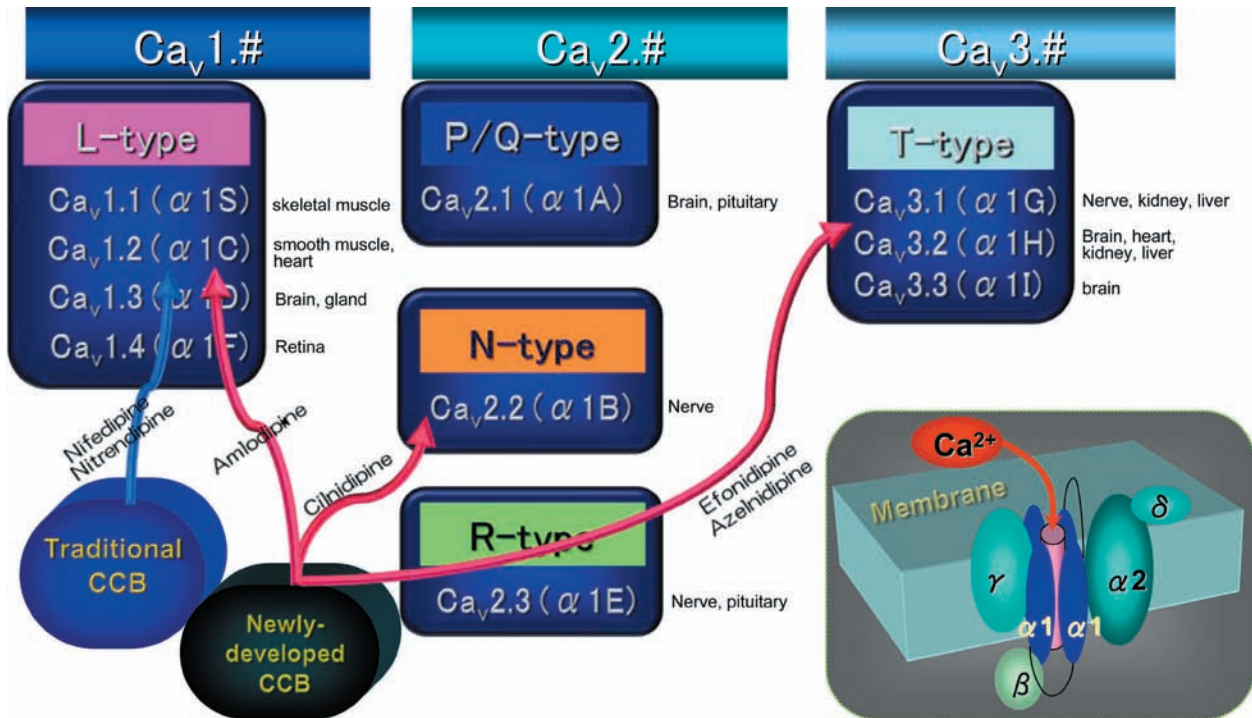


Fig. 1 Classification of voltage-dependent Ca channels. CCB; calcium channel blocker.

tially reduces blood pressure. In the renal microvasculature, however, the vasodilator response to L-type CCBs is observed only in preglomerular microvessels (e.g., afferent arterioles), whereas efferent arterioles are refractory to the dilator action of these agents.^{4–8} The renal microvascular response thus supports speculation that glomerular hypertension may develop following the administration of these agents.

In contrast, T-type Ca channels have been identified as an important molecular target in various organs, and they function as a part of physiological and/or pathophysiological activities.¹⁴ In the cardiac sinus node, T-type Ca channels participate in the generation of the pacemaker potential.¹⁵ In the kidney, several Ca channel subtypes are reported to be present, including L-type, T-type, N-type and P/Q-type Ca channels.^{16,17} Recently, much interest has been focused on the role of T-type Ca channels in the kidney. It has been demonstrated that the blockade of T-type Ca channels exerts a beneficial action on kidney function in chronic kidney disease (CKD), including reduction in proteinuria^{18–22} and improvement in kidney survival.²³ Collectively, the role of Ca channels may differ depending on the subtype expression within the kidney, and the specific blockade of Ca channel subtypes would be anticipated to exert a salutary action on the renal microvasculature.

In this article, we review the role of Ca channel subtypes in renal physiology. Furthermore, the effects of the

blockade of these Ca channels on CKD are also surveyed.

1. Ca Channel Subtypes

Voltage-dependent Ca channels are classified into L-, P/Q-, N-, R- and T-type subtypes based on their pharmacological and electrophysiological properties; they are made up of heteromeric multisubunits, including $\alpha 1$, $\alpha 2$, β , δ and γ (skeletal muscle).¹³ Among these, the $\alpha 1$ subunit possesses the main characteristics of the Ca channel, such as the ion-conducting pore, ion selectivity and voltage sensitivity, and is encoded by the CACNA1 gene family, which consists of 10 genes (**Fig. 1**). In the kidney, a number of Ca channels with various $\alpha 1$ subunits, including $\text{Ca}_v2.1$ ($\alpha 1A$), $\text{Ca}_v1.2$ ($\alpha 1C$), $\text{Ca}_v1.3$ ($\alpha 1D$), $\text{Ca}_v3.1$ ($\alpha 1G$) and $\text{Ca}_v3.2$ ($\alpha 1H$), are expressed and function as L-type ($\text{Ca}_v1.2$, $\text{Ca}_v1.3$), T-type ($\text{Ca}_v3.1$, $\text{Ca}_v3.2$) and P/Q-type ($\text{Ca}_v2.1$) Ca channels;¹³ precise or organized electrophysiological analyses, however, have not been conducted because the kidney contains divergent cell populations. Furthermore, the kidney is supplied with numerous nerve endings that possess N-type ($\alpha 1B$) Ca channels. Interestingly, P- ($\text{Ca}_v2.1a$) and Q-type ($\text{Ca}_v2.1b$) Ca channel subunits are splice variants of a single gene (i.e., CACNA1A) and are expressed in the afferent arteriole.¹⁶ Although splice variants have been demonstrated in neuronal and cardiac cells²⁴ as well as

in vascular smooth muscle cells from atherosclerotic tissues,²⁵ whether these variants affect the renal function remains undetermined.

2. Characterization of Renal Microvessels

Afferent and efferent arterioles exist adjoining the glomerulus; they adjust their vascular tone in response to various vasoactive stimuli. The fact that glomerular filtration exhibits divergent changes depending on the vasoactive stimuli applied implies that the responsiveness of afferent and efferent arterioles to these stimuli differs. For example, atrial natriuretic peptide causes afferent arteriolar dilation and efferent arteriolar constriction.^{26,27} Furthermore, elevated renal perfusion pressure²⁸, endothelin²⁹ and high K levels³⁰ elicit predominant constriction of the afferent arteriole.

It has been demonstrated that voltage-dependent Ca channels functionally prevail in the afferent arteriole.^{4,7,30-33} High K-induced membrane depolarization selectively constricts the afferent arteriole, whereas the efferent arteriole is relatively insensitive to such depolarization.^{30,32} Furthermore, Ca channel agonists (e.g., Bay K-8644), which directly activate voltage-dependent Ca channels, cause preferential afferent arteriolar constriction.³³ Carmines, *et al.*³² directly assessed the intracellular Ca concentration ($[Ca^{2+}]_i$) of isolated rabbit glomeruli with attached afferent and efferent arterioles. They demonstrated that high K-induced depolarization elevated $[Ca^{2+}]_i$ in afferent but not in efferent arterioles. They also demonstrated that the inhibition of voltage-dependent Ca channels by nifedipine completely prevented the high K-induced rise in $[Ca^{2+}]_i$.

When administered *in vivo*, CCBs, including nifedipine,^{4,34} nicardipine³⁵ and verapamil,^{4,7} cause a greater increase in glomerular filtration rate than that in renal plasma flow, resulting in an elevated filtration fraction. These observations suggest predominant action on the afferent arteriole. However, in the *in vivo* setting, systemic blood pressure is decreased, which may confound the effect of CCBs on renal arterioles. To eliminate the pressure-induced changes in vascular tone, Loutzenhiser, *et al.*^{5,36-38} used the isolated perfused rat normal kidney model. This model provides constant renal perfusion pressure, whereby the myogenic tone of renal microvessels is unaltered. In a series of the experiments, Loutzenhiser, *et al.* found that under angiotensin II- or norepinephrine-induced vasoconstrictor tone, CCBs including nifedipine, nisoldipine, diltiazem and amlodipine caused greater increases in glomerular filtration rate than those in renal plasma flow, resulting in exaggerated increases in filtration fraction.^{5,36-38} Thus, these observations again support the finding that CCBs act predominantly on the renal preglomerular vessels.

Recent advances in laboratory techniques have facilitated more detailed direct observation of the renal micro-

circulation.^{27-30,39-45} Casellas and Navar⁴³ developed an *in vitro* technique that allows direct visualization of the juxtamedullary nephron circulation. In their experiments, both verapamil and diltiazem potently inhibited afferent arteriolar vasoconstriction, whereas efferent arterioles were relatively refractory to the vasodilator action of these agents.⁴⁶ Similarly, Ito, *et al.*^{41,42} developed the isolated renal cortical microvessel model and found that nifedipine predominantly dilated the afferent arteriole.⁴⁷ Loutzenhiser and Epstein developed a model of the isolated perfused hydronephrotic kidney that facilitated direct observation of the renal microvasculature under defined *in vitro* conditions.²⁷⁻³⁰ Using this model, we demonstrated that both dihydropyridine-class (e.g., nifedipine, nicardipine and amlodipine) and benzothiazepine-class (e.g., diltiazem) CCBs reversed the angiotensin II-induced constriction of the afferent arteriole, whereas the efferent arteriole was refractory to the vasodilator action of these antagonists.^{48,49} Furthermore, using intravital pencil-lens CCD camera videomicroscopy, we observed that nifedipine caused predominant dilation of the afferent arteriole in the canine kidney (**Fig. 2A**).⁵⁰ Of note, we also have shown that nifedipine elevates the filtration fraction (i.e., glomerular filtration rate/renal plasma flow) in dogs⁵⁰ (**Fig. 2B**). Since this parameter nearly parallels the glomerular capillary pressure, our findings suggest a potential risk for the development of glomerular hypertension as a result of nifedipine administration.

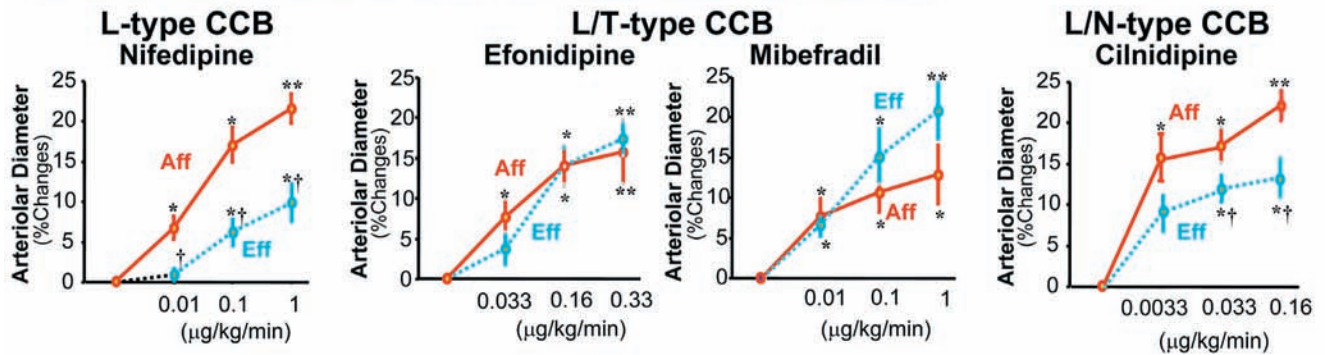
The preferential afferent arteriolar action of CCBs suggests the predominant distribution of L-type Ca channels in this vessel (**Fig. 3**). Indeed, Hansen, *et al.*¹⁷ have demonstrated that mRNA encoding $Ca_v1.2$ ($\alpha1C$) L-type Ca channel subunits is expressed in afferent arterioles from rabbit cortical preglomerular arterioles. In contrast, this subunit was not found in cortical efferent arterioles, although it was present in juxtamedullary efferent arterioles. Similarly, Ono, *et al.* [personal communication] have recently demonstrated that $Ca_v1.2$ is prevalent in the rat afferent arteriole, whereas the efferent arteriole lacks this subunit. These observations thus endorse the functional evidence indicating preferential activity of L-type Ca channels in the afferent, but not in the efferent, arteriole.

In summary, it is reasonable to conclude that voltage-dependent Ca channels predominate in the afferent arteriole; in contrast, these channels are sparse or functionally silent in the efferent arteriole.

3. Role of T-type and N-type Ca Channels in Renal Microvessels

Unlike the conventional types of CCBs, novel CCBs developed in Japan (e.g., manidipine and efonidipine) are reported to dilate both afferent and efferent arterioles.^{8,10} Using microdissected renal arterioles, Arima, *et*

A Differing action of CCB on renal microcirculation



B Effects of CCB on filtration fraction

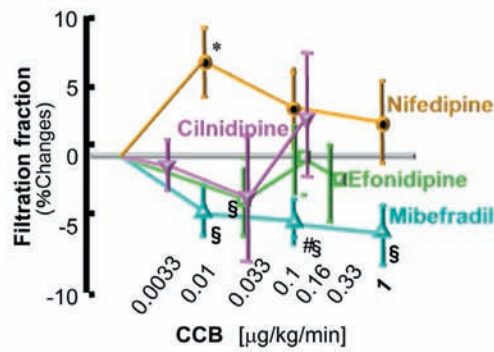


Fig. 2 Effects of various Ca channel blockers on *in vivo* renal microvessels and renal hemodynamics in dogs.

(A) CCBs with preferential blockade of L-type Ca channels (nifedipine) cause predominant afferent arteriolar action, whereas CCBs with blocking activity toward L-/T-type Ca channels (efonidipine and mibefradil) dilate both afferent and efferent arterioles. Cilnidipine, an L-/N-type Ca channel blocker, dilates both microvessels, although the response is greater in the afferent arteriole (from Honda M, *et al.*⁵⁰). (B) The changes in filtration fraction, a marker for glomerular pressure, vary depending on the CCB used (from Honda M, *et al.*⁵⁰). #, $p=0.05$ vs. baseline; *, $p<0.05$ vs. baseline; **, $p<0.01$ vs. baseline; §, $p<0.05$ vs. nifedipine; †, $p<0.05$ vs. afferent arterioles.

*al.*⁴⁷ reported that manidipine caused efferent as well as afferent arteriolar dilation. Furthermore, Takabatake, *et al.*⁵¹ reported that in a rat micropuncture study, efonidipine reduced pre- and postglomerular capillary resistance. Finally, we demonstrated that several CCBs, including manidipine, nilvadipine, benidipine and efonidipine, cause substantial dilation of efferent arterioles in the isolated perfused rat hydronephrotic kidney.^{48,52,53} Since traditional CCBs act on L-type voltage-dependent Ca channels, and these channels are predominant in the afferent arteriole,^{30,53} the effects on the efferent arteriole by these CCBs are most likely attributed to additional actions of these CCBs, not due to the class effects of these agents.

Recently, a pharmacological study has demonstrated that efonidipine possesses blocking activity toward T-type, as well as L-type, voltage-dependent Ca channels.⁵⁴ T-type Ca channels are distributed substantially in several parts of the microvasculature, including mesenteric and cremaster arterioles, and the blockade of these

channels by selective T-type CCBs, e.g., mibefradil, inhibits the vasoconstriction of these arterioles. In the renal microvasculature, Hansen, *et al.*¹⁷ demonstrated that T-type Ca channels are prevalent in juxtamedullary efferent arterioles, as well as in afferent arterioles of superficial and juxtamedullary nephrons. Furthermore, using *in situ* hybridization, Ono, *et al.* recently identified the $Ca_v3.1$ subunit (an $\alpha 1$ subunit of T-type Ca channels) in superficial efferent as well as in afferent arterioles [personal communication]. Similarly, using the micropuncture technique, Nakamura, *et al.*⁵⁵ found that mibefradil decreased both afferent and efferent arteriolar resistance in kidneys from spontaneously hypertensive rat (SHR) kidneys. Recently, Ozawa, *et al.*⁵⁶ directly visualized efferent arteriolar dilation by some CCBs that possess blocking activity toward T-type Ca channels. Both mibefradil and nickel chloride potently reverse the angiotensin II-induced constriction of efferent arterioles in the isolated perfused hydronephrotic rat kidney model. Furthermore, the intravital pencil-type CCD camera tech-

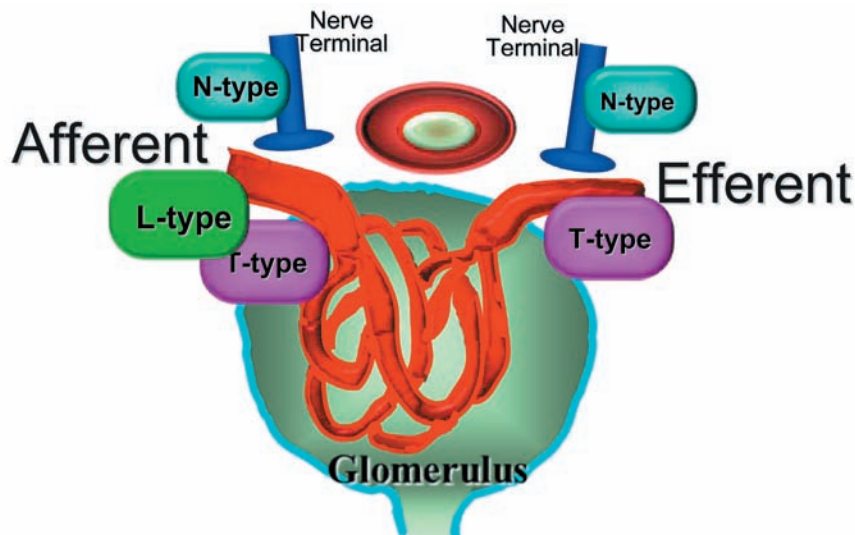


Fig. 3 Schematic illustrating the localization of Ca channels in the kidney.

nique revealed that efonidipine and mibefradil provoke efferent as well as afferent arteriolar dilation in dog kidneys *in vivo*⁵⁰ (**Fig. 2A**). Collectively, these novel findings strongly suggest a critical role for T-type Ca channels in mediating the efferent arteriolar tone.

N-type Ca channels are present at sympathetic nerve terminals that are distributed along afferent and efferent arterioles. It has been reported that cilnidipine, an N-type CCB, reduced both afferent and efferent arteriolar resistance in a renal micropuncture study of nitro-L-arginine methyl ester-treated SHR⁵⁷ and dilated both arterioles in the *in vivo* hydronephrotic kidney.⁵⁸ Similarly, using an intravital CCD camera, we found that cilnidipine causes substantial vasodilation of efferent, as well as afferent, arterioles in the canine kidney *in vivo* (**Fig. 2A**). This class of CCB is characterized by a unique pharmacological action, i.e., inhibitory action on norepinephrine secretion^{59,60} and neurally stimulated renal vasoconstriction.⁶¹ It is noteworthy that, in the *in vitro* isolated perfused hydronephrotic kidney, cilnidipine elicits predominant action in the afferent arteriole.⁶² It was inferred, therefore, that the integrity of the sympathetic nerve is required for the full activity of N-type CCBs.

The vasodilator action of T-type and N-type CCBs on renal microvessels favors reduced glomerular capillary pressure. Indeed, we have shown that both T-type (efonidipine and mibefradil) and N-type CCBs (cilnidipine) reduce or tend to decrease the filtration fraction, an observation contrasting the effects of nifedipine (**Fig. 2B**).⁵⁰

4. Role of CCBs in the Progression of Renal Injury

The glomerular hemodynamic effects of L-type CCBs suggest that these CCBs fail to correct glomerular hypertension in certain experimental conditions. The overall

effect of L-type CCBs on glomerular hemodynamics is determined by the balance between the reduction in afferent arteriolar resistance and the fall in systemic blood pressure, and the changes in these two factors may vary depending on the experimental settings, the magnitude of depressor activity, and the types of CCBs used. For example, verapamil is reported to reduce proteinuria and protect against renal injury in remnant kidney models.^{63,64} In contrast, there have been several reports suggesting deleterious effects of dihydropyridine-class CCBs in renal diseases.^{9-12,65-67} Wenzel, *et al.*⁶⁶ demonstrated that nitrendipine actually increased proteinuria and glomerulosclerosis in a two-kidney, one-clip model of hypertension.

In contrast, CCBs that act on both L-type and T-type Ca channels and dilate afferent and efferent arterioles may alleviate glomerular hypertension and could exert salutary actions on the progression of renal injury. Shudo, *et al.*^{18,68,69} reported that efonidipine acutely decreased proteinuria in spontaneously hypertensive rats, whereas systemic blood pressure was only partially reduced. Additionally, mibefradil potently prevents the development of renal injury in SHR⁵⁵ and DOCA hypertensive rats.²⁰ Likewise, cilnidipine, an L-type and N-type CCB, has been reported to suppress the elevation in blood pressure and blunt the progression of renal injury in Dahl salt-sensitive rats⁷⁰ and to ameliorate glomerular injury and proteinuria in Dahl rats fed on a high-sucrose diet.⁷¹

Our previous studies demonstrated that 8-week-treatment with CCBs including nifedipine and efonidipine showed contrasting effects for these antagonists.⁵² Despite the same reduction in systemic blood pressure, efonidipine markedly prevented an increase in proteinuria, whereas nifedipine did not prevent an increase in

proteinuria in subtotaly nephrectomized SHR, a model of CKD with hypertension.⁷² Furthermore, the histopathological changes and serum creatinine levels were also ameliorated by efonidipine but not by nifedipine. Of note, efonidipine reduces proteinuria to the same level as enalapril, and both drugs cause afferent and efferent arteriolar dilation. Thus, the renal protective effect of efonidipine may be attributed, at least in part, to the glomerular hemodynamic action of this agent, since efonidipine is anticipated to reduce glomerular capillary pressure in the same way that enalapril does.

5. Mechanisms for T-type CCB-induced Renal Protection

In addition to hemodynamic factors, multiple mechanisms appear to contribute to the ability of T-type CCBs to protect the kidney. It has been reported that CCBs suppress mesangial cell proliferation by inhibiting activator protein-1 (AP-1)⁷³ and that CCBs modulate gene transcriptions involved in proinflammatory changes (interleukin 1 β and granulocyte/monocyte colony stimulating factors).⁷⁴ Efonidipine, a T-type Ca antagonist, has been shown to suppress the phorbol myristate acetate (PMA)-induced activation of nuclear factor kappa B (NF- κ B) in cultured human mesangial cells.⁷⁵ Furthermore, CCBs could act as free radical scavengers.⁷⁶

Rossier, *et al.*⁷⁷ found that aldosterone release provoked by angiotensin II and KCl was inhibited by mibefradil but not by nifedipine, suggesting an important contribution of T-type Ca channels to aldosterone release. Similarly, Somekawa, *et al.*⁷⁸ recently demonstrated that efonidipine downregulates the expression of aldosterone synthetase, CYP11B2, in rat adrenal cells. Indeed, plasma aldosterone levels are reported to be lower in patients with hypertension⁷⁹ or chronic glomerulonephritis treated with efonidipine rather than with amlodipine.⁸⁰ Since aldosterone is reported to promote renal injury,⁸¹ the blockade of aldosterone release would be anticipated to exert salutary action on the progression of renal injury.

We have recently shown that Rho kinase activation participates in the progression of renal injury in subtotal nephrectomized rats, a model of chronic renal failure.⁸² Conversely, the inhibition of Rho kinase should therefore confer benefit in the treatment of renal disease^{83–85} in addition to its hypotensive action.⁸⁶ Of interest, our recent study demonstrated that T-type CCBs prevent renal Rho kinase activation induced by subtotal nephrectomy and alleviate the progression of kidney disease (**Fig. 4**).⁸⁷ Furthermore, the expression of T-type Ca channels is upregulated in kidneys from rats with chronic kidney disease.⁸⁷ Since Rho kinase is known to elicit the inflammatory process as well as the enhancement of vascular tone,⁸⁸ the blockade of T-type Ca channels is expected to act as a tool for the prevention of CKD.

In summary, T-type Ca channels play an important role in the development of chronic kidney disease, which action is mediated by multifaceted pathways, including hemodynamic, hormonal and inflammatory factors.

6. Clinical Aspects of the Role of CCBs

The divergent action of L-type and T-type CCBs on renal microcirculation and kidney injury in animal models may be extrapolated to human CKD. Previous clinical trials, including ALLHAT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial)⁸⁹ and INSIGHT (Intervention as a Goal in Hypertension Treatment),⁹⁰ showed equivocal effects of L-type CCBs (amlodipine and nifedipine) on the progression of CKD, compared with ACE inhibitors or diuretics (**Table 1**). In contrast, AASK (African American Study of Kidney Disease and Hypertension)⁹¹ demonstrated that the amlodipine-treated group manifested an elevation in glomerular filtration rate (GFR) at 3 months but exhibited a greater reduction in GFR than the ACE inhibitor-treated group in African American patients without diabetes mellitus (non-DM) with CKD. Furthermore, the addition of felodipine to ACE inhibitor therapy conferred no additive benefit in non-DM patients with CKD (REIN-2; Ramipril Efficacy in Nephropathy trial-2).⁹² Additionally, IDNT (Irbesartan Diabetic Nephropathy Trial), in which the effect of and irbesartan on the progression of CKD was evaluated, revealed that amlodipine [an angiotensin receptor blocker (ARB)] potentially prevented the progression of CKD, whereas amlodipine failed to offer renal protection in type 2 diabetic patients with CKD.⁹³ Similarly, the GUARD (Gauging Albuminuria Reduction with Lotrel in Diabetic Patients with Hypertension) trial showed that the reduction in albuminuria was less in the amlodipine-treated group than in the diuretics-treated group.⁹⁴ Head-to-head comparison of amlodipine vs. valsartan also unveiled an increase in albuminuria in Japanese type 2 DM patients treated with amlodipine (SMART; Shiga Microalbuminuria Reduction Trial).⁹⁵ In concert, these observations suggest reduced or no protective action of the L-type CCB in the progression of CKD.

One caveat is that the adequate control of blood pressure would be anticipated to alleviate the progression of CKD. In the sub-analysis of IDNT, the relative risk for the development of end-stage renal disease was reported to be lower in patients with systolic blood pressure below 121 mmHg, and the risk in the amlodipine-treated group tended to parallel the levels of systolic blood pressure.⁹³ Furthermore, in the NICE Combi (Nifedipine and Candesartan Combination) study, in which the effects of the maximal dose of candesartan and combination therapy with candesartan and controlled-release nifedipine on microalbuminuria were compared, the combination treatment group manifested significantly reduced albu-

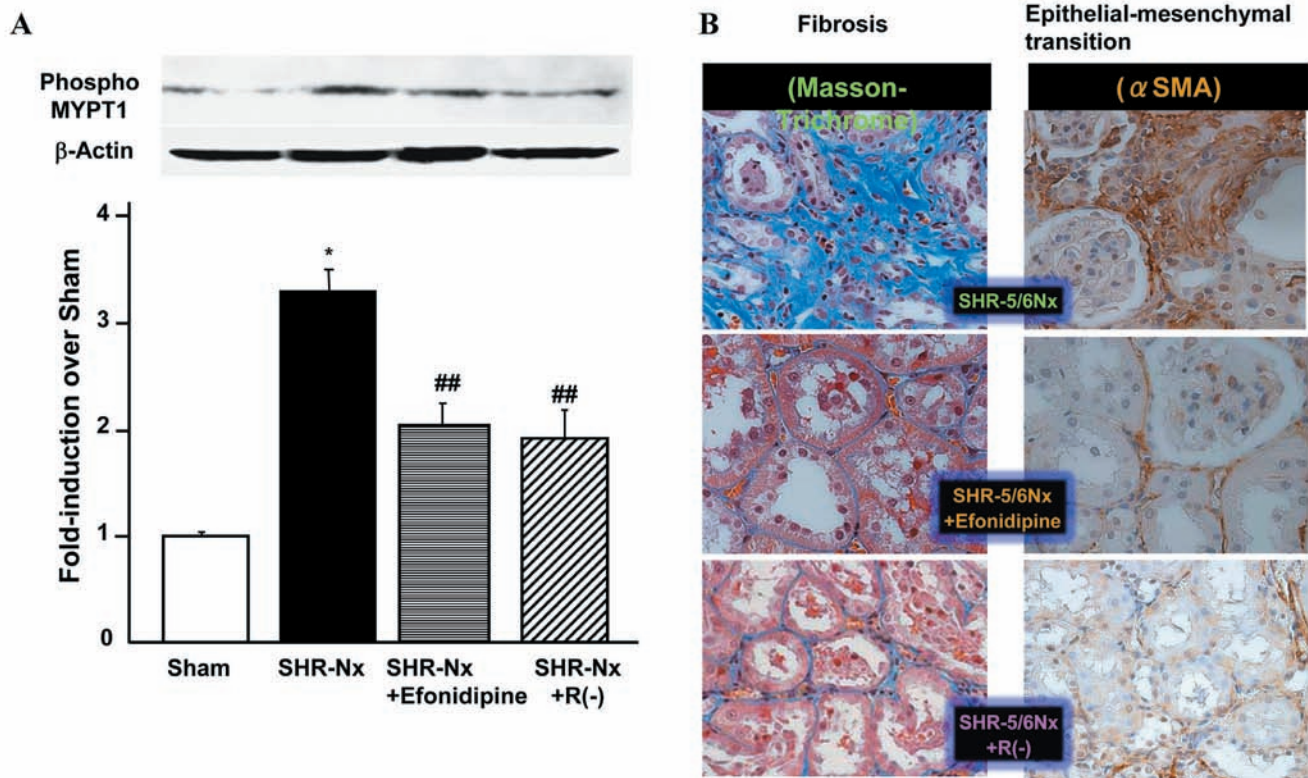


Fig. 4 Effects of efonidipine on Rho kinase activity and renal histology in subtotally nephrectomized spontaneously hypertensive rats (SHR-Nx).

(A) The phosphorylation level of MYPT1, as a marker for Rho kinase activity, in renal cortex of SHR-Nx. * $P < 0.05$ vs. Sham, ## $P < 0.01$ vs. SHR-Nx. (B) Effects of Ca channel blockers on the expression of α -smooth muscle action (SMA) and interstitial fibrosis in kidneys from SHR-Nx. R(-) indicates an R(-)-enantiomer of efonidipine that selectively blocks T-type Ca channels but has no effect on L-type Ca channels. Data adopted with modification from Sugano N, *et al.*⁸⁷

Table 1 Renal protective effects of L-type CCB

	CCB	Control		Observation period	Results
ALLHAT ⁸⁹	Amlodipine	Diuretics, ACE inhibitors, β -blockers	Non-DM DM	6 years	eGFR preserved
INSIGHT ⁹⁰	Nifedipine-GITS	Diuretics	Non-DM DM	4 years	Nifedipine-GITS better than diuretics in maintaining eGFR
AASK ⁹¹	Amlodipine	ACE inhibitors, β -blockers	Non-DM	4 years	Greater reduction in eGFR with Amlodipine than ACE with inhibitors
REIN-2 ⁹²	ACE inhibitors + Felodipine	ACE inhibitors	Non-DM	4 years	Renal events did not reduce despite addition of felodipine
IDNT ⁹³	Amlodipine	ARB	DM	5 years	Renal events more frequent with amlodipine than with ARB
GUARD ⁹⁴	ACE inhibitors + Amlodipine	ACE inhibitors + Diuretics	DM	1 years	Reduction in albuminuria less with amlodipine than with diuretics
SMART ⁹⁵	Amlodipine	Valsartan	DM	24 weeks	Albuminuria increased with amlodipine

GITS; gastrointestinal therapeutic system.

Table 2 Renal protective effects of L/N- and L/T-type CCB

Study name	CCB	Control	Subjects	Results
Hayashi K, <i>et al.</i> ²¹	Efonidipine (L/T-type)	ACE inhibitors	Non-DM	Same proteinuria-reducing effects CCr well preserved with efonidipine
Ishimitsu T, <i>et al.</i> ⁸⁰	Efonidipine (L/T-type)	Amlodipine (L-type)	Chronic glomerulonephritis	Proteinuria-reducing effect better with efonidipine
Nakamura T, <i>et al.</i> ⁹⁷	Azelnidipine (L/T-type)	Amlodipine (L-type)	Mild CKD	Proteinuria-reducing effect better with azelnidipine
Ogawa S, <i>et al.</i> ⁹⁸	Azelnidipine (L/T-type)	Nifedipine-CR (L-type)	DM	Proteinuria-reducing effect better with azelnidipine
Tsuchihashi T, <i>et al.</i> ⁹⁹	Cilnidipine (L/N-type)	Other CCBs	Essential hypertension Renal hypertension	Proteinuria decreased No changes in proteinuria
Fujita T, <i>et al.</i> ¹⁰⁰ (Carter study)	ARB + Cilnidipine (L/N-type)	ARB + Amlodipine (L-type)	Non-DM DM	Proteinuria-reducing effect better with cilnidipine No difference
Abe M, <i>et al.</i> ¹⁰¹	ARB + Benidipine (L/T/N-type)	ARB + Amlodipine (L-type)	CKD stage3-5 Non-DM, DM	Proteinuria-reducing effect better with benidipine
Omae K, <i>et al.</i> ²³	L/T-type CCB Benidipine Barnidipine Manidipine Nilvadipine Efonidipine	L-type CCB Nifedipine Amlodipine Nicardipine Nitrendipine	CKD (non-DM)	Better outcome of renal events with L/T-type CCB

CCr; creatinine clearance.

minuria with greater reductions in systemic blood pressure.⁹⁶ Accordingly, these observations lend support to the formulation that strict blood pressure control offers renal protection that does not depend on the type of anti-hypertensive agent used.

In contrast, a growing body of clinical evidence has shown that CCBs with blocking activity toward T-type Ca channels provide salutary action in protection against renal injury (**Table 2**). We previously demonstrated that efonidipine was as potent as ACE inhibitors in reducing proteinuria.²¹ Furthermore, efonidipine significantly reduced proteinuria even in a subset of patients in which mean blood pressures failed to decrease below 100 mmHg. Ishimitsu, *et al.*⁸⁰ also reported that switching from amlodipine to efonidipine caused a significant decrease in proteinuria in patients with chronic glomerulonephritis. Finally, comparison of azelnidipine (an L/T-type CCB), **Table 3** with amlodipine or nifedipine (L-type CCBs) showed greater reductions in proteinuria by azelnidipine in patients with CKD⁹⁷ and DM,⁹⁸ respectively.

Cilnidipine is a representative N-type CCB(**Table 3**) and dilates both afferent and efferent arterioles through the inhibition of N-type Ca channels in nerve terminals innervating renal afferent and efferent arterioles. It has been reported that cilnidipine exerts antiproteinuric action when administered in patients with essential hypertension.⁹⁹ Recently, Fujita, *et al.*¹⁰⁰ demonstrated that

add-on therapy with cilnidipine reduces proteinuria more potently than that with amlodipine in CKD patients treated with renin-angiotensin blocking agents (CARTER study, **Table 2**). They suggest that the beneficial action of cilnidipine on proteinuria is mediated by the inhibition of sympathetic nerve activity and the resultant amelioration of glomerular hypertension. Of note, in a subset of the DM nephropathy group, they failed to show a significant decrease in proteinuria with cilnidipine. Since the salutary action of cilnidipine on proteinuria appears to be attributed to the blockade of N-type Ca channels, it is reasonable to speculate that the failure to alleviate proteinuria can be ascribed to the impaired integrity of sympathetic nerve terminals in DM. In contrast, Abe, *et al.*¹⁰¹ recently demonstrated that during treatment with ARB, benidipine, but not amlodipine, potently reduces proteinuria in DM as well as in non-DM patients. Based on the pharmacological properties of benidipine (i.e., blocking action on L/T/N-type Ca channels), this study underscores a critical role of T-type Ca channel blockade not only in non-DM but also in DM patients.

Very recently, Omae, *et al.*²³ evaluated the long-term effects of CCBs with different Ca channel subtype activities on the development of end-stage renal disease in 107 non-DM CKD patients (**Table 2**). This prospective study used L-type CCBs (nifedipine, amlodipine, nicardipine and nitrendipine), L/T-type CCBs (benidipine,

Table 3 Characteristics of Ca channel blockers and their clinical significance

Drugs	Blocking activity		
	L-type	T-type	N-type
Nifedipine	+	-	-
Amlodipine	+	-	?
Efonidipine	+	+	-
Nilvadipine	+	+	-
Azelnidipine	+	+	-
Benidipine	+	+	+
Cilnidipine	+	-	+

Clinical significance			
Heart	Contractility ↓	Heart rate ↓	Heart rate ↓
Sympathetic nervous system	Pulse rate ↑	Pulse rate ↓	Pulse rate ↓
Kidney	Glomerular pressure ↑	Glomerular pressure ↓	Glomerular pressure ↓
Adrenal		Aldosterone secretion ↓	

Data regarding channel blocking activity were adopted with modification from Furukawa T, *et al*. Eur J Pharmacol 2009; 613: 100–107.

barnidipine, manidipine, nilvadipine and efonidipine, **Table 3**) and non-dihydropyridines (diltiazem and verapamil) and found that the use of L-type CCBs was associated with an increase in proteinuria. Furthermore, the use of L/T-type CCBs was associated with favorable outcomes, as assessed by the changes in eGFR and the renal survival rate.

In summary, the results obtained from various clinical trials demonstrate that the effects of CCBs with L-type Ca channel specific blocking activity on renal outcomes vary depending on several factors, including underlying renal disease, the blood pressure achieved and the duration of the study. In contrast, CCBs with L/T-type blocking activity consistently offer favorable action on the development of CKD. Although the role of N-type Ca channel blockade in CKD appears intriguing, its actual effect may depend on the integrity of the underlying sympathetic nervous system.

Concluding Remarks

Pharmacological characterization of Ca channel subtypes and their blockers allows understanding of the role of these channels in the kidney. It is now established that the blockade of T-type or N-type Ca channels exerts renal protective action by abrogating glomerular hypertension through vasodilator action on both afferent and efferent arterioles. Additionally, T-type CCBs may provide beneficial action through multiple non-hemodynamic mechanisms that act to suppress inflammatory processes and the renin-angiotensin-aldosterone system.

Such multifaceted action of T-type CCBs serves to protect against renal injury, and their use is anticipated to increase in the treatment of hypertension with CKD. Although these subclasses of CCB are clinically available only in Japan and South Korea, a growing body of evidence regarding their efficacy should facilitate the spread of knowledge on these novel types of CCBs.^{102,103}

References

- Heller J, Horacek V: The effect of two different calcium antagonists on the glomerular haemodynamics in dogs. Eur J Physiol 1990; **415**: 751–755.
- Dietz JR, Davis JO, Freeman RH, Villarreal D, Echtenkamp SF: Effects of intrarenal infusion of calcium entry blockers in anesthetized dogs. Hypertension 1983; **5**: 482–488.
- Roy M, Guthrie GP, Holladay FP, Kotchen TA: Effects of verapamil on renin and aldosterone in the dog and rat. Am J Physiol 1983; **245**: E410–E416.
- Carmines PK, Navar LG: Disparate effects of Ca channel blockers on afferent and efferent arteriolar responses to ANG II. Am J Physiol 1989; **256**: F1015–F1020.
- Loutzenhiser R, Epstein M, Horton C, Sonke P: Reversal by the calcium antagonist nisoldipine of norepinephrine-induced reduction of GFR: evidence for preferential antagonism of preglomerular vasoconstriction. J Pharmacol Exp Ther 1984; **232**: 382–387.
- Loutzenhiser R, Epstein M: Renal hemodynamic effects of calcium antagonists. In: Epstein M, Loutzenhiser R (eds) Calcium Antagonists and the Kidney. Philadelphia, Hanley & Belfus, 1990, pp 33–74.
- Fleming JT, Parekh N, Steinhausen M: Calcium antagonists preferentially dilate preglomerular vessels of hydronephrotic kidney. Am J Physiol 1987; **253**: F1157–F1163.
- Nagahama T, Hayashi K, Fujiwara K, Ozawa Y, Saruta T: Charac-

- terization of the renal action of prandipine in the rat. *Arzneimittel Forschung* 2000; **50**: 248–253.
9. Bidani AK, Schwartz MM, Lewis EJ: Renal autoregulation and vulnerability to hypertensive injury in remnant kidney. *Am J Physiol* 1987; **252**: F1003–F1010.
 10. Griffin KA, Picken M, Bidani AK: Method of renal mass reduction is a critical determinant of subsequent hypertension and glomerular injury. *J Am Soc Nephrol* 1994; **4**: 2023–2031.
 11. Griffin KA, Picken MM, Bidani AK: Deleterious effects of calcium channel blockers on pressures transmission and glomerular injury in rat remnant kidneys. *J Clin Invest* 1995; **96**: 793–800.
 12. Griffin KA, Picken MM, Bakris GL, Bidani AK: Class differences in the effects of calcium channel blockers in the rat remnant kidney model. *Kidney Int* 1999; **55**: 1849–1860.
 13. Ertel EA, Campbell KP, Harpold MM, *et al*: Nomenclature of voltage-gated calcium channels. *Neuron* 2000; **25**: 533–535.
 14. Perez-Reyes E: Molecular physiology of low-voltage-activated T-type calcium channels. *Physiol Rev* 2003; **83**: 117–161.
 15. Ono K, Iijima T: Pathophysiological significance of T-type Ca²⁺ channels: properties and functional roles of T-type Ca²⁺ channels in cardiac pacemaking. *J Pharmacol Sci* 2005; **99**: 197–204.
 16. Hansen PB, Jensen BL, Andreasen D, Friis UG, Skott O: Vascular smooth muscle cells express the alpha(1A) subunit of a P-/Q-type voltage-dependent Ca(2+) channel, and it is functionally important in renal afferent arterioles. *Circ Res* 2000; **87**: 896–902.
 17. Hansen PB, Jensen BL, Andreasen D, Skott O: Differential expression of T- and L-type voltage-dependent calcium channels in renal resistance vessels. *Circ Res* 2001; **89**: 630–638.
 18. Shudo C, Masuda Y, Sugita H, Furukawa S, Hayashi K, Hirata H, Tanaka S, Tomita K: Renal protective effect of efonidipine hydrochloride (NZ-105), a new calcium antagonist, in spontaneously hypertensive rats. *Gen Pharmacol* 1994; **25**: 1567–1575.
 19. Fujiwara K, Kanno Y, Hayashi K, Takenaka T, Saruta T: Renal protective effects of efonidipine in partially nephrectomized spontaneously hypertensive rats. *Clin Exp Hypertens* 1998; **20**: 295–312.
 20. Karam H, Clozel J-P, Bruneval P, Gonzalez M-F, Ménard J: Contrasting effects of selective T- and L-type calcium channel blockade on glomerular damage in DOCA hypertensive rats. *Hypertension* 1999; **34**: 673–678.
 21. Hayashi K, Kumagai H, Saruta T: Effects of efonidipine and ACE inhibitors on proteinuria in human hypertension with renal impairment. *Am J Hypertens* 2003; **16**: 116–122.
 22. Baylis C, Qiu C, Engels K: Comparison of L-type and mixed L- and T-type calcium channel blockers on kidney injury caused by deoxycorticosterone-salt hypertension in rats. *Am J Kidney Dis* 2001; **38**: 1292–1297.
 23. Omae K, Ogawa T, Nitta K: Influence of T-calcium channel blocker treatment on deterioration of renal function in chronic kidney disease. *Heart Vessels* 2009; **24**: 301–307.
 24. Jurkat-Rott K, Lehmann-Horn F: The impact of splice isoforms on voltage-gated calcium channel α_1 subunits. *J Physiol* 2003; **554**: 609–619.
 25. Tiwari S, Zhang Y, Heller J, Abernethy DR, Soldatov NM: Atherosclerosis-related molecular alteration of the human Ca_v1.2 calcium channel α_1C subunit. *Proc Natl Acad Sci USA* 2006; **103**: 17024–17029.
 26. Marin-Grez M, Fleming JT, Steinhausen M: Atrial natriuretic peptide causes pre-glomerular vasodilatation and post-glomerular vasoconstriction in rat kidney. *Nature* 1986; **324**: 473–476.
 27. Loutzenhiser R, Hayashi K, Epstein M: A trial natriuretic peptide reverses afferent arteriolar vasoconstriction and potentiates efferent arteriolar vasoconstriction in the isolated perfused rat kidney. *J Pharmacol Exp Ther* 1988; **246**: 522–528.
 28. Hayashi K, Epstein M, Loutzenhiser R: Pressure-induced vasoconstriction of renal microvessels in normotensive and hypertensive rats: studies in the isolated perfused hydronephrotic kidney. *Circ Res* 1989; **65**: 1475–1484.
 29. Loutzenhiser R, Epstein M, Hayashi K, Horton C: Direct visualization of effects of endothelin on the renal microvasculature. *Am J Physiol* 1990; **258**: F61–F68.
 30. Loutzenhiser R, Hayashi K, Epstein M: Divergent effects of KCl-induced depolarization on afferent and efferent arterioles. *Am J Physiol* 1989; **257**: F561–F564.
 31. Bührle CP, Nobiling R, Taugner R: Intracellular recordings from renin-positive cells of the afferent glomerular arteriole. *Am J Physiol* 1985; **249**: F272–F281.
 32. Carmines PK, Fower BC, Bell PD: Segmental distinct effects of depolarization on intracellular [Ca²⁺] in renal arterioles. *Am J Physiol* 1993; **265**: F677–F685.
 33. Steinhausen M, Baehr M: Vasomotion and vasoconstriction induced by a Ca agonist in the split hydronephrotic kidney. *Prog Appl Microcirc* 1989; **14**: 25–29.
 34. Reams GP, Bauer JH: Acute and chronic effects of calcium antagonists on the essential hypertensive kidney. In: Epstein M, Loutzenhiser R (eds) *Calcium Antagonists and the Kidney*. Philadelphia, Hanley & Belfus, 1990, pp 247–256.
 35. Ichikawa I: Will angiotensin II receptor antagonists be renoprotective in humans? *Kidney Int* 1996; **50**: 684–692.
 36. Loutzenhiser R, Horton C, Epstein M: Effects of diltiazem and manganese on renal hemodynamics: studies in the isolated perfused rat kidney. *Nephron*. 1985; **39**: 382–388.
 37. Loutzenhiser R, Epstein M, Fischetti F, Horton C: Effects of amlodipine on renal hemodynamics. *Am J Cardiol* 1989; **64**: 1221–1271.
 38. Loutzenhiser R, Epstein M: Modification of the renal hemodynamic response to vasoconstrictors by calcium antagonists. *Am J Nephrol* 1987; **7** (Suppl 1): 7–16.
 39. Edwards RM: Segmental effects of norepinephrine and angiotensin II on isolated renal microvessels. *Am J Physiol*. 1983; **244**: F526–F534.
 40. Yuan B, Robinette JB, Conger JD: Effects of angiotensin II and norepinephrine on isolated rat afferent and efferent arterioles. *Am J Physiol* 1990; **258**: F741–F750.
 41. Ito S, Carretero OA: An *in vitro* approach to the study of macula densa-mediated glomerular hemodynamics. *Kidney Int* 1990; **38**: 1206–1210.
 42. Ito S, Ren Y: Evidence for the role of nitric oxide in macula densa control of glomerular hemodynamics. *J Clin Invest* 1993; **92**: 1093–1098.
 43. Casellas D, Navar LG: *In vitro* perfusion of juxtamedullary nephrons in rats. *Am J Physiol* 1984; **246**: F349–F358.
 44. Steinhausen M, Snoei H, Parekh N, Baker R, Johnson PC: Hydronephrosis: A new method to visualize vas afferens, efferens, and glomerular network. *Kidney Int* 1983; **23**: 794–806.
 45. Yamamoto T, Hayashi K, Matsuda H, *et al*: *In vivo* visualization of tubuloglomerular feedback- and angiotensin II-induced renal vasoconstriction in dogs by an intravitral pencil lens-probe video-microscope. *Kidney Int* 2001; **60**: 364–369.
 46. Carmines PK, Navar LG: Disparate effects of Ca channel blockers on afferent and efferent arteriolar responses to ANG II. *Am J Physiol* 1989; **256**: F1015–F1020.
 47. Arima S, Ito S, Omata K, Tsunoda K, Yaoita H, Abe K: Diverse effects of calcium antagonists on glomerular hemodynamics. *Kidney Int Suppl* 1996; **55**: S132–S134.
 48. Hayashi K, Nagahama T, Oka K, Epstein M, Saruta T: Disparate effects of calcium antagonists on renal microcirculation. *Hypertens Res* 1996; **19**: 31–36.
 49. Saruta T, Kanno Y, Hayashi K, Konishi K: Antihypertensive agents and renal protection: Calcium channel blockers. *Kidney Int* 49 (Suppl) 1996; **55**: S52–S56.
 50. Honda M, Hayashi K, Matsuda H, *et al*: Divergent renal vasodilator action of L- and T-type calcium antagonists *in vivo*. *J Hypertens* 2001; **9**: 2031–2037.

51. Takabatake T, Ohta H, Sasaki T, Satoh S, Ohta K, Ise T, Kobayashi K: Renal effects of manidipine hydrochloride. A new calcium antagonist in hypertensive patients. *Eur J Clin Pharmacol* 1993; **45**: 321–325.
52. Hayashi K, Ozawa Y, Saruta T, Epstein M: Renal hemodynamic effects of calcium antagonists. In: Epstein M, ed, *Calcium Antagonists in Clinical Medicine* (3rd ed.). Hanley & Belfus, pp559–578, 2002.
53. Ozawa Y, Hayashi K, Nagahama T, Fujiwara K, Wakino S, Saruta T: Renal afferent and efferent arteriolar dilation by nilvadipine: studies in the isolated perfused hydronephrotic kidney. *J Cardiovasc Pharmacol* 1999; **33**: 243–247.
54. Masumiya H, Shijuku T, Tanaka H, Shigenobu K: Inhibition of myocardial L- and T-type Ca²⁺ currents by efonidipine: possible mechanism for chronotropic effect. *Eur J Pharmacol* 1998; **349**: 351–357.
55. Nakamura Y, Ono H, Frohlich ED: Differential effects of T- and L-type calcium antagonists on glomerular dynamics in spontaneously hypertensive rats. *Hypertension* 1999; **34**: 273–278.
56. Ozawa Y, Hayashi K, Nagahama T, Fujiwara K, Saruta T: Effect of T-type selective calcium antagonist on renal microcirculation: studies in the isolated perfused hydronephrotic kidney. *Hypertension* 2001; **38**: 343–347.
57. Zhou X, Ono H, Ono Y, Frohlich ED: N- and L-type calcium channel antagonist improves glomerular dynamics, reverses severe nephrosclerosis, and inhibits apoptosis and proliferation in an L-NAME/SHR model. *J Hypertens* 2002; **20**: 993–1000.
58. Kimura K, Suzuki N, Ohba S, Mise N, Miyashita K, Tojo A, Goto A, Omata M: Effects of amlodipine, a calcium channel blocker, on rat renal arterioles. *Curr Ther Res* 1997; **58**: 375–380.
59. Hosono M, Fujii S, Hiruma T, Watanabe K, Hayashi Y, Ohnishi H, Takata Y, Kato H: Inhibitory effect of cilnidipine on vascular sympathetic neurotransmission and subsequent vasoconstriction in spontaneously hypertensive rats. *Jpn J Pharmacol* 1995; **69**: 127–134.
60. Nap A, Mathy MJ, Balt JC, Pfaffendorf M, van Zwieten PA: The evaluation of the N-type channel blocking properties of cilnidipine and other voltage-dependent calcium antagonists. *Fundam Clin Pharmacol* 2004; **18**: 309–319.
61. Takahara A, Dohmoto H, Hisa H, Satoh S, Yoshimoto R: Cilnidipine attenuates renal nerve stimulation-induced renal vasoconstriction and antinatriuresis in anesthetized dogs. *Jpn J Pharmacol* 1997; **75**: 27–32.
62. Hayashi K, Wakino S, Sugano N, Ozawa Y, Homma K, Saruta T: Ca²⁺ channel subtypes and pharmacology in the kidney. *Circ Res* 2007; **100**: 342–353.
63. Yoshioka T, Shiraga H, Yoshida Y, Fogo A, Glick AD, Deen WM, Hoyer JR, Ichikawa I: “Intact nephrons” as the primary origin of proteinuria in chronic renal disease. Study in the rat model of subtotal nephrectomy. *J Clin Invest* 1988; **82**: 1614–1623.
64. Harris DC, Hammond WS, Burke TJ, Schrier RW: Verapamil protects against progression of experimental chronic renal disease. *Kidney Int* 1987; **31**: 41–46.
65. Dworkin LD, Tolbert E, Recht PA, Hersch JC, Feiner H, Levin RI: Effects of amlodipine on glomerular filtration, growth, and injury in experimental hypertension. *Hypertension* 1996; **27**: 245–250.
66. Wenzel UO, Troschau G, Schoeppe W, Helmchen U, Schwietzer G: Adverse effect of the calcium channel blocker nitrendipine on nephrosclerosis in rats with renovascular hypertension. *Hypertension* 1992; **20**: 233–241.
67. Pelayo JC, Westoo JY: Impaired autoregulation of glomerular capillary hydrostatic pressure in the rat remnant nephron. *J Clin Invest* 1991; **88**: 101–105.
68. Shudo C, Masuda Y, Sugita H: Beneficial effect of efonidipine hydrochloride (NZ-105) on proteinuria in aged spontaneously hypertensive rats (SHR). *Pharm Sci* 1995; **1**: 333–335.
69. Shudo C, Masuda Y, Sugita H, *et al*: Effects of efonidipine, nicardipine and captopril on proteinuria in aged spontaneously hypertensive rats. *Arzneimittel Forschung* 1996; **46**: 852–854.
70. Konda T, Enomoto A, Takahara A, Yamamoto H: Effects of L/N-type calcium channel antagonist, cilnidipine on progressive renal injuries in Dahl salt-sensitive rats. *Biol Pharm Bull* 2006; **29**: 933–937.
71. Konda T, Enomoto A, Matsushita J, Takahara A, Moriyama T: The N- and L-type calcium channel blocker cilnidipine suppresses renal injury in Dahl rats fed a high-sucrose diet, an experimental model of metabolic syndrome. *Nephron Physiol* 2005; **101**: 1–13.
72. Fujiwara K, Hayashi K, Kanno Y, Takenaka T, Saruta T: Renal protective effects of efonidipine hydrochloride in subtotally nephrectomized spontaneously hypertensive rats. *Clin Exp Hypertens* 1998; **23**: 295–312.
73. Sugiura T, Imai E, Moriyama T, Horio M, Hori M: Calcium channel blockers inhibit proliferation and matrix production in rat mesangial cells: possible mechanism of suppression of AP-1 and CREB activities. *Nephron* 2000; **85**: 71–80.
74. Roth M, Keul R, Emmons LR, Horl WH, Block LH: Manidipine regulates the transcription of cytokine genes. *Proc Natl Acad Sci USA* 1992; **89**: 4071–4075.
75. Hayashi M, Yamaji Y, Nakazato Y, Saruta T: The effects of calcium channel blockers on nuclear factor kappa B activation in the mesangial cells. *Hypertens Res* 2000; **23**: 521–525.
76. Fukuo K, Yang J, Yasuda O, *et al*: Nifedipine indirectly upregulates superoxide dismutase expression in endothelial cells via vascular smooth muscle cell-dependent pathways. *Circulation* 2002; **106**: 356–361.
77. Rossier MF, Ertel EA, Vallotton MB, Capponi AM: Inhibitory action of mibefradil on calcium signaling and aldosterone synthesis in bovine adrenal glomerulosa cells. *J Pharmacol Exp Ther* 1998; **287**: 824–831.
78. Somekawa S, Imagawa K, Naya N, *et al*: Regulation of aldosterone and cortisol production by the transcriptional repressor neuron restrictive silencer factor. *Endocrinology* 2009; **150**: 3110–3117.
79. Tanaka T, Tsutamoto T, Sakai H, Fuji M, Yamamoto T, Horie M: Comparison of the effects of efonidipine and amlodipine on aldosterone in patients with hypertension. *Hypertens Res* 2007; **30**: 691–697.
80. Ishimitsu T, Kameda T, Akashiba A, *et al*: Efonidipine reduces proteinuria and plasma aldosterone in patients with chronic glomerulonephritis. *Hypertens Res* 2007; **30**: 621–626.
81. Rudolph AE, Rocha R, McMahon EG: Aldosterone target organ protection by eplerenone. *Mol Cell Endocrinol* 2004; **217**: 229–238.
82. Kanda T, Wakino S, Hayashi K, Homma K, Ozawa Y, Saruta T: Effect of fasudil on Rho-kinase and nephropathy in subtotally nephrectomized spontaneously hypertensive rats. *Kidney Int* 2003; **64**: 2009–2019.
83. Nishikimi T, Akimoto K, Wang X, *et al*: Fasudil, a Rho-kinase inhibitor, attenuates glomerulosclerosis in Dahl salt-sensitive rats. *J Hypertens* 2004; **22**: 1787–1796.
84. Nishikimi T, Matsuoka H: Molecular mechanisms and therapeutic strategies of chronic renal injury: renoprotective effect of Rho-kinase inhibitor in hypertensive glomerulosclerosis. *J Pharmacol Sci* 2006; **100**: 22–28.
85. Ruperez M, Sanchez-Lopez E, Blanco-Colio LM, Esteban V, Rodriguez-Vita J, Plaza JJ, Egido J, Ruiz-Ortega M: The Rho-kinase pathway regulates angiotensin II-induced renal damage. *Kidney Int Suppl* 2005; **99**: S39–S45.
86. Uehata M, Ishizaki T, Satoh H, *et al*: Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* 1997; **389**: 990–994.
87. Sugano N, Wakino S, Kanda T, *et al*: T-type calcium channel blockade as a therapeutic strategy against renal injury in rats with

- subtotal nephrectomy. *Kidney Int* 2008; **73**: 826–834.
88. Zhao D, Pothoulakis C: Rho GTPases as therapeutic targets for the treatment of inflammatory diseases. *Expert Opin Ther Targets* 2003; **7**: 583–592.
 89. The ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group: Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretics. *JAMA* 2003; **288**: 2981–2997.
 90. de Leeuw PW, Ruilope LM, Palmer CR, Brown MJ, Castaigne A, Mancia G, Rosenthal T, Wagener G: Clinical significance of renal function in hypertensive patients at high risk: results from the INSIGHT trial. *Arch Intern Med* 2004; **164**: 2459–2464.
 91. Agodoa LY, Appel L, Bakris GL, *et al*: African American Study of Kidney Disease and Hypertension (AASK) Study Group: Effect of ramipril vs. amlodipine on renal outcomes in hypertensive nephrosclerosis: a randomized controlled trial. *JAMA* 2002; **285**: 2719–2728.
 92. Ruggenti P, Perna A, Loriga G, *et al*: REIN-2 Study Group: Blood-pressure control for renoprotection in patients with non-diabetic chronic renal disease (REIN-2): multicentre, randomised controlled trial. *Lancet* 2005; **365**: 939–946.
 93. Pohl MA, Blumenthal S, Cordonnier DJ, *et al*: Independent and additive impact of blood pressure control and angiotensin II receptor blockade on renal outcomes in the irbesartan diabetic nephropathy trial: clinical implications and limitations. *J Am Soc Nephrol* 2005; **16**: 3027–337.
 94. Bakris GL, Toto RD, McCullough PA, Rocha R, Purkayastha D, Davis P; GUARD (Gauging Albuminuria Reduction With Lotrel in Diabetic Patients With Hypertension) Study Investigators: Effects of different ACE inhibitor combinations on albuminuria: results of the GUARD study. *Kidney Int* 2008; **73**: 1303–1309.
 95. Shiga Microalbuminuria Reduction Trial (SMART) Group, Uzu T, Sawaguchi M, Maegawa H, Kashiwagi A: Impact of renin-angiotensin system inhibition on microalbuminuria in type 2 diabetes: a post hoc analysis of the Shiga Microalbuminuria Reduction Trial (SMART). *Hypertens Res* 2008; **31**: 1171–1176.
 96. Hasebe N, Kikuchi K; NICE Combi Study Group: Controlled-release nifedipine and candesartan low-dose combination therapy in patients with essential hypertension: the NICE Combi (Nifedipine and Candesartan Combination) Study. *J Hypertens* 2005; **23**: 445–453.
 97. Nakamura T, Sugaya T, Kawagoe Y, Suzuki T, Ueda Y, Koide H, Inoue T, Node K: Azelnidipine reduces urinary protein excretion and urinary liver-type fatty acid binding protein in patients with hypertensive chronic kidney disease. *Am J Med Sci* 2007; **333**: 321–326.
 98. Ogawa S, Mori T, Ito S: Combination therapy with renin-angiotensin system inhibitors and the calcium channel blocker azelnidipine decreases plasma inflammatory markers and urinary oxidative stress markers in patients with diabetic nephropathy. *Hypertens Res* 2008; **31**: 1147–1155.
 99. Tsuchihashi T, Ueno M, Tominaga M, Kajioka T, Onaka U, Eto K, Goto K: Anti-proteinuric effect of an N-type calcium channel blocker, cilnidipine. *Clin Exp Hypertens* 2005; **27**: 583–591.
 100. Fujita T, Ando K, Nishimura H, Ideura T, Yasuda G, Isshiki M, Takahashi K; Cilnidipine versus Amlodipine Randomised Trial for Evaluation in Renal Disease (CARTER) Study Investigators: *Kidney Int* 2007; **72**: 1543–1549.
 101. Abe M, Okada K, Maruyama T, Maruyama N, Matsumoto K: Comparison of the antiproteinuric effects of the calcium channel blockers benidipine and amlodipine administered in combination with angiotensin receptor blockers to hypertensive patients with stage 3–5 chronic kidney disease. *Hypertens Res* 2009; **32**: 270–275.
 102. Hart P, Bakris GL: Calcium antagonists: Do they equally protect against kidney injury? *Kidney Int* 2008; **73**: 795–796.
 103. Ge W, Ren J: Combined L-/T-type calcium channel blockers: ready for prime time. *Hypertension* 2009; **53**: 592–594.