Ca\textsuperscript{2+} Channel Subtypes and Pharmacology in the Kidney

Koichi Hayashi, Shu Wakino, Naoki Sugano, Yuri Ozawa, Koichiro Homma, Takao Saruta

Abstract—A large body of evidence has accrued indicating that voltage-gated Ca\textsuperscript{2+} channel subtypes, including L-, T-, N-, and P/Q-type, are present within renal vascular and tubular tissues, and the blockade of these Ca\textsuperscript{2+} channels produces diverse actions on renal microcirculation. Because nifedipine acts exclusively on L-type Ca\textsuperscript{2+} channels, the observation that nifedipine predominantly dilates afferent arterioles implicates intrarenal heterogeneity in the distribution of L-type Ca\textsuperscript{2+} channels and suggests that it potentially causes glomerular hypertension. In contrast, recently developed Ca\textsuperscript{2+} channel blockers (CCBs), including mibefradil and efonidipine, exert blocking action on L-type and T-type Ca\textsuperscript{2+} channels and elicit vasodilatation of afferent and efferent arterioles, which suggests the presence of T-type Ca\textsuperscript{2+} channels in both arterioles and the distinct impact on intraglomerular pressure. Recently, aldosterone has been established as an aggravating factor in kidney disease, and T-type Ca\textsuperscript{2+} channels mediate aldosterone release as well as its effect on renal efferent arteriolar tone. Furthermore, T-type CCBs are reported to exert inhibitory action on inflammatory process and renin secretion. Similarly, N-type Ca\textsuperscript{2+} channels are present in nerve terminals, and the inhibition of neurotransmitter release by N-type CCBs (eg, cilnidipine) elicits dilation of afferent and efferent arterioles and reduces glomerular pressure. Collectively, the kidney is endowed with a variety of Ca\textsuperscript{2+} channel subtypes, and the inhibition of these channels by their specific CCBs leads to variable impact on renal microcirculation. Furthermore, multifaceted activity of CCBs on T- and N-type Ca\textsuperscript{2+} channels may offer additive benefits through nonhemodynamic mechanisms in the progression of chronic kidney disease. (Circ Res. 2007;100:342-353.)

Key Words: afferent arteriole ■ efferent arteriole ■ Ca\textsuperscript{2+} channel blockers ■ renal microcirculation ■ voltage-dependent Ca\textsuperscript{2+} channels ■ renal disease ■ efonidipine ■ mibefradil

The kidney is supplied with a large amount of blood from the heart and is committed to multifaceted functions that are requisite for the preservation of body fluid and electrolyte homeostasis. Furthermore, the kidney is a major target organ for the complications in pathological conditions such as hypertension and diabetes, which would increase the risk for cardiovascular events. Numerous antihypertensive agents have been developed to blunt the progression of chronic kidney disease, including angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists. Ca\textsuperscript{2+} channel blockers (CCBs) are also used widely as a first-line antihypertensive agent, and the inhibition of L-type Ca\textsuperscript{2+} channels with CCBs, including nifedipine, diltiazem, and nitrendipine, elicits marked increases in glomerular filtration rate and renal blood flow.\textsuperscript{1-4} These observations indicate substantial distribution of L-type Ca\textsuperscript{2+} channels within the renal vascular bed. Of note, it has been demonstrated that these CCBs elevate filtration fraction,\textsuperscript{1-4} a marker for glomerular capillary pressure. Because glomerular filtration is controlled by preglomerular (afferent) and postglomerular (efferent) arterioles as well as the mesangial ultrafiltration, these CCBs may act predominantly on the preglomerular arterioles.

Recently, a growing body of evidence has accumulated showing important roles of T-type Ca\textsuperscript{2+} channels in various organs, such as cardiac sinus node and adrenal gland, which serve to generate pacemaker potential and release aldosterone, respectively.\textsuperscript{5} Furthermore, newly developed CCBs, including efonidipine, nilvadipine, and mibefradil, have been demonstrated to possess blocking activity on T-type as well as L-type Ca\textsuperscript{2+} channels.\textsuperscript{6-9} Interestingly, these CCBs exert renal microvascular action distinct from conventional CCBs (eg, nifedipine); these agents cause a lower increase in filtration fraction\textsuperscript{10-12} and greater proteinuria-sparing action.\textsuperscript{13,14} Furthermore, the blockade of other Ca\textsuperscript{2+} channel subtypes (eg, N-type) is reported to exert unique action that leads to reduction in glomerular hypertension.\textsuperscript{15} Such renal action would allow us to speculate that T-type/N-type Ca\textsuperscript{2+} channels participate in the regulation of the renal microvascular tone and that the distribution of these Ca\textsuperscript{2+} channels differs from that of L-type Ca\textsuperscript{2+} channels. The putative intrarenal distribution of these Ca\textsuperscript{2+} channels implicates the kidney as a unique organ that would facilitate the characterization of Ca\textsuperscript{2+} channel subtypes.

The purpose of this review is to provide an overview of the role of Ca\textsuperscript{2+} channels in the kidney, with special references to Ca\textsuperscript{2+} channels subtypes, including L-type, T-type, and N-type Ca\textsuperscript{2+} channels. Furthermore, we review the current under-
standing of the effect of CCBs in the renal microcirculation and the pathophysiological process of renal injury.

**Ca²⁺ Channel Subtypes**

Voltage-gated Ca²⁺ channels are classified into L-, P/Q-, N-, R-, and T-type subtypes by their pharmacological and electrophysiological properties and comprise heteromeric multi-subunits, including α₁, α₂, β, δ, and γ (skeletal muscle). Among these, the α₁ subunit possesses main characteristics of the channel, such as ion-conducting pore, ion selectivity, and voltage sensitivity, and is encoded by CACNA1 gene family consisting of 10 genes (Figure 1). In the kidney, a number of Ca²⁺ channels comprising various α₁ subunits, including Ca²⁺V2.1 (α₁A), Ca²⁺V1.2 (α₁C), Ca²⁺V1.3 (α₁D), Ca²⁺V3.1 (α₁G), and Ca²⁺V3.2 (α₁H), are expressed, and function as L-type (Ca²⁺V1.2, Ca²⁺V1.3), T-type (Ca²⁺V3.1, Ca²⁺V3.2), and P/Q-type (Ca²⁺V2.1) Ca²⁺ channels; 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 contain N-type (α₁H) Ca²⁺ channels. Interestingly, P- (Ca²⁺V2.1a) and Q-type Ca²⁺ channel subunits (Ca²⁺V2.1b) are splice variants of a single gene (ie, CACNA1A) and are expressed in the afferent arteriole. Although splice variants have been identified in neuronal and cardiac cells, as well as in vascular smooth muscle cells from atherosclerotic tissues, whether these variants affect the renal function remains undetermined.

**Effect of CCBs on Renal Function and Microvessels**

Afferent and efferent arterioles exist adjoining the glomerulus and adjust their vascular tone in response to various vasoactive stimuli. The responsiveness of these arterioles, however, should differ with respect to efficient adjustment of glomerular filtration. Indeed, atrial natriuretic peptide causes afferent arteriolar dilation and efferent arteriolar constriction. Furthermore, elevated renal perfusion pressure, endotelin, and high potassium elicit predominant constriction of the afferent arteriole.

When administered in vivo, nifedipine causes a greater increase in glomerular filtration rate than that in renal plasma flow, resulting in elevated filtration fraction. Furthermore, nicardipine and verapamil are reported to increase filtration fraction. These observations strongly suggest predominant action on the afferent arteriole. In the in vivo settings, however, 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 and colleagues used the isolated perfused rat normal kidney model. This model allows constant renal perfusion pressure, whereby the myogenic tone of renal microvessels is unaltered. In a series of their experiments, they found that under angiotensin II- or norepinephrine-induced vasoconstrictor tone, CCBs including nifedipine, nisoldipine, diltiazem, and amiodipine caused greater increases in glomerular filtration rate than those in renal plasma flow, resulting in exaggerated increases in filtration fraction. Thus, these observations again support the formulation that the CCB acts predominantly on the renal preglomerular vessels.

Recent advance in renal physiology facilitates more direct observation of renal microcirculation. Ca²⁺ sellas and Navar developed an in vitro technique that allows direct visualization of the juxtamedullary nephron circulation. In their experiments, both verapamil and diltiazem potently inhibited the afferent arteriolar vasconstriction, whereas efferent arterioles were relatively refractory to the vasodilator action of these agents. Similarly, Ito and colleagues developed the isolated renal cortical microvessel model and found that nifedipine predominantly dilated the afferent arteriole.

Loutzenhiser, Epstein, and colleagues developed a model of the isolated perfused hydropnephrotic kidney that facilitates direct observation of the renal microvasculature under defined in vitro conditions. Using this model, we demonstrated that both dihydropyridine class (eg, nifedipine, nicardipine, and amlodipine) and benzothiazepine class (eg, 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. Furthermore, we have observed that nifedipine...
causes predominant dilation of the afferent arteriole in the canine kidney, using the intravital pencil-lens charge-coupled device camera videomicroscopy (Figure 2C) (see below).10 Alternatively, direct visualization of the renal microcirculation with the isolated perfused hydronephrotic kidney24 and isolated microvessels41 demonstrates that high K–induced membrane depolarization selectively constricts the afferent arteriole, whereas the effluent arteriole is relatively insensitive to the depolarization. Furthermore, a Ca2+/H1 agonist (eg, Bay K-8644), which directly activates voltage-dependent Ca2+/H1 channels, causes preferential afferent arteriolar constriction.42

The preferential afferent arteriolar action of the CCB suggests predominant distribution of L-type Ca2+ channels in this vessel. Indeed, Hansen et al43 have demonstrated that the mRNA encoding Ca2+/H1 L-type Ca2+/H1 channel subunits is expressed in afferent arterioles from rabbit cortical preglomerular arterioles (Table). In contrast, no subunit was found in cortical effluent arterioles, although these channel subunits were expressed at juxtamedullary effluent arterioles. Similarly, it has been demonstrated that Ca1.2 preferentially prevails at the rat afferent arteriole, whereas the effluent arteriole lacks in this subunit (K. Ono, personal communication, 2005). These observations thus endorse the functional evidence indicating preferential activity of L-type Ca2+ channels at the afferent, but not effluent, arteriole.

**Effect of T-Type CCBs on Renal Microvessels**

In contrast to preferential action of the conventional types of CCBs on preglomerular arterioles, a growing body of evidence has been accumulated demonstrating that certain types of CCBs may affect postglomerular as well as preglomerular arterioles.

Intrarenal Localization of Calcium Channel Subtypes

<table>
<thead>
<tr>
<th>Juxtamedullary</th>
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<tr>
<td><strong>L-type</strong></td>
<td></td>
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<tr>
<td>Ca1.2(α1D)</td>
<td>+343</td>
</tr>
<tr>
<td>Ca2.1(α1D)</td>
<td>+17,103</td>
</tr>
<tr>
<td><strong>P/Q-type</strong></td>
<td></td>
</tr>
<tr>
<td>Ca2.1(α1D)</td>
<td>+17,103</td>
</tr>
<tr>
<td><strong>T-type</strong></td>
<td></td>
</tr>
<tr>
<td>Ca3.1(α1D)</td>
<td>+43</td>
</tr>
<tr>
<td>Ca3.2(α1D)</td>
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Superscripted numbers represent the applicable references.

Figure 2. Effects of various Ca2+ channel blockers on in vivo renal microvessels and renal hemodynamics. A, Changes in filtration fraction, a marker for glomerular pressure, vary depending on the CCBs used (from Honda et al10). B, Direct in vivo visualization of renal microcirculation with the use of intravital pencil-type charge-coupled device videomicroscopy (from Matsuda et al43). C, CCBs with preferential blockade of L-type Ca2+ channels cause predominant afferent arteriolar action (nifedipine), whereas CCBs with blocking activity on L-/T-type Ca2+ channels dilate both afferent and effluent arterioles (efonidipine and mibefradil). Cilnidipine with L-/N-type Ca2+ channel–blocking action dilates both microvessels, although the response is greater in the afferent arteriole (from Honda et al10). #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.
vascular tone. Takabatake et al found that the intravenous administration of manidipine caused a greater increase in renal plasma flow than that in glomerular filtration rate in spontaneously hypertensive rats (SHRs), resulting in decreased filtration fraction. Furthermore, nilvadipine is reported to increase renal plasma flow without any changes in glomerular filtration rate in humans. Yokoyama et al also reported that efonidipine potently increased renal plasma flow more markedly than glomerular filtration rate, causing a decrease in filtration fraction. Collectively, the observations obtained in vivo strongly suggest that these CCBs decrease efferent arteriolar resistance. Furthermore, the assessment of renal arteriolar resistance with the use of renal function curves indicates an efferent arteriolar dilation by bendipidine in human nondiabetic nephropathy.

Very recently, a growing body of direct evidence for efferent arteriolar action of certain types of CCBs has accumulated. Tojo et al have reported that manidipine elicits both afferent and efferent arteriolar dilation in the in vivo hydronephrotic kidney model, although the magnitude of the efferent arteriolar dilation is still less than that of the afferent arteriolar dilation. Using the microdissected renal arterioles, Arima et al also reported that manidipine caused efferent as well as afferent arteriolar dilation. Furthermore, Kawabata et al reported in a rat micropuncture study that efonidipine reduced both pre- and postglomerular capillary resistance. Finally, we have demonstrated that several CCBs, including nilvadipine, manidipine, bendipidine, efonidipine, mibebradil, and arandipine, cause substantial dilation of efferent arterioles in the isolated perfused rat hydronephrotic kidney (Figure 3A).

There exists some possibility that the results obtained from the hydronephrotic kidney model might be distorted because of the nature of its experimental setting. To circumvent this possibility, we further extended our premise, with the use of the intravital pencil-lens charge-coupled device camera videomicroscopy (Figure 2B). This experimental technique is unique because in vivo, in situ, and relatively intact renal microcirculation is accessible by simply introducing the pencil-lens probe into the renal cortex, without disrupting the renal microvascular responsiveness to tubuloglomerular feedback mechanism or angiotensin II. Using this experimental technique, we confirmed heterogeneity in the action of CCBs on the renal microcirculation. Thus, nifedipine elicited predominant dilation of the afferent arteriole, whereas both efonidipine and mibebradil caused marked dilation of efferent, as well as afferent, arterioles (Figure 2C).

Finally, cilnidipine elicited substantial dilation of the efferent arteriole, although the vasodilator response was greater in the afferent arteriole. These findings parallel the observation that filtration fraction is elevated with nifedipine, is unaltered with cilnidipine, tends to decrease with efonidipine, and is reduced with mibebradil (Figure 2A).

Collectively, these results strongly support our formulation that the renal microvascular effects of CCBs vary depending on the types of the antagonists used.

Several pharmacological studies demonstrate that certain types of CCBs possess blocking activity not only on L-type but also on other types of Ca²⁺ channels. Nickel chloride is a well-known CCB that inhibits the T-type Ca²⁺ current, although the selectivity for T-type and L-type Ca²⁺ current is not high. Mibebradil has been developed and is used as a selective T-type CCB, although it has been withdrawn from the market because of adverse effects related to drug interaction. A series of the subsequent studies have revealed that numerous CCBs available for clinical use exert blocking.
action on both L-type and T-type Ca\(^{2+}\) current, including nilvadipine, manidipine, and efonidipine.\(^6,7,60\) Recently, Furukawa and colleagues\(^66,61\) have surveyed the blocking activity of various CCBs on L-/T-/N-type Ca\(^{2+}\) current in Xenopus oocytes. They demonstrate predominant action of nifedipine, nitrendipine, and nimodipine on L-type Ca\(^{2+}\) channels, whereas these agents caused only modest inhibition of T-type Ca\(^{2+}\) channels. In contrast, efonidipine, benidipine, and manidipine potently inhibited the T-type as well as L-type Ca\(^{2+}\) channels.

The nature of the potent inhibitory action of T-type CCBs may bear on the efferent arteriolar action. In several microvasculature, including mesenteric\(^62\) and cremaster arterioles, a T-type Ca\(^{2+}\) channel subunit at superficial efferent, as well as afferent juxtamedullary nephrons (Table). Furthermore, the presence of T-type Ca\(^{2+}\) channels are distributed substantially, and the blockade of these channels by mibefradil, a selective T-type CCB, inhibits the vasoconstriction of these arterioles. The effect of mibefradil, however, may be attributed to the blocking action on L-type Ca\(^{2+}\) channels.\(^64\) In the renal microvasculature, Hansen et al\(^43\) have demonstrated that T-type Ca\(^{2+}\) channels, as assessed by \(\alpha_{\text{Ca}}\) 3.1 (an \(\alpha_{\text{Ca}}\) subunit of T-type Ca\(^{2+}\) channels), prevail at juxtamedullary efferent arterioles, as well as afferent arterioles of superficial and juxtamedullary nephrons (Table). Furthermore, the presence of a Ca\(^{3+}\) 3.1 subunit at superficial efferent, as well as afferent arterioles, has recently been found with the use of in situ hybridization (K. Ono, personal communication, 2005). Consistent with these observations, Nakamura et al\(^65\) found that mibefradil decreased both afferent and efferent arteriolar resistance in SHR kidneys, using the micropuncture technique. Ozawa et al\(^45\) have directly visualized the efferent arteriolar tone. Consequently, these novel findings strongly suggest a critical role of T-type Ca\(^{3+}\) channels in mediating the efferent arteriolar tone.

Some controversy remains regarding the role of T-type Ca\(^{2+}\) channels in vascular tone. Recently, mice deficient in Ca\(^{3+}\) 3.1 T-type Ca\(^{2+}\) channels manifest normal contractile responses but reduced relaxation in response to acetylcholine.\(^59\) Moosmang et al\(^64\) have also demonstrated that mibefradil has no effect on blood pressure or peripheral resistance in conditional knockout mice of Ca\(^{3+}\) 1.2 L-type Ca\(^{2+}\) channels. Indeed, we have found that R(−)-enantiomer of efonidipine, which possesses more selective blocking activity on T-type Ca\(^{2+}\) channels than efonidipine,\(^71-73\) fails to reduce blood pressure in hypertensive rats.\(^74\) Nevertheless, angiotensin II upregulates the Ca\(^{3+}\) 3.1 T-type Ca\(^{2+}\) channels in vascular smooth muscle cells.\(^74\) Furthermore, we and other laboratories have found that the Ca\(^{3+}\) 3.1 subunit is substantially expressed in the kidney,\(^43,74,75\) where angiotensin II is abundantly present. Finally, Ozawa et al\(^45\) showed that mibefradil completely inhibited the nifedipine-resistant renal efferent arteriolar tone preconstricted by angiotensin II. It requires further investigations, however, to determine whether T-type Ca\(^{2+}\) channels, particularly Ca\(^{3+}\) 3.1 (\(\alpha_{\text{Ca}}\) 10), serve to control the renal microvascular tone.

Besides afferent and efferent arterioles, T-type Ca\(^{2+}\) channels are reported to be present in other renal vascular segments. Hansen et al\(^43\) have demonstrated that Ca\(^{3+}\) 3.1 and Ca\(^{3+}\) 3.2 are present in vasa recta (Table). In combination with the previous finding that efonidipine causes efferent arteriolar dilation,\(^10,38,39\) the elevated luminal pressure at the vasa recta would diminish tubular sodium reabsorption and, therefore, may contribute to the greater natriuresis by efonidipine than by nifedipine.\(^55\)

**Mechanism for T-Type CCB-Induced Efferent Arteriolar Vasodilation**

A recent pharmacological study has indicated that efonidipine possesses the blocking activity on T-type, as well as L-type, voltage-dependent Ca\(^{2+}\) channels.\(^6,80\) Although T-type Ca\(^{2+}\) channels are closely associated with pacemaking potentials,\(^57\) the role of these Ca\(^{3+}\) channel subtypes in the vasculature remains poorly understood. Furthermore, the mechanism whereby T-type Ca\(^{2+}\) channel activity modifies the intracellular vasoconstrictor signaling pathway, and thus dilates efferent arterioles, remains unknown.

Angiotensin II–induced vasoconstriction of renal arterioles involves 2 main intracellular signaling pathways, protein kinase C (PKC) and inositol-1,4,5-trisphosphate (IP\(_3\))–induced intracellular Ca\(^{2+}\) release.\(^76,77\) In the efferent arteriole, we previously demonstrated that during angiotensin II stimulation, both PKC- and IP\(_3\)-associated vasoconstrictor mechanisms were activated in an additive manner.\(^55,64\) In this regard, mibefradil is reported to inhibit the PKC-mediated signaling pathway and prevent the vascular smooth muscle contraction in the vascular smooth muscle cell.\(^78\) Furthermore, Sipido et al\(^79\) reported that T-type channel activation facilitated Ca\(^{2+}\) release from sarcoplasmic reticulum in cardiac myocytes. We therefore examined the interactions between these intracellular mechanisms and mibefradil-induced vasodilation. Thus, the PKC-mediated pathway is relatively refractory to the vasodilator action of mibefradil. In the presence of thapsigargin, whereby angiotensin II should stimulate the PKC-mediated vasoconstrictor pathway dominantly,\(^76,77\) mibefradil had only a modest effect on the efferent arteriolar constriction. In contrast, in the presence of staurosporine, the angiotensin II–induced vasoconstriction of efferent arterioles is highly sensitive to the vasodilator action of mibefradil. Because staurosporine prevents the PKC-mediated constrictor mechanism, the major remaining vasoconstrictor mechanism of angiotensin II should be an IP\(_3\)-mediated Ca\(^{2+}\) release pathway.\(^76,77\) In concert, these observations are consistent with the view that the IP\(_3\)-mediated pathway constitutes an important target for the action of mibefradil during the angiotensin II–induced arteriolar constriction.

Although the link between T-type Ca\(^{2+}\) channels and sarcoplasmic Ca\(^{2+}\) release remains determined, recent investigations suggest an intimate interaction between the
plasma membrane and the endoplasmic reticulum, which is proposed as a conformational coupling model.80 Thus, T-type Ca\(^{2+}\) channels within the plasma membrane may communicate with the sarcoplasmic Ca\(^{2+}\) regulation. Indeed, T-type channel activation is reported to facilitate Ca\(^{2+}\) release from sarcoplasmic reticulum in cardiac myocytes.79,81 Further studies are required to determine how T-type Ca\(^{2+}\) channels interact with this constrictor mechanism.

Additional mechanisms for the efferent arteriolar dilation by recently developed CCBs merit comment. It has been reported that T-type Ca\(^{2+}\) channel activation stimulates renin release. Wagner et al82 have demonstrated that mibebradil suppresses renin release. This observation raises the possibility that T-type Ca\(^{2+}\) channel blockade inhibits angiotensin II production and, therefore, would be anticipated to contribute in part to the efferent arteriolar vasodilation. Furthermore, Arima and colleagues83,84 have recently demonstrated that aldosterone causes efferent arteriolar constriction that involves T-type Ca\(^{2+}\) channel–mediated vasomotor tone. Finally, we have recently found that mibebradil and efomidipine prevent the angiotensin II–induced stimulation of the Rho kinase pathway in vascular smooth muscle cells.85 Because Rho-kinase enhances the vascular tone of the efferent arteriole,86–89 it is anticipated that Rho-kinase mediates the efferent arteriolar tone induced by T-type Ca\(^{2+}\) channels.

**Role of N-Type and P/Q-Type Ca\(^{2+}\) Channels in Renal Microvascular Tone**

Besides L-type and T-type CCBs, several CCBs possessing both L- and N-type Ca\(^{2+}\) channel–blocking activity have been developed.91,92 and cilnidipine is clinically available in Japan.93,94 This class of the CCB is unique because of its pharmacological characteristics, ie, the inhibitory action on norepinephrine secretion95,96 and neurally stimulated renal vasoconstriction.97 As shown in Figure 3A, both cilnidipine and pranidipine elicit predominant action on the afferent arteriole in the in vitro isolated perfused hydrenephrotic kidney.98 In contrast, we also found that cilnidipine caused substantial vasodilation of efferent, as well as afferent, arterioles in the canine kidney in vivo (Figure 2C). Similar findings have been reported in a study using the in vivo hydrenephrotic kidney model99 (Figure 3B) and in a renal micropuncture study showing decreases in both afferent and efferent arteriolar resistance in nitro-L-arginine methyl ester–treated SHRs.15 Because the sympathetic nerve is distributed along afferent and efferent arterioles, the inhibition of N-type Ca\(^{2+}\) channels would dilate both arterioles. Indeed, amloidipine, which possesses the inhibitory action on L-type and N-type Ca\(^{2+}\) channels,61,100 is reported to cause substantial dilation of efferent, as well as afferent, arterioles in the in vivo hydrenephrotic kidney,101 whereas it predominantly dilates the afferent arteriole in the in vitro isolated perfused hydrenephrotic kidney (Figure 3B).90 Furthermore, the ability of bendipine and mibebradil to dilate the efferent arteriole is exaggerated in the in vivo hydrenephrotic kidney (K. Kimura, personal communication, 1994) and in the canine kidney in vivo,10 respectively, when compared with the action observed in the in vitro isolated hydrenephrotic kidney.45,102 In contrast, no such enhancement is observed with nifedipine or manidipine, neither of which possesses N-type Ca\(^{2+}\) channel–blocking activity. The apparently discrepant observations in the in vivo and in vitro experimental settings suggest the requirement of the integrity of sympathetic nerves for the full action of N-type CCBs.

Several recent studies extend our knowledge regarding the distribution of P/Q-type Ca\(^{2+}\) channels, which have been reported to prevail abundantly in neuronal cells. Hansen and colleagues17,103 demonstrated the presence of Ca\(_{\text{V}1.2}\) (\(\alpha_{1c}\)) in rat preglomerular arterioles, using RT-PCR and immunostaining (Table). They further showed that the functional role of P/Q-type Ca\(^{2+}\) channels in mediating the KCl-induced constriction of the afferent arteriole.17 In concert, available evidence indicates that several Ca\(^{2+}\) channel subtypes are present in the kidney and serve to modulate the renal microvascular tone.

**Ca\(^{2+}\) Channel Subtypes in Renal Tubules**

It has been demonstrated that Ca\(_{\text{V}3.1}\) (\(\alpha_\text{v}_3\)) is expressed in cells of the distal tubules as well as outer and inner medullary collecting ducts (Table).104 Although this channel subtype is localized more predominantly on the basolateral membrane than apical membrane, physiological roles of this channel remain undetermined.

Andreasen et al175 examined the nephron localization of Ca\(_{\text{V}3.3}\) (\(\alpha_\text{v}_3\)) in the inner medullary collecting ducts, distal collecting ducts and connecting tubules, particularly on the apical site (Table). On the basis of the fact that epithelial sodium channels are localized to the same cells, depolarization induced by sodium absorption through epithelial sodium channels could activate apical T-type Ca\(^{2+}\) channels.

A recent investigation by Brunette et al105 has indicated that several CCBs, including diltiazem, mibebradil, and \(\omega\)-conotoxin MVIIC (a P/Q-type CCB), inhibit the Ca\(^{2+}\) transport through the membrane from the distal tubule. They suggest that these channels belong to the L-type, T-type, and P/Q-type Ca\(^{2+}\) channels. The functional significance of these channels requires further investigation. Ca\(^{2+}\) overload induced by ischemia106 and chronic renal injury107 teleologically may be prevented by the blockade of these Ca\(^{2+}\) channels.

**Hemodynamic Effects of L-/T-/N-Type CCBs in Renal Injury**

Based on the renal microcirculatory action, it is inferred that whereas the depressor action of traditional CCBs favors an attenuation of glomerular hypertension and the subsequent renal protection,98,108–110 the predominant activity on preglomerular vessels might cause glomerular hypertension that could finally be associated with the progression of kidney diseases.111–115 Thus, the changes in these 2 factors may vary depending on the experimental settings, magnitude of depressor activity, and types of the CCBs used. Thus, verapamil is reported to reduce proteinuria and protect against renal injury in remnant kidney models.116,117 Furthermore, Dworkin and colleagues118–120 demonstrated that nifedipine reduced both urinary protein excretion and glomerular injury in subtotally nephrectomized rats, uninephrectomized SHRs, and deoxy-corticosterone acetate (DOCA)-salt hypertensive rats, despite
the persistent glomerular hypertension. Contrasting results have also been reported indicating deleterious effects of dihydropyridine class CCBs in kidney diseases.\textsuperscript{111–114,118,121,122} Wenzel et al\textsuperscript{121} demonstrated that nitrendipine increased proteinuria and glomerulosclerosis in a 2-kidney, 1-clip model of hypertension. Furthermore, Dworkin et al\textsuperscript{118} found that amldipine did not exhibit renoprotective action in DOCA-salt hypertensive rats. In the ALLHAT (\textit{Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial}), glomerular filtration rate was well preserved in patients on amlodipine therapy during the trial period, when compared with patients on lisinopril or chlorthalidone treatment.\textsuperscript{123} In a large-scale clinical trial evaluating the renal protective effect of antihypertensive agents in African-Americans with hypertensive kidney disease (AASK), however, amlodipine is less effective in retarding the decline of renal function than ramipril.\textsuperscript{124} Thus, the ability of the CCBs with predominant activity on afferent arterioles may vary depending on the level of blood pressure achieved.

In contrast, novel CCBs acting on both afferent and efferent arterioles theoretically correct glomerular hypertension and could exert salutary actions on the progression of renal injury. Shudo and colleagues\textsuperscript{125–127} reported that efonidipine acutely decreased proteinuria in spontaneously hypertensive rats, whereas systemic blood pressure was only partially reduced. Furthermore, Fujimaki et al\textsuperscript{128} found that manidipine exerted salutary action on renal structure in uninephrectomized spontaneously hypertensive rats. Mibe-fradil has also been reported to mitigate renal injury in SHRs\textsuperscript{65} and DOCA hypertensive rats.\textsuperscript{129,130} A similar beneficial effect was observed with arandipine,\textsuperscript{50} which possessed the blocking activity on T-type Ca\textsuperscript{2+} channels.\textsuperscript{66} In this regard, we previously demonstrated that 8-week treatment with efonidipine markedly prevented the increase in proteinuria, whereas nifedipine did not reduce it despite the same reduction in systemic blood pressure.\textsuperscript{13} Of note, efonidipine reduced proteinuria to the same level as enalapril, which causes both afferent and efferent arteriolar dilation. The salutary action of the T-type CCB is also evident in a clinical setting. Thus, in patients with nondiabetic kidney disease, 12-month treatment with efonidipine reduced proteinuria to the same level as that with angiotensin-converting enzyme inhibitors.\textsuperscript{131} Of importance, in the patients in whom mean systemic blood pressure did not achieve a level below 100 mm Hg, proteinuria was significantly decreased. Although obviously the beneficial effects of T-type CCBs are not totally ascribed to glomerular hemodynamic action, the proteinuria-reducing effect of T-type CCBs would suggest an important role of a glomerular hemodynamic factor (ie, efferent arteriolar dilation) in blunting the progression of renal disease.

Analogous to the action of T-type CCBs, N-type CCBs would modulate glomerular capillary pressure and subsequently provide beneficial action in renal disease. Cilnidipine has been reported to suppress the elevation in blood pressure and blunt the progression of renal injury in Dahl salt-sensitive rats\textsuperscript{132} and ameliorate glomerular injury and proteinuria in Dahl rats with high-sucrose diet.\textsuperscript{133} Furthermore, this CCB exerts antiproteinuric action in patients with essential hypertension.\textsuperscript{134}

A caveat is in order, however, because preferential afferent arteriolar dilation following renal injury\textsuperscript{135} may influence the action of CCBs. Thus, the ability of afferent arteriole–selective CCBs to ameliorate glomerular hypertension may depend largely on whether systemic hypertension is corrected in this circumstance. In contrast, the CCBs acting on efferent arterioles would predispose glomerular capillary pressure to reduce in addition to hypotensive action. Consequently, the contrasting effects of these antihypertensive agents on proteinuria may be unique in chronic kidney disease.

**Nonhemodynamic Effects of L-/T-/N-Type CCBs in Renal Injury**

Besides glomerular capillary pressure, multiple mechanisms appear to contribute to the ability of CCBs to protect against renal injury. For example, CCBs (nifedipine and cilnidipine) are reported to prevent mesangial cell proliferation by inhibiting activator protein-1.\textsuperscript{1,136} CCBs are also shown to suppress the cell cycle transition from the G1 to S phase (benidpine)\textsuperscript{137} and modulate gene transcriptions that are involved in proinflammatory changes, such as interleukin-1\beta and granulocyte/macrophage colony stimulating factors (manidipine).\textsuperscript{138} Furthermore, CCBs could act as free radical scavengers (nifedipine, amlodipine, nilvadipine).\textsuperscript{139–141} Finally, CCBs (nifedipine and bendipine) inhibit the apoptotic or necrotic processes induced by tumor necrosis factor-\alpha or cycloheximide.\textsuperscript{142}

Although the observations described above lend support to the premise that the CCB exerts salutary actions in renal injury process, it remains undetermined whether these effects are mediated by the blockade of specific Ca\textsuperscript{2+} channel subtypes. It has been shown that T-type Ca\textsuperscript{2+} channels participate importantly in cell differentiation and proliferation\textsuperscript{143} and contribute to the platelet-derived growth factor–induced vascular smooth muscle cell migration.\textsuperscript{144} These pathophysiologic processes could reflect inflammatory tissue injury and may emerge in kidney disease. Indeed, efonidipine has been shown to suppress the A23187+ phorbol myristate acetate–induced activation of nuclear factor-\kappaB in cultured human mesangial cells.\textsuperscript{145} In contrast, L-type CCBs (verapamil and nifedipine) have no effect on this activation. Furthermore, Baylis et al\textsuperscript{130} demonstrated that 4 to 5 weeks of administration of mibefradil to DOCA-salt rats ameliorated proteinuria and glomerular damage, whereas amlodipine failed to improve renal injury. Of note, both amlodipine and mibebradil exerted similar antihypertensive actions and decreased glomerular capillary pressure to the same level. Based on the properties of these CCBs, it appears that the T-type CCB confers greater benefit, distinct from that provided by the L-type CCB.

Recent investigations have demonstrated that Rho-kinase participates in the progression of various types of renal disease,\textsuperscript{146,147} including subtotally nephrectomized rats,\textsuperscript{148} Dahl salt–sensitive rats,\textsuperscript{149} DOCA-salt rats,\textsuperscript{150} and angiotensin II–infused renal injury.\textsuperscript{151} We have found that mibebradil and efonidipine suppress the angiotensin II–stimulated Rho-kinase activity more pronouncedly than nifedipine in vascular
smooth muscle cells. Furthermore, \( R^- \)-enantiomer of efonidipine (a more selective T-type CCB) downregulates GTP-RhoA (an upstream molecule for Rho-kinase). When administered to subtotally nephrectomized rats, \( R^- \)-enantiomer of efonidipine fails to reduce blood pressure but markedly suppresses proteinuria and tubulointerstitial changes. Although the possibility remains that this CCB affects glomerular capillary pressure and subsequently ameliorates renal pathological damages, the striking improvement in the renal tubulointerstitial fibrosis suggests that direct salutary actions of the T-type CCB contribute to the amelioration of tubulointerstitial injury rather than the glomerular hemodynamic action. Finally, it has recently been demonstrated that the angiotensin II signaling pathway enhances T-type \( \text{Ca}^{2+} \) current and upregulates CaV3.1 in cardiomyocytes. We also have found that angiotensin II enhances the expression of CaV3.1 in vascular smooth muscle cells and mesangial cells. Furthermore, the increased expression of CaV3.1 is observed in the renal tissue from subtotally nephrectomized rats, which intrarenal angiotensin II contributes to the progression of renal injury. This observation therefore allows speculation that the T-type \( \text{Ca}^{2+} \) channel blockade offers more pronounced renal protective effect in chronic kidney disease.

A growing body of evidence has accumulated indicating that aldosterone promotes renal injury. Conversely, the blockade of aldosterone action exerts salutary effect on the progression of renal injury in both humans and experimental animals. Rossier et al. found that the aldosterone release provoked by angiotensin II and KCl was inhibited by mibefradil but not by nicardipine, suggesting an important contribution of T-type \( \text{Ca}^{2+} \) channels to aldosterone release. Lotshaw has reported similar results, showing an important role of T-type \( \text{Ca}^{2+} \) channels in mediating the aldosterone secretion from adrenal glomerulosa cells. Furthermore it has recently been demonstrated that efonidipine is more potent than nifedipine in inhibiting the angiotensin II–and KCl-induced aldosterone secretion from H295R cells. Of note, aldosterone is reported to induce renal injury partly through the activation of Rho-kinase, and the Rho-kinase pathway is suppressed by a selective T-type CCB, \( R^- \)-enantiomer of efonidipine. Collectively, the T-type CCB serves to mitigate the renal injury in which aldosterone play a role as a deteriorating factor.

The pharmacological action of N-type CCBs in kidney disease merits comment. Because N-type CCBs inhibit sympathetic nerve activity, this type of the CCB could exert beneficial action in chronic kidney disease, in which sympathetic nervous system activity is increased. Indeed, Konda et al. demonstrated that 8-week treatment with cilnidipine decreased plasma concentrations of norepinephrine and renin activity and caused reductions in blood pressure.
pressure and improvement in glomerular sclerosis in Dahl salt-sensitive rats. Furthermore, they compared the effects of cilnidipine with those of amlodipine on the progression of renal injury in Dahl salt-sensitive rats fed a high-sucrose diet and found that cilnidipine provided superior protection against renal damage compared with amlodipine. Although amloidipine also possesses N-type Ca2+ channel–blocking activity, the elevated levels of urinary norepinephrine excretion and renal renin mRNA expression suggest that the N-type blocking activity of amloidipine is not as potent as that of cilnidipine. Another N-type CCB, prandipine, is also shown to reduce blood pressure and exert antiproteinuric and renoprotective actions in subtotally nephrectomized rats and Dahl salt-sensitive rats. 

Although prandipine does not dilate the efferent arteriole in the in vitro isolated perfused hydropneumonic kidney, the property of N-type Ca2+ channel–blocking action within this CCB could lead to the direct alterations in glomerular hemodynamics.

Concluding Remarks

Substantial advances have been made regarding our knowledge of the renal distribution of Ca2+ channel subtypes. Furthermore, characterization of the CCB facilitates the renal action of this class of the agent (Figure 4). It is established that L-type CCBs cause predominant dilation of the afferent arteriole. In contrast, a large amount of evidence has accrued indicating that T-type CCBs exert renal protective action by ameliorating glomerular microcirculation with the property of vasodilator action on both afferent and efferent arterioles. Additionally, the blockade of T-type Ca2+ channels will be important to the pathogenesis of renal injury and may constitute a potential target for the treatment of hypertensive kidney.

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Disclosures

None.

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