Heterogeneity of L- and T-Channels in the Vasculature
Rationale for the Efficacy of Combined L- and T-Blockade

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Abstract—Clinical studies suggest that T-type Ca$^{2+}$ channel blockade may have incremental benefits over conventional L-channel blockade, particularly in microvascular disorders. This study examined functional vasomotor differences in L- and T-channel blockade between large and small vessels and compared the abundance of the L- and T-type channels in these vessels. The inhibition of endothelin-1 and potassium-induced vascular contractile responses by L-channel blockers (verapamil and nifedipine) was compared with combined L- and T-channel blockers (mibefradil and efonidipine) in large (rat aorta) and small (rat mesenteric and human subcutaneous) vessels using wire myography. All 4 of the Ca$^{2+}$ channel blockers inhibited contractile responses to a similar extent in large rat vessels; however, in rat microvessels, the combined L- and T-channel blockers produced significantly greater inhibition of contraction than L-channel blockers alone. The significance of this differential T-channel effect in microvessels was further supported by the following: (1) a greater abundance of T-channels compared with L-channels in microvessels but not in large vessels; (2) demonstration of divergent Ca$^{2+}$ channel blocker responses in human microvessels; (3) incremental inhibition of constrictor responses with combined L- and T-Ca$^{2+}$ channel blockers despite maximal L-channel blockade; (4) the use of structurally diverse Ca$^{2+}$ channel blockers with varied affinity for L- and T-channels; (5) the use of pharmacodynamically and therapeutically appropriate Ca$^{2+}$ channel blocker concentrations; (6) confirmation of contractile agonist independent responses; and (7) exclusion of an endothelium-dependent mechanism. We propose that T-type channels play an important role in regulating contractile responses in the microvasculature and, therefore, are a potential therapeutic target. (Hypertension. 2009;53:00-00.)

Key Words: calcium ■ calcium channel blockers ■ vessels ■ vasoconstriction ■ vasculature

Calcium (Ca$^{2+}$) channel blockers (CCBs) have a major therapeutic role in the management of cardiovascular disorders, particularly as antihypertensive and antianginal agents. These clinical effects are mediated via the inhibition of transmembrane Ca$^{2+}$ channels on vascular smooth muscle cells, thus reducing Ca$^{2+}$ ion influx, resulting in reduced vascular smooth muscle contraction and vascular tone. Although chemically diverse, clinically established CCBs such as nifedipine (a dihydropyridine CCB) and verapamil (a phenylalkylamine CCB) mediate their effects via a common mechanism, namely the inhibition of the long-acting voltage-dependent Ca$^{2+}$ channels (L-channel). In contrast, some new-generation CCBs, eg, mibefradil and efonidipine, have additional effects, such as inhibition of the transient Ca$^{2+}$ channel (T-channel).

Although the clinical benefits of L-channel blockade are well established, the benefits of T-channel blockade remain less clear. Previous clinical studies have suggested that T-channel blockade may have incremental antianginal benefits over L-channel blockade alone. For example, the Posicor Reduction of Ischemia During Exercise Study demonstrated improved exercise treadmill parameters with mibefradil compared with diltiazem (a benzothiazepine L-CCB). Moreover, in patients with microvascular dysfunction, on background verapamil therapy, a single dose of mibefradil substantially improved coronary angiographic flow. Similarly, the antihypertensive literature reports unique benefits of efonidipine in reducing proteinuria in hypertensive patients with renal impairment, unlike conventional L-CCBs. Consistent with this renal effect, Hayashi et al have demonstrated that T-CCBs dilate both afferent and efferent glomerular arterioles, whereas L-CCBs dilate only the afferent microvessels, thereby explaining the apparent differential benefits of efonidipine compared with conventional L-CCBs in reducing proteinuria.

The above clinical benefits may be attributable to differential vascular expression of L- and T-channels. Gustafsson et al demonstrated the presence of T-channel mRNA but an absence of detectable levels of L-channel mRNA in microvessels <40 μm in diameter. However, in contrast to these findings, Moosmang et al demonstrated that mibefradil’s blood pressure lowering and increased hindlimb perfusion effects were absent in an L-channel conditional knockout mouse model, thereby proposing that mibefradil’s vascular effects were mediated via the L-channel only.
The above clinical, pathophysiological, and genetic studies suggest that there may be both species differences and perhaps a differential distribution of L- and T-channels in the vasculature. However, these findings have received little attention and, hence, there are no detailed comparative studies evaluating segmental heterogeneity in vascular Ca^{2+} channels. Thus, the primary objectives of this study were to determine, in human and rat models, whether there were functional differences in responses to L- and T-channel blockade based on vessels size and to identify the relative distribution of these channels in the large and small vessels.

Methods

To achieve the above objectives, both “functional” vasomotor studies using an in vitro myograph model and “structural” Ca^{2+} channel quantification studies using quantitative western blot analysis were undertaken. These studies were performed on rat vascular tissues derived from large (thoracic aorta) or small (mesenteric) vascular segments. Adult male Sprague-Dawley rats, aged 8 weeks and weighing ~400 g, were euthanized under fluorothane anesthesia and 2-mm segments from the above vessels excised.

In addition to the rat vessels, human subcutaneous microvessels were obtained from patients undergoing elective lower abdominal surgery to determine the applicability of the microvascular vasomotor findings in a human model. Patients without a history of cardiovascular disease and not prescribed vasoactive agents were recruited preoperatively, and written consent was obtained. These investigations were approved by the institutional animal and human ethics committees, respectively.

Functional Vasomotor Studies

Myograph Preparation

Rat aorta (large) and mesenteric (small) vessels were mounted in a wire myograph (multi myograph model 610 M, Danish Myo Technology) and the resting tension normalized using the procedure described by Mulvany and Aalkjaer. This normalizing method provides a resting tension equivalent to a vessel circumference of 90% of the value at 100 mmHg intraluminal pressure. Accordingly, comparisons between vessels of different sizes can be undertaken because they have proportionate resting tensions. The vessels were continually bathed in Krebs solution at 37°C and gassed with Carbogen (95% oxygen, 5% carbon dioxide). The Krebs solution was of the following composition (mmol/L): NaCl (118), KH_{2}PO_{4} (1.18), NaHCO_{3} (25), MgCl_{2} (1.05), CaCl_{2} (2.54), EDTA (0.01), and glucose (5.56; pH 7.4). After a 30-minute equilibration, baseline contractile responses to a depolarizing solution, potassium physiological salt solution was obtained by replacing the NaCl in Krebs with iso-osmolar potassium chloride (KCl). The mean of the final 2 potassium physiological salt solution responses was used as a reference value for other contractile responses.

After establishing a concentration-response curve to phenylephrine, vessels were precontracted to 75% of the maximal response and endothelial integrity assessed with incremental doses of an endothelium-dependent vasodilator (bradykinin [BK] 0.001 to 3.000 μmol/L) for human microvessels; acetylcholine [ACH] 0.001 to 30.000 μmol/L for rat vessels). The endothelium was considered intact if the contractile response was reduced >80% by the endothelium-dependent vasodilator. To determine the influence of the endothelium on the CCB responses, the endothelium was removed in selected vessels, and loss of endothelial integrity was confirmed using ACh.

Study Protocol

The experimental protocol used a paired-sample design with 1 vascular segment incubated in the study CCB and the other in the drug vehicle, thereby providing both a temporal and vehicle control.

The vascular rings were incubated for a 30-minute period with an L-CCB (verapamil or nifedipine), a combined L- and T-channel blocker (efonidipine or mibefradil), or the corresponding vehicle control. After this incubation period, vasoconstrictor agents (endothelin-1 [Et-1] or KCl) were administered and contractile responses recorded using Chart 5 (ADI Instruments).

The study involved 4 series of experiments. The first set of experiments explored the vasomotor effects of various CCB concentration ranges. Concentrations equivalent to the therapeutic plasma levels in CCB clinical studies were initially used. These included verapamil 1 μmol/L (Abbott), nifedipine 1 μmol/L (Sigma-Aldrich), mibefradil 1 μmol/L (Sigma-Aldrich), and efonidipine 0.021 μmol/L (Nissan Chemical Industries, Ltd). Ten-fold higher and lower CCB concentrations were then used to assess the comparative concentration ranges.

The second series of experiments assessed the effect of the various CCBs at their respective plasma therapeutic concentrations on receptor-mediated vasoconstrictor responses. In these experiments, incremental doses of Et-1 were administered and concentration-response curves obtained. Et-1 was selected as the agonist because of its sustained contractile responses and clinical relevance.

In a third series of experiments, the effect of administering an L- and T-type channel blocker on constrictor responses, in vessels exposed to maximal L-channel blockade, was assessed. This later experiment endeavored to “pharmacologically reproduce” the L-channel conditional knockout model established by Moosmang et al and to shed light on the use of combined L- and T-channel blockade in patients on existing CCB therapy.

Finally, the effect of the various CCBs at their therapeutic plasma concentrations on depolarizing-mediated constrictor responses with 87 mmol/L of KCl was assessed.

Data Analysis

The inhibition of Et-1 and KCl contractions in response to the CCBs were expressed as a percentage of the mean potassium physiological salt solution responses. For Et-1, constrictor responses, sigmoid curves of best fit were constructed using nonlinear regression (GraphPad Prism, version 4.0a) with the EC_{50} and the concentration for maximal response (E_{max}) subsequently derived. For KCl-mediated depolarization responses, initial contraction was calculated for each CCB. Comparisons between CCBs in EC_{50}, E_{max}, and initial contraction was assessed using ANOVA with Bonferroni correction. Data were presented as means±SEM, *P*<0.05 was considered statistically significant, and “n” refers to the number of samples taken from independent experimental units.

Structural Quantification of Pore-Forming Subunits of Ca^{2+} Channels

Quantitative western blot analysis was used to identify the abundance of the pore-forming subunits of L- (Ca_{1,2}) and T- (Ca_{3,1,3.2}) channels in the large and small vessels. Specific antibodies included the following: (1) anti-Ca_{1,2}, which recognizes the α1C subunit of the L-channel (polyclonal, rabbit IgG, Chemicon); (2) anti-Ca_{3,1}, which recognizes the α1G subunit of the T-channel (polyclonal, rabbit IgG, Sigma-Aldrich); and (3) anti-Ca_{3,2}, which recognizes the α1H subunit of the T-channel (polyclonal, rabbit IgG, Sigma-Aldrich).

Protein Extraction

The α1 subunits of the L-channel are known to be highly sensitive to proteases; therefore, to prevent degradation, all of the protein samples were extracted in the presence of Calpain Inhibitor 1 (Calbiochem) and broad-spectrum protease inhibitor Complete Mini tablets (Roche Applied Sciences). The vessels were extracted in an aqueous solution containing a 1:10 dilution of the calpain/protease inhibitor mix, 1 mmol/L of diithiothreitol, 50 mmol/L of Tris (pH 8.6), 30% glycerol, 0.001% bromophenol blue, and 2% sodium dodecyl sulfate. After extraction, the samples were heated to 70°C for 5 minutes.
Western Blotting Procedure
Vessel samples were analyzed by SDS-PAGE, coomassie brilliant blue staining, and western blot. Densitometric scanning of stained gels and western blots enabled us to adjust sample volumes to ensure equal loading of samples and, importantly, to ensure that samples were in the linear range for quantitative western blot analysis. Total protein extracts were analyzed using 7.5% mini-gels and run with SDS-PAGE at 200 volts for 1 hour. Proteins were electrophoretically transferred onto 0.2-μm nitrocellulose membranes, followed by blocking of nonspecific antibody interacting sites using 5% nonfat dried milk powder in Tris-buffered saline (25 mmol/L of Tris-HCl [pH 7.5] and 150 mmol/L of NaCl) containing 0.01% Tween 20 (TBS-T), solution for 1 hour. Antibody detection of the pore-forming subunit of voltage-sensitive Ca\(^{2+}\) channels was carried out in solutions containing 1% nonfat dried milk powder in Tris-buffered saline with 0.01% Tween 20 containing the anti-Ca\(^{2+}\)-1.2 antibody (1:500 dilution), anti-Ca\(^{2+}\)-3.1 antibody (1:500), or anti-Ca\(^{2+}\)-3.2 antibody (1:20000) each for 1 hour. Membranes were washed and then incubated with an antirabbit IgG-horseradish peroxidase–conjugated secondary antibody for 1 hour (1:10 000 dilution). Blots were then briefly exposed to enhanced chemiluminescence reagents and signals detected using autoradiographic film.

Protein Quantification
To quantify the relative abundance of the pore-forming subunit of each Ca\(^{2+}\) channel present in the large and small vessels, the autoradiographic exposures of the western blots were scanned (BioRad GS-710 Imaging Densitometer) and the channel signal in each lane determined with the program QuantityOne. The coomassie blue stained gel was also scanned to identify equivalent protein loading in each lane. The abundance of all of the Ca\(^{2+}\) channels was represented as the ratio of optical density for T/L channels to account for minor differences in protein loading. Statistical differences were calculated using Student t tests with P<0.05 taken to be significant and “n” referring to the number of samples taken from independent experimental units.

Results

Functional Vasomotor Studies

Ca\(^{2+}\) Channel Blocker Concentrations
The initial experiments exploring the CCB concentration ranges were undertaken in rat mesenteric vessels (n=5 per CCB) with Et-1-induced constrictor responses. As illustrated in Figure 1, the maximal inhibition produced by the therapeutic plasma level equivalent concentration for verapamil and nifedipine was similar to that produced by the 10-fold higher concentration, suggesting near maximal effects for the L-CCBs at the therapeutic plasma level (E\(_{max}\): verapamil 1 μmol/L=82±6%, 10 μmol/L=72±3%, P<0.05; nifedipine 1 μmol/L=76±3%, 10 μmol/L=67±2%, P<0.05). For the combined L- and T-CCBs, the therapeutic plasma concentrations used were also near maximal (E\(_{max}\): efonidipine 0.021 μmol/L=45±2%, 0.21 μmol/L=41±4%, P<0.05; mibefradil 1 μmol/L=36±4%, 10 μmol/L=29±2%, P<0.05). Furthermore, there were no significant differences in the Et-1 EC\(_{50}\)s across the CCB concentration ranges for any of the CCBs. Hence, comparisons between the exclusive L-channel blockers (verapamil and nifedipine) with the combined L- and T-channel blockers (efonidipine and mibefradil) are both of pharmacodynamic and therapeutic relevance.

Receptor-Mediated Depolarization

Rat Aortic Vessel Responses
The aortic rings had a mean diameter of 2055±35 μm and intact endothelium-dependent vasodilator responses with a mean maximal ACh relaxation of 91±1% (n=7). As shown in Figure 2A, pretreatment with verapamil, nifedipine, efonidipine, or mibefradil significantly reduced Et-1 contractile responses compared with control (E\(_{max}\): 83±6%, 79±5%, 96±3%, and 100±4% versus control 159±6%, respectively; P<0.05). However, as shown in the Table, there is no difference between the CCBs in the extent of inhibition of the maximal Et-1 contractile responses.

Rat Microvascular Responses
Rat mesenteric microvessels had a mean diameter of 304±7 μm and intact endothelium-dependent vasodilator responses with a mean maximal ACh relaxation of 87±2% (n=6). Pretreatment
with verapamil, nifedipine, efonidipine, or mibefradil inhibited Et-1 contractile responses compared with control (E_max: 82 ± 6%, 76 ± 3%, 45 ± 2%, and 36 ± 4% versus control 115 ± 3%, respectively; P < 0.05; see Figure 2B). As shown in the Table, the combined L- and T-CCBs (efonidipine and mibefradil) inhibited Et-1 contractile responses almost twice as effectively as the L-CCBs (verapamil and nifedipine) in these microvessels.

In 6 independent experiments, the endothelium was removed from mesenteric microvessels (mean vessel diameter: 314 ± 13 μm) by gentle rubbing against the lumen of the vessel, and impaired endothelium-dependent vasodilator responses were confirmed using ACh. Pretreatment with verapamil, nifedipine, efonidipine, or mibefradil in these endothelium-denuded vessels inhibited Et-1 contractile responses (E_max: 88 ± 4%, 83 ± 5%, 47 ± 1%, and 45 ± 3%, respectively, versus control 140 ± 2%; P < 0.05; see Figure 2C). As shown in Figure 2C, the combined L- and T-CCBs (efonidipine and mibefradil) inhibited Et-1 contractile responses almost twice as effectively as the L-CCBs (verapamil and nifedipine) in these microvessels.

**Human Subcutaneous Microvascular Responses**

The 17 subjects (55 ± 4 years; 11 women) recruited to the study had no known history of cardiovascular disease, although several had cardiovascular risk factors, including hypercholesterolemia (29%), hypertension (24%), cigarette smoking (29%), and diabetes mellitus (12%). No patient was being prescribed vasodilator or statin therapy.

Subcutaneous microvessels were obtained during noncardiac surgery and mounted in the myograph. The mean vessel diameter (at resting normalized tension) was 289 ± 14 μm, and endothelium-dependent vasodilator responses to BK were intact in all of the vessels with a mean maximal BK relaxation of 87 ± 5%. The human microvascular responses to the CCBs were similar to those of the rat microvessels (Figure 3), with a significantly greater inhibitory effect on Et-1-mediated contractile responses by the combined L- and T-CCBs compared with L-channel blockade alone (P < 0.05; Table).

**Inhibitory Effect of Efonidipine in Rat Microvessels With Maximal L-Channel Blockade**

In rat mesenteric vessels pretreated with maximal L-channel blockers (either verapamil 10 μmol/L or nifedipine 10 μmol/L), efonidipine was administered to ascertain whether there was incremental inhibition of the constrictor response with the combined L- and T-CCBs. Despite complete verapamil or nifedipine-mediated L-channel blockade, efonidipine produced incremental inhibition of Et-1 constrictor responses, suggesting that mechanisms other than L-channel blockade were involved (E_max difference relative to control: verapamil alone, −49 ± 3%; verapamil/efonidipine, −64 ± 3%; nifedipine alone, −48 ± 6%;

![Figure 2](image1.png)  
**Figure 2.** Et-1-mediated developed tension in rat aortic and mesenteric vessels in the presence of CCBs. Concentration-response curves to Et-1 after 30-minute incubation with verapamil (1 μmol/L, ■), nifedipine (1 μmol/L, □), efonidipine (0.021 μmol/L, ●), mibefradil (1 μmol/L, ○), or control/solvent vehicle (†). A, In rat aorta, there was a significant inhibition of the Et-1 E_max between the control and each of the CCBs, although the extent of inhibition was not different between the CCBs (n = 7). B, In rat mesenteric microvessels, there was also a significant inhibition of the Et-1 E_max by each of the CCBs; however, the combined L- and T-channel blockers produced greater inhibition than the L-channel blockers (n = 6). C, In endothelium-denuded rat mesenteric microvessels, the differential inhibitory effects of the combined L- and T-channel blockers compared with the L-channel blockers remained evident (n = 6).

![Table](image2.png)  
**Table. Change in Et-1 E_max by Various CCBs**

<table>
<thead>
<tr>
<th></th>
<th>L-Channel CCB</th>
<th>L- and T-Channel CCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔE_max Relative Control</td>
<td>Verapamil</td>
<td>Nifedipine</td>
</tr>
<tr>
<td>Rat aorta</td>
<td>−69 ± 1%</td>
<td>−75 ± 11%</td>
</tr>
<tr>
<td>Rat microvessel*</td>
<td>−37 ± 7%</td>
<td>−43 ± 9%</td>
</tr>
<tr>
<td>Human microvessel*</td>
<td>−40 ± 7%</td>
<td>−56 ± 8%</td>
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</table>

*Data show the significant difference in reducing E_max by combined L- and T-channel blockers compared with L-channel blockers (ANOVA, P < 0.05).

![Figure 3](image3.png)  
**Figure 3.** Effectiveness of L- and combined L- and T-channel blockade in human subcutaneous microvessels. Concentration-response curves to Et-1 in human subcutaneous microvessels after 30-minute incubation with verapamil (1 μmol/L, ■), nifedipine (1 μmol/L, □), efonidipine (0.021 μmol/L, ●), mibefradil (1 μmol/L, ○), or control/solvent vehicle (†). There was a significant inhibition of the Et-1 E_max between the control (123 ± 8%) and each of the CCBs, verapamil (81 ± 4%), nifedipine (66 ± 1%), efonidipine (38 ± 2%), and mibefradil (30 ± 4%) (P < 0.05 vs control, **P < 0.05 vs L-channel blockade; n = 6).
nifedipine/efonidipine, −67±2%; *P<0.05; n=5; Figure 4, only verapamil data shown).

**High Potassium-Mediated Depolarization**

*Rat Aorta Vessel Responses*

As shown in Figure 5A, pretreatment with verapamil, nifedipine, efonidipine, or mibefradil significantly reduced the initial KCl contractile responses compared with control (KCl: 20±1%, 20±2%, 17±2%, 18±1%, respectively; *P<0.05; n=4). However, there was no difference between the CCBs in the extent of inhibition of contraction.

*Rat Mesenteric Vessel Responses*

As expected, pretreatment with verapamil, nifedipine, efonidipine, or mibefradil significantly inhibited initial KCl contractile responses compared with control (KCl: 12±1%, 12±1%, 2±1%, and 3±2%, respectively; *P<0.05; n=4; see Figure 5B). Furthermore, as observed with receptor-mediated responses, the combined L- and T-CCBs (efonidipine and mibefradil) inhibited KCl contractile responses to a greater extent than L-channel blockade alone (verapamil and nifedipine) in these microvessels.

**Structural Ca^{2+} Channel Quantification Studies**

**Western Blotting Validation**

To ensure accurate comparisons in quantitative western blotting, linear range experiments were performed for each tissue type and antibody. Sample loading beyond 10 μL was identified to be at saturation point, with the linear range for each tissue type between 5 and 10 μL and with *r*² values ranging from 0.98 to 1.00 (Figure 6).

**Rat Vascular L- and T-Channel Abundance**

Quantitative western blot analysis of Ca_{1.2} L-channel protein, Ca_{3.1} T-channel protein, and Ca_{3.2} T-channel protein in rat aorta revealed no relative differences in the amounts of channel expressed in these tissues. However, analysis of the rat mesenteric vessels identified a significant increase in the optical density of Ca_{3.1} T-channel (0.90±0.05 OD) and Ca_{3.2} T-channel (1.23±0.18 OD) compared with the Ca_{1.2} L-channel (0.47±0.17 OD). Using ratiometric analysis, these values equate to a significant increase of 112±38% expression of the Ca_{3.1} T-channel and a significant increase of 163±48% expression of the Ca_{3.2} T-channel (n=4; Figure 7).

**Discussion**

The above experiments demonstrate segmental heterogeneity in vascular responses between conventional L-CCBs (verapamil and nifedipine) and newer agents with combined L- and T-Ca^{2+} channel-blocking properties (efonidipine and mibefradil). Specifically, we have identified that there were no differences between CCBs in the inhibition of contractile responses in the large conduit vessels; however, in the microvasculature of both humans and rats, combined L- and T-channel blockers were far more effective at attenuating contraction. The significance of this observation is further supported by the following: (1) a relative increase in the abundance of the pore-forming subunits Ca_{3.1} and Ca_{3.2} of the T-channel compared with the Ca_{1.2} subunit of the L-channel in the microvessels only (Figure 7); (2) consistent results in both human and animal models (Figures 2B and 3); (3) reproducibility with mechanistically different vasoconstrictor stimuli (ie, receptor-mediated Et-1 or depolarization-mediated KCl; Figures 2 and 5); (4) incremental inhibition of contractile responses by efonidipine in the presence of maximal L-channel blockade (Figure 4); (5) pairing of CCBs with similar chemical structure (verapamil: phenylalkylamine; nifedipine and efonidipine: dihydro-
pyridine; and mibefradil: benzimidazole); (6) the use of CCB concentrations that are near maximal for all of the CCBs and equivalent to their therapeutic plasma levels (Figure 1); and (7) exclusion of an endothelium-dependent mechanism (Figure 2B and 2C). These functional and structural findings suggest that T-channels may play a significant role in human microvascular tone and provides a mechanism whereby combined L- and T-CCBs may have additional therapeutic benefits over conventional L-channel blockers.

Role of T-Channels in the Vasculature

Efonidipine9,10 and mibefradil11 inhibit T-channel currents significantly more than the L-channel currents in isolated vascular smooth muscle preparations. Other investigators have also suggested that mibefradil may be more effective in the microvasculature. Kung et al12 demonstrated that mibefradil dilates endothelin-contracted porcine small coronary vessels more effectively than larger coronary arteries. VanBavel et al13 showed that mibefradil is more potent than verapamil in inhibiting myogenic tone in rat cremasteric muscle arterioles.

Recently, Moosmang et al,7 using a conditional L-channel knockout mouse model, demonstrated a loss of mibefradil’s vasomotor effects, including impairment of its blood pressure–lowering effect and a reduction in its inhibition of vasoconstrictor effects in a hindlimb perfusion model. Appropriately, these researchers concluded that, in their mouse model, vasomotor effects of mibefradil were mediated exclusively via the L-channel. We have pharmacologically reproduced the inactivation of L-channels using maximal concentrations of the L-channel blockers (Figure 4). Our functional data with human and rat microvessels indicate that L-channel blockade inhibits contractile responses by approximately half. However, the addition of T-channel blockade in these microvessels results in a further 30% reduction in contractile responses. These data support the notion that there are species differences among both rodent models and humans.
Clinical Implications
The new-generation CCBs, which have combined L- and T-channel–blocking properties, appear to have incremental clinical benefits over the conventional L-CCBs. As demonstrated in this study, these 2 groups of CCB agents differ in their pharmacodynamic responses at the small resistance vessel level. Because these vessels play a pivotal role in the regulation of blood pressure, renal perfusion, and coronary blood flow, additional benefits of the newer agents could be expected in disorders relating to these circulations. For example, in a comparison of the antihypertensive effects of diltiazem (an L-channel blocker) and mibebradil, the combined L- and T-channel blocker reduced blood pressure to a greater extent than the L-channel blocker.14 Furthermore, efonidipine has also been shown to have these same antihypertensive effects.15

Studies of the glomerular microcirculation have demonstrated L-channels in the afferent but not the efferent arteriole, whereas T-channels are found in both types of glomerular microvessels.16 Thus, L-channel blockers predominantly dilate the afferent arteriole and may produce glomerular hypertension, whereas T-channel blockade does not. This may explain why efonidipine and mibebradil have been shown to reduce proteinuria in hypertensive patients with renal impairment, whereas L-channel blockers do not.5

The coronary slow-flow phenomenon is a coronary microvascular disorder characterized by the delayed passage of contrast during angiography reflecting the increased downstream resistance.17 Patients with this disorder on maintenance verapamil therapy still exhibit the angiographic phenomenon. However, the addition of mibebradil acutely improves angiographic flow.3 Furthermore, mibebradil was shown to alleviate the angina associated with this microvascular disorder.1 Mibebradil has also been shown to reduce myocardial ischemia more effectively than diltiazem in atherosclerotic coronary artery disease.2 Our combination CCB experiments are consistent with these clinical findings such that the addition of efonidipine in the presence of maximal L-channel blockade produced incremental inhibition of microvascular constrictor responses.

Perspectives
The incremental microcirculatory benefits of the combined L- and T-channel blockers, efonidipine and mibebradil, over the conventional L-channel blockers are likely attributable to their additional T-channel blocking properties and the increased presence of T-channels compared with L-channels in the microvasculature of the rat. However, further studies aimed at quantifying T- and L-channels in the human vasculature and also defining the precise role of the T-channel with the newer CCBs and their role in the regulation of vascular tone are required. We believe that these studies provide a rational explanation for the beneficial effects of combined L- and T-channel blockers and provide the necessary data to warrant further investigation of the therapeutic value of these agents in cardiovascular conditions, particularly in those involving increased microvascular resistance.

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Disclosures
None.

References