Beneficial Effects of the Dual L- and T-Type Ca^{2+} Channel Blocker Efonidipine on Cardiomyopathic Hamsters

Shinsuke Suzuki, MD[†]; Tomoko Ohkusa, MD[†]; Katsushige Ono, MD^{*};
Takashi Sato, MD; Masa-aki Yoshida, MD; Masafumi Yano, MD;
Satoshi Takebayashi, MD^{*}; Masunori Matsuzaki, MD

Background The T-type Ca^{2+} channel (TCC) is activated, and abnormalities of the TCC may be related to the pathogenesis of Ca^{2+} overload, in cardiomyopathic hamster hearts. The aims of the present study were to investigate the alteration in expression of the TCC and to examine the effects of a dual L- and T-type Ca^{2+} channel blocker, efonidipine (EFO), on cardiac function and TCC during development of heart failure in UM-X7.1 cardiomyopathic hamsters.

Methods and Results UM-X7.1 and golden hamsters were examined, and EFO was administered at the age of 20 weeks for 4 weeks. Cardiac function was examined, the expression of TCC 1G was measured, and ventricular myocytes were subjected to a patch-clamp study. At 24 weeks, vehicle-treated UM-X7.1 hamsters exhibited significant increases in left ventricular (LV) size, with marked decreases in ejection fraction (LVEF) compared with golden hamsters. In the UM-X7.1 group, the expression of TCC 1G increased during development of heart failure compared with the golden hamster group. In the UM-X7.1 group, EFO treatment significantly attenuated the decrease of LVEF without affecting blood pressure compared with the vehicle group. EFO treatment decreased heart rate (by ~10%) in both groups. In the golden hamster group, EFO treatment did not affect LV function. The TCC current in ventricular myocytes was significantly increased in UM-X7.1, and was inhibited by EFO in a dose-dependent manner.

Conclusions In cardiomyopathic hamster hearts, abnormalities in the TCC may be at least in part related to the pathogenesis of abnormal Ca^{2+} homeostasis, and TCC-blocker treatment may decrease the TCC current, resulting in an improvement of cardiac function. TCC blocker therapy might be a new strategy for certain types of heart failure. (Circ J 2007; 71: 1970–1976)

Key Words: Ca^{2+} channel; Ca^{2+} channel blocker; Efonidipine; Heart failure; T-type Ca^{2+} channel

Voltage-gated Ca^{2+} channels play a critical role in transmembrane signaling in excitable cells. They are involved in diverse cellular functions, including excitation–contraction coupling, neurotransmitter release, and gene expression. In the cardiovascular system, only L- and T-type Ca^{2+} channels (LCC and TCC) are present and they differ by their structure, electrophysiological and pharmacological properties, function, and distribution.

Cardiac TCC have been implicated in cardiac hypertrophy.^{1,2} Under normal conditions, TCC currents are not expressed in adult ventricular myocytes; however, unusual expression of TCC currents has been found in hypertrophied ventricular myocytes.^{1,2} In BIO 14.6 cardiomyopathic hamsters, an abnormal increase in TCC current density may be responsible for Ca^{2+} overload in the heart,³ and in the

post-infarction remodeled rat left ventricle, the TCC gene and TCC current can be re-expressed.⁴ Recently, angiotensin II and endothelin-1 were reported to increase TCC currents in hypertrophied and failing myocardium, respectively.^{5,6} Thus, TCC channels appear to take part in the signaling pathway that controls differentiation and proliferation.

The UM-X7.1 cardiomyopathic hamster is known to develop a genetically determined cardiomyopathy, with development of progressive and ultimately fatal congestive heart failure (HF).⁷ The myocardium of the UM-X7.1 cardiomyopathic hamster shows evidence of intracellular Ca^{2+} overload, and it has been suggested that the excess free intracellular Ca^{2+} plays an important role in the pathogenesis of this disease.⁸ It has been postulated that increased influx of Ca^{2+} via voltage-sensitive Ca^{2+} channels may cause Ca^{2+} overload. Some studies have reported an increase of dihydropyridine binding sites in the cardiomyopathic hamster hearts, but others report conflicting findings.^{9,10} Both hemodynamic and clinical deterioration have been reported during short- and long-term therapy with verapamil, nifedipine, diltiazem, or nicardipine in patients and animals with chronic HF.^{11–13} These adverse effects might result from the cardiodepressant nature of LCC blockers and from their tendency to bring about neurohormonal activation. Therefore, LCC blockers have limited beneficial effects or even detrimental effects in the treat-

(Received June 18, 2007; revised manuscript received July 27, 2007; accepted August 7, 2007)

Division of Cardiology, Department of Medicine and Clinical Science, Yamaguchi University Graduate School of Medicine, Ube, *Department of Cardiovascular Science, Oita University School of Medicine, Oita, Japan

[†]These authors equally contributed to this work.

Mailing address: Tomoko Ohkusa, MD, Division of Cardiology, Department of Medicine and Clinical Science, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube 755-8505, Japan. E-mail: ohkusa@yamaguchi-u.ac.jp

ment of HF.

Our aim was to investigate the alteration in the expression of the TCC, and to examine the effects of efonidipine hydrochloride (EFO: dual LCC and TCC blocker) on cardiac function and TCC during the development of HF in UM-X7.1 cardiomyopathic hamsters.

Methods

Animals

Cardiomyopathic (UM-X7.1) hamsters (kindly provided by Dr Lemanski, SUNY Health Science Center, Syracuse, NY, and inbred in our laboratory) and sex- and age-matched normal golden hamsters (controls; Japan SLC Inc, Hamamatsu, Japan) were used as experimental animals. They were all maintained under identical conditions on a normal laboratory diet with ad libitum access to tap water. EFO was administered with diet (0.1% in diet) from the age of 20 weeks for 4 weeks. Our choice of this dose and timing of administration of EFO was based on a pilot study in which the results showed that the serum level of EFO significantly increased without producing a significant decrease in blood pressure in the hamsters. Also, according to our previous report,⁷ cardiac function in the UM-X7.1 hamster is impaired at the age of 18–20 weeks. The care of the animals and the protocols conformed with the Guiding Principles in the Care and Use of Animals and were in accord with guidelines laid down by the Animal Ethics Committee of Yamaguchi University Graduate School of Medicine. The investigation also conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

General Protocols and Study Objectives

To enable us to determine the left ventricular (LV) dimensions and assess the contractile state in vivo, transthoracic echocardiography was performed as previously described⁷ (echocardiograph model SSD-1000, Aloka, Tokyo, Japan, with a 10-MHz sector scan probe). After the echocardiographic examination was completed, the hamsters were given additional pentobarbital sodium (30 mg/kg, im) and the chest was quickly opened. The heart was excised from each hamster and immersed in ice-cold 0.9% NaCl. After atrial tissue, visible fat, and connective tissue had all been removed, the biventricular tissue was quickly frozen in liquid nitrogen and stored at -80°C until use. Blood samples were obtained at the time of death. Serum was prepared and frozen at -20°C for subsequent analysis of B-type natriuretic peptide (BNP) measured by radio immunoassay, according to previously described methods.¹⁴

Quantitative Reverse Transcription (RT) and Polymerase Chain Reaction (PCR) Amplification (RT-PCR)

Total cellular RNA was isolated from each frozen tissue sample by the method of acid guanidinium thiocyanate/phenol/chloroform extraction, then stored at -80°C . cDNA was prepared using a Takara RNA PCR Kit (Takara, Tokyo, Japan), as previously described.¹⁵ The PCR was performed as follows: to 20 μL of the RT reaction mixture was added 2 μL of 0.1 mol/L forward primer, 2 μL of 0.1 mol/L reverse primer, 8 μL of 10 $\times$ amplification buffer (100 mmol/L Tris-HCl, pH 8.3, 500 mmol/L KCl), 12 μL of 25 mmol/L MgCl_2 , 55 μL of H_2O , 0.5 μL of [$-^{32}\text{P}$]dCTP (Amersham), and 0.5 μL (2.5 U/100 μL) of Taq polymerase. The primers for the am-

plification of the TCC 1G and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed from published sequences. For the TCC 1G, they were based on rat sequences:¹⁶ at positions 3,906–3,924 (sense primer, 5'-TCA GAG CCT GAT TTC TTT-3') and 3,837–3,855 (antisense primer, 5'-CAG GAG ACG AAA CCT TGA-3'). For GAPDH they were based on human sequences:¹⁷ at positions 102–125 (sense primer, 5'-CTT CAT TGA CCT CAA CTA CAT GGT-3') and 805–828 (antisense primer, 5'-CTC AGT GTA GCC CAG GAT GCC CTT-3'). Next, 100 μL of reaction mixture was overlaid with 20 μL mineral oil, and cycling was performed by means of a thermal cycler (Perkin Elmer/Cetus, San Diego, CA, USA) using the following parameters: denaturation at 95°C for 1 min, annealing at 55°C for 45 s, extension at 72°C for 45 s, followed by a final incubation at 72°C for 10 min.

The optimal number of amplification cycles needed to allow quantitation of TCC 1G and GAPDH gene PCR products was determined. The PCR products for each cycle were subjected to 5% polyacrylamide gel electrophoresis and autoradiography, and the associated radioactivity was measured using an imaging analyzer (model BAS-2000; Fuji Photo Film Co, Tokyo, Japan). The optimal number of cycles was found to be 35 for both the TCC 1G and 24 for GAPDH.

The relative radioactivity associated with the TCC 1G PCR products in each sample was calculated by dividing the radioactivity associated with the TCC 1G PCR products by the radioactivity associated with the GAPDH gene product (internal control; amplified simultaneously). Each level of RT-PCR product was obtained as the average of duplicate data.

Immunologic Quantification of TCC 1G

Whole ventricular tissue was homogenized twice for 20 s each time, using a Brinkmann Polytron, in 20 mmol/L Tris maleate containing 0.3 mol/L sucrose, 0.1 mol/L KCl, 5 mg/L leupeptin, and 0.1 mmol/L phenylmethyl sulfonyl fluoride, at pH 7.0. The homogenate was centrifuged at 5,500 g for 20 min at 4°C . The supernatant was frozen in liquid nitrogen and stored at -80°C until used.

Immunoblot analysis was performed as previously described⁷ with some modifications. The membrane protein (50 μg protein/lane) was electrophoresed on 6% SDS-polyacrylamide gels. The proteins in the gel were transferred to a protran nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). The membranes were then treated with 5% nonfat dry milk in phosphate-buffer, incubated with anti-Cav3.1 antibody (1:1,000) (Alomone labs, Jerusalem, Israel) solution, and incubated further with peroxidase-conjugated secondary antibody (1:1,000 dilution).

The amount of protein recognized by the antibodies was quantified by means of an ECL immunoblotting detection system (Amersham, Bucks, UK), the membrane being exposed to X-ray film. Quantitative densitometry of immunoblots was performed using a microcomputer imaging device (AE-6900M, ATTO, Tokyo, Japan). The relative activity associated with TCC 1G protein in each sample was normalized to the value of a 4-week-old golden hamster, which was set to a value of 1.

Cell Isolation and Electrophysiological Recordings

UM-X7.1 and golden hamsters at the age of 20 weeks were heparinized (3 IU/g, IP) and anaesthetized with pentobarbital (50 mg/kg). LV myocytes were isolated enzymati-

Table 1 Effect of EFO on Somatic and Cardiac Growth

	UM-X7.1		Golden hamster	
	Untreated (n=7)	EFO (n=7)	Untreated (n=5)	EFO (n=5)
Body weight (g)				
20w	113±8 [§]	114±6 [§]	163±11	160±12
24w	115±9 [§]	127±6 ^{§,*,#}	188±12	168±11 [#]
Biventricular weight (mg)				
20w	344±29	350±22	334±32	338±30
24w	364±45	384±24 [*]	387±47	367±19
Biventricular weight/body weight (mg/g)				
20w	3.0±0.2 [§]	3.1±0.2 [§]	2.0±0.1	2.0±0.2
24w	3.2±0.3 [§]	3.0±0.2 [§]	2.1±0.2	2.2±0.1

Data are mean±SD.

[§]p<0.05 vs golden hamster, *p<0.05 vs baseline, #p<0.05 vs untreated group.

EFO, efonidipine; w, weeks.

Fig 1. Expression of T-type Ca^{2+} channel (TCC) 1G mRNA (A) and protein (B) significantly increased at 24 weeks in UM-X7.1 hamsters compared with age-matched golden hamsters. The relative radioactivity associated with TCC 1G polymerase chain reaction (PCR) products in each sample was calculated by dividing the radioactivity associated with the TCC 1G PCR products by the radioactivity associated with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene product (internal control; amplified simultaneously). The relative activity associated with TCC 1G protein in each sample was normalized to the value of a 4-week-old golden hamster, which was set to a value of 1.

cally from both groups of hamsters using a modified procedure of Wang et al.¹⁸ The cells were kept at room temperature and were studied within 6 h after isolation.

The ventricular myocytes from the UM-X7.1 and golden hamsters were subjected to a patch-clamp study (EPC 9, HEKA Elektronik, Lambrecht, Germany), as described previously.¹⁹ Ca^{2+} currents were recorded in an Na^{+} -free bath solution with the use of tetrodotoxin (TTX) (Sankyo Co, Tokyo, Japan) containing 140 mmol/L TEA-Cl, 1.8 mmol/L CaCl_2 , 2 mmol/L MgCl_2 , 10 mmol/L glucose, 10 mmol/L HEPES and 0.05 mmol/L TTX (pH 7.4 adjusted with tetraethylammonium hydroxide), and a pipette solution containing 130 mmol/L CsCl, 5 mmol/L MgATP, 5 mmol/L creatine phosphate, 0.5 mmol/L Na_2GTP , 10 mmol/L HEPES, and 10 mmol/L EGTA (pH 7.2 adjusted with CsOH). EFO was used to confirm the characters of I_{CaT} .

Statistical Analysis

All data are presented as mean±standard deviation. Comparisons were performed by 2-way analysis of variance. Differences were taken to be significant at $p<0.05$.

Results

Somatic and Cardiac Growth

Table 1 shows the morphological characteristics of the hamsters. Although the body weight of the UM-X7.1 hamsters was significantly smaller at each stage than that of the control hamsters, the biventricular-to-body weight ratio was significantly higher in the UM-X7.1 group. At 24 weeks, severe congestive HF associated with significant cardiac hypertrophy occurred in untreated UM-X7.1 hamsters.

The EFO-treated UM-X7.1 hamsters showed an increase in body weight with no remarkable increase in the biventricular-to-body weight ratio compared with 20-week-old UM-X7.1 hamsters (baseline). In the golden hamsters, although EFO treatment decreased body weight, it did not affect the ratio of biventricular weight to body weight.

Expression of TCC 1G

Fig 1 shows the relative expression level of TCC 1G (protein and mRNA) ($n=6-8$ for each group). In the UM-X7.1 hamsters, the expression of TCC 1G mRNA and protein increased during development of HF, compared with the golden hamsters (Figs 1A,B). The expression of TCC 1G mRNA and protein in the UM-X7.1 hamsters at the age of

24 weeks was significantly high compared with age-matched golden hamsters (Figs 1A,B). As shown in Fig 2, EFO treatment did not affect the expression of TCC 1G mRNA in either the golden and UM-X7.1 hamster groups.

Cardiac Function

Tables 2 and 3 show the changes in LV dimensions, contractile characteristics, blood pressure and heart rate in the UM-X7.1 and golden hamster groups. After 4 weeks (at 24 weeks of age), both the LV end-diastolic dimension (LVEDd) and LV end-systolic dimension (LVESd) were increased in the untreated UM-X7.1 hamsters and LV motion was reduced compared with baseline. In the UM-X7.1 hamsters, there was no significant difference in the LVEDd of untreated and EFO-treated animals at the 4-week treatment stage (Table 2). However, the LVESd was slightly, although not significantly, reduced and the LVEF was significantly increased in the EFO-treated group, compared with the untreated group at the 4-week treatment stage (Table 2). On the other hand, among the golden hamsters, EFO-treatment did not show significant changes in LVEDd, LVESd, or LVEF after 4 weeks of treatment (Table 2). EFO-treatment did not produce significant alterations in blood pressure in either UM-X7.1 or golden hamsters (Table 3). At 24 weeks of age, heart rate was significantly increased in UM-X7.1 hamsters compared with the golden hamsters. EFO treatment produced approximately 10% decrease in heart rate in both groups (Table 3).

Changes in BNP Level

To evaluate the severity of congestive HF, we measured

the serum BNP levels ($n=6$ for each group) (Fig 3). At 20 weeks of age, the BNP level was slightly, although not significantly, increased in the UM-X7.1 hamster group (35.2 ± 6.0 pg/ml) compared with the golden hamsters (26.3 ± 2.5 pg/ml). After 4 weeks, the BNP levels in untreated UM-X7.1 hamsters (40.5 ± 11.5 pg/ml) were significantly increased compared with the golden hamsters. However, EFO-treatment significantly decreased the BNP level in UM-X7.1 hamsters (30.5 ± 3.3 pg/ml) as compared with the untreated group, and there was no significant difference in BNP level compared with the EFO-treated golden group.

Fig 2. Effects of efonidipine (EFO) on the expression of T-type Ca^{2+} channel (TCC) 1G mRNA. Efonidipine treatment did not affect the expression of TCC 1G mRNA in either group. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 2 Echo Geometry and Cardiac Function

	UM-X7.1		Golden hamster	
	Untreated (n=7)	EFO (n=7)	Untreated (n=5)	EFO (n=5)
LVEDd (mm)				
20w	5.5±0.3 [§]	5.2±0.3 [§]	4.2±0.2	4.1±0.1
24w	6.4±0.4 ^{§,*}	6.4±0.3 ^{§,*}	4.6±0.2	4.3±0.1
LVESd (mm)				
20w	4.4±0.2 [§]	4.2±0.3 [§]	2.3±0.1	2.2±0.2
24w	5.3±0.5 ^{§,**}	5.0±0.3 ^{§,*}	2.4±0.4	2.2±0.4
EF (%)				
20w	47±3 [§]	45±5 [§]	79±6	75±9
24w	43±7 [§]	50±5 ^{§,#}	82±6	83±8

Data are mean±SD.

[§] $p<0.05$ vs golden hamster, * $p<0.05$, ** $p<0.01$ vs baseline, # $p<0.05$ vs untreated group.

LVEDd, left ventricular end-diastolic diameter; LVESd, left ventricular end-systolic diameter; EF, ejection fraction. Other abbreviations see in Table 1.

Table 3 Changes in HR and BP

	UM-X7.1		Golden hamster	
	Untreated (n=7)	EFO (n=7)	Untreated (n=5)	EFO (n=5)
BP (mmHg)				
20w	109±12/84±11	100±20/88±10	121±22/72±28	118±21/81±20
24w	93±25/63±23	110±21/81±20	123±7/82±12	106±13/70±10
HR (beats/min)				
20w	432±14	430±15	421±14	425±14
24w	438±27 [§]	406±42 ^{§,#}	397±26	362±23 [#]

Data are mean±SD.

[§] $p<0.05$ vs golden hamster, # $p<0.05$ vs untreated group.

HR, heart rate; BP, blood pressure. Other abbreviations see in Table 1.

Fig 3. Changes in the level of brain natriuretic peptide (BNP). After treatment with efonidipine (EFO) for 4 weeks, the BNP level in UM-X7.1 hamsters was significantly decreased compared with untreated UM-X7.1 hamsters. There was no significant difference in BNP level compared with EFO-treated golden group.

Fig 4. Recording of I_{CaL} and I_{CaT} . (A) Typical whole I_{Ca} family from a holding potential (HP) of -100 mV to various test potentials from a control golden hamster cardiomyocyte. I_{Ca} families from HPs of -100 mV (a), an HP of -40 mV (b), and their current subtraction (c). (B) Typical whole I_{Ca} family in a cardiomyocyte from a UM-X7.1 hamster. I_{Ca} families from a HP of -100 mV (a), a HP of -40 mV (b), and their current subtraction to isolate I_{CaT} (c). I_{Ca} families from a HP of -40 mV (Ab and Bb) represent I_{CaL} , and I_{Ca} families obtained by current subtraction (Ac namely Aa–Ab; Bc namely Ba–Bb) represent I_{CaT} .

Fig 5. Characteristics of I_{CaT} obtained from cardiomyopathic cardiomyocytes. (A) Current–voltage (I – V) relations of the peak Ca^{2+} currents, I_{CaL} and I_{CaT} , in cardiomyocytes obtained from the UM-X7.1 hamster illustrated in Fig 4 and (B) I_{CaT} traces with or without efonidipine ($0.33 \mu\text{mol/L}$ and $0.66 \mu\text{mol/L}$). I_{CaT} were recorded at a stimulation frequency of 0.2 Hz as described in Fig 4. HP, holding potential.

Ca^{2+} Channel Currents in Cardiomyocytes

I_{CaT} and I_{CaL} in Hypertrophied Myocytes Two components of the Ca^{2+} channel currents were recorded in myocytes obtained from the UM-X7.1 hamsters. Fig 4 shows representative Ca^{2+} currents obtained from a golden hamster myocyte and an UM-X7.1 hamster myocyte at the age of 20 weeks with the use of $50 \mu\text{mol/L}$ TTX. Subtraction of the currents shows the presence of a low-voltage-activated I_{CaT} in addition to a high-voltage-activated I_{CaL} in an UM-X7.1 hamster (Fig 4Bc). However, no I_{CaT} was detected in golden hamsters, as shown in Fig 4Ac ($n=5$, including datum in Fig 4A). The average current density (the maximum peak current) of I_{CaT} from UM-X7.1 hamster myocytes was -4.2 ± 0.5 pA/pF, which was appreciably larger than the average current density of I_{CaL} obtained from a holding potential of -40 mV (-3.8 ± 0.2 pA/pF, $n=6$). Moreover, we have observed I_{CaT} not only in LV but also in RV myocytes obtained from UM-X7.1 hamsters. Importantly, the I_{CaT} density in the LV (ibid.) and RV (-3.9 ± 0.6 pA/pF, $n=4$) are statistically identical. Fig 5A illustrates the current–voltage relationships (I – V curves) of I_{CaL} and I_{CaT} obtained from the UM-X7.1 hamster illustrated in Fig 4B. The I – V curve for I_{CaT} shows that activation of the current starts at approximately -70 mV, and reaches the maximum at -20 mV of the membrane potentials.

Fig 5B shows the effects of EFO on I_{CaT} in an LV myocyte from an UM-X7.1 hamster. At the concentrations of 0.33 $\mu\text{mol/L}$ and 0.66 $\mu\text{mol/L}$, EFO blocks the I_{CaT} via a tonic blocking action, demonstrating that the I_{CaT} expressed in a myocyte of an UM-X7.1 hamster is highly sensitive to the dual I_{CaL} and I_{CaT} blocker, EFO.

Discussion

This study provides data on the over-expression of the TCC and current during the development of HF, and the beneficial effects of EFO on cardiac dysfunction in the UM-X7.1 cardiomyopathic hamster. We demonstrated that (1) in this cardiomyopathic hamster model, TCC 1G mRNA expression markedly increased during development of HF; (2) in the UM-X7.1 group, untreated hamsters exhibited significant increase in LV size with marked decreases in EF, whereas EFO significantly increased LVEF without affecting blood pressure and decreased heart rate by approximately 10%; (3) EFO did not affect the expression of TCC 1G mRNA; and (4) the T-type Ca^{2+} current in ventricular myocytes was significantly increased in the cardiomyopathic hamster, and was inhibited by EFO in a dose-dependent manner. These results suggest a new therapeutic strategy using dual LCC and TCC blockers for certain types of HF.

TCC are generally observed in diverse cell types, including embryonic and neonatal ventricular myocytes that are known to disappear throughout postnatal development.⁶ As our data shows, in the golden hamsters the expression of the TCC (mRNA and protein) in cardiomyocytes was low, whereas in the UM-X7.1 cardiomyopathic hamster hearts the TCC was re-expressed during the development of HF. In the cardiomyopathic hamster, rat and feline, it is postulated that excessive Ca^{2+} overload may be related to the abnormal expression of the TCC.¹⁻³

We used the UM-X7.1 hamster to demonstrate that cardiomyopathic myocytes express I_{CaT} , whereas it was absent in myocytes isolated from the hearts of control golden hamsters. In this study, we carefully evaluated I_{CaT} , avoiding possible contamination with I_{Na} or $I_{Ca(TTX)}$ by using a Na^+ -free solution with an extremely high concentration of TTX (50 $\mu\text{mol/L}$), and were able to show that I_{CaT} was insensitive to TTX. Furthermore, the contribution of I_{CaT} to the whole I_{Ca} was confirmed by using a dual LCC and TCC blocker (ie, EFO). The electrophysiological and pharmacological properties of the re-expressed I_{CaT} were similar to those reported previously in terms of voltage-dependency in activation-properties, inactivation properties, and EFO sensitivity.^{20,21} Thus the present findings indicate that I_{CaT} remodeling is associated with cardiomyopathy and is consistent with previous studies in different animal species.¹⁻³

Little is known about the pathophysiological role of I_{CaT} . It is widely speculated that intracellular Ca^{2+} overload induced by an increased influx of Ca^{2+} via re-expressed I_{CaT} may lead to cellular dysfunction in the cardiomyopathic heart.¹⁻³ Nevertheless, we have not been able to conclude that re-expression of I_{CaT} is deleterious for pathophysiological cardiac states. Mulder et al reported increased survival after long-term treatment with mibefradil, a selective TCC blocker, in HF induced in rats by coronary artery ligation.²² However, the MACH-1 study demonstrated that mibefradil did not affect the usual outcome in congestive HF.²³ Based on our study results, the use of EFO in the UM-X7.1 hamsters did not modify the mRNA levels of TCC 1G, but it

has been shown that EFO improves cardiac function and protects against abnormal secretion of BNP by the cardiomyocytes of UM-X7.1 hamsters, which strongly suggests that I_{CaT} plays an important role in cardiac cell function. In our study, EFO decreased the heart rate in UM-X7.1 and golden hamsters, which was consistent with Veniant's data in which mibefradil decreased heart rate even in animals with compromised cardiac function.²⁴ Colzel et al reported a direct effect of TCC blocker on sinoatrial rate.²⁵ In addition, it was reported that a TCC blocker has no effect on activation of the sympathetic nervous system secondary to vasodilatation.²⁶ Based on those results, we consider that the beneficial effects of EFO in the treatment of HF may be related not only to modulation of intracellular Ca^{2+} homeostasis, but also to a direct effect on the sinoatrial rate, an absence of activation of the sympathetic nervous system, or both.

In conclusion, the dual LCC and TCC blocker, EFO, could be a beneficial and valuable clinical therapeutic tool for certain types of cardiomyopathic HF. The selective TCC blocker, mibefradil, was introduced to the clinical market as an antihypertensive agent, but because of its propensity to cause serious adverse drug interactions via potent cytochrome P450 inhibition, it has been withdrawn.²⁷ Therefore, to investigate the precise difference between a selective TCC blocker, such as mibefradil, and a dual TCC and LCC blocker, such as EFO, for treatment of HF, we would not try to treat UM-X7.1 cardiomyopathic hamsters with mibefradil. Further experimental studies are required to clarify the point.

Acknowledgments

This study was supported by in part by a grant-in-aid for scientific research in Japan (C17590738) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. Nuss HB, Houser SR. T-type Ca^{2+} current is expressed in hypertrophied adult feline left ventricular myocytes. *Circ Res* 1993; **73**: 777–782.
2. Martinez ML, Heredia MP, Delgado C. Expression of T-type Ca^{2+} channels in ventricular cells from hypertrophied rat hearts. *J Mol Cell Cardiol* 1999; **31**: 1617–1625.
3. Sen L, Smith TW. T-type Ca^{2+} channels are abnormal in genetically determined cardiomyopathic hamster hearts. *Circ Res* 1994; **75**: 149–155.
4. Huang B, Qin D, Deng L, Boutjdir M, El-Sherif N. Reexpression of T-type Ca^{2+} channel gene and current in post-infarction remodeled rat left ventricle. *Cardiovasc Res* 2000; **46**: 442–449.
5. Izumi T, Kihara Y, Sarai N, Yoneda T, Iwanaga Y, Inagaki K, et al. Reinduction of T-type calcium channels by endothelin-1 in failing hearts in vivo and in adult rat ventricular myocytes in vitro. *Circulation* 2003; **108**: 2530–2535.
6. Ferron L, Capuano V, Ruchon Y, Deroubaix E, Coulombe A, Renaud JF. Angiotensin II signaling pathways mediate expression of cardiac T-type calcium channels. *Circ Res* 2003; **93**: 1241–1248.
7. Ueyama T, Ohkusa T, Hisamatsu Y, Nakamura Y, Yamamoto T, Yano M, et al. Alteration in cardiac SR Ca^{2+} -release channels during development of heart failure in cardiomyopathic hamsters. *Am J Physiol* 1998; **274**: H1–H7.
8. Tawada-Iwata Y, Imanaga T, Yoshida A, Takahashi M, Nakamura H, Shigekawa M. Increased mechanical extraction of T-tubule/junctional SR from cardiomyopathic hamster heart. *Am J Physiol* 1993; **264**: H1447–H1453.
9. Wanger JA, Reynolds LJ, Weisman HF, Dudeck P, Weisfeld ML, Snyder SH. Calcium antagonist receptors in cardiomyopathic hamster: Selective increase in heart, muscle, brain. *Science* 1986; **232**: 515–517.
10. Bazan E, Sole MJ, Schwartz A, Johnson CL. Dihydropyridine receptor binding sites in the cardiomyopathic hamster heart are unchanged from control. *J Mol Cell Cardiol* 1991; **23**: 111–117.

11. Packer M, Kessler PD, Lee WH. Calcium channel blockade in the management of severe chronic congestive heart failure: A bridge too far. *Circulation* 1987; **75**(Suppl V): V-56–V-64.
12. Elkayam U, Amin J, Mehra A, Vasquez J, Weber L, Rahimtoola AH. A prospective, randomized, double-blind, crossover study to compare the efficacy and safety of chronic nifedipine therapy with that of isosorbide dinitrate and their combination in the treatment of chronic congestive heart failure. *Circulation* 1990; **82**: 1954–1961.
13. Venin M, Clozel JP, Hess P, Wolfgang R. Ro 40–5967, in contrast to diltiazem, does not reduce left ventricular contractility in rats with chronic myocardial infarction. *J Cardiovasc Pharmacol* 1991; **17**: 277–284.
14. Cingolani OH, Yang XP, Cavaşin MA, Carretero OA. Increased systolic performance with diastolic dysfunction in adult spontaneous hypertensive rats. *Hypertension* 2003; **41**: 249–254.
15. Yamada J, Ohkusa T, Nao T, Ueyama T, Yano M, Kobayashi S, et al. Up-regulation of inositol 1,4,5 trisphosphate receptor expression in atrial tissue in patients with chronic atrial fibrillation. *J Am Coll Cardiol* 2001; **37**: 1111–1119.
16. McRory JE, Santi CM, Hamming KSC, Mezeyova J, Sutton KG, Baillie DL, et al. Molecular and functional characterization of a family of rat brain T-type calcium channels. *J Biol Chem* 2001; **276**: 3999–4011.
17. Tso JY, Sun XH, Kao T, Reece KS, Wu R. Isolation and characterization of rat and human glyceraldehydes-3-phosphate dehydrogenase cDNAs: Genomic complexity and molecular evolution of the gene. *Nucleic Acids Res* 1985; **13**: 2485–2502.
18. Wang DW, Kiyosue T, Shigematsu S, Arita M. Abnormalities of K⁺ and Ca²⁺ currents in ventricular myocytes from rats with chronic diabetes. *Am J Physiol* 1995; **269**: H1288–H1296.
19. Lee TS, Ono K, Hadama T, Uchida Y, Arita M. Roles of α_1 and α_2 subunits derived from cardiac L-type Ca²⁺ channels on voltage-dependent facilitation mechanisms. *Jpn J Physiol* 2001; **51**: 337–344.
20. Perez-Reyes E. Molecular physiology of low-voltage-activated T-type calcium channels. *Physiol Rev* 2003; **83**: 117–161.
21. Takebayashi S, Li Y, Kaku T, Inagaki S, Hashimoto Y, Kimura K, et al. Remodeling excitation-contraction coupling of hypertrophied ventricular myocytes is dependent on T-type calcium channels expression. *Biochem Biophys Res Commun* 2006; **345**: 766–773.
22. Mulder P, Richard V, Compagnon P, Henry JP, Lallemand F, Clozel JP, et al. Increased survival after long-term treatment with mibefradil, a selective T-channel calcium antagonist, in heart failure. *J Am Coll Cardiol* 1997; **29**: 416–421.
23. Levine TB, Bernink PJ, Caspi A, Elkayam U, Geltman EM, Greenberg B, et al. Effect of mibefradil, a T-type calcium channel blocker, on morbidity and mortality in moderate to severe congestive heart failure: The MACH-1 Study. *Circulation* 2000; **101**: 758–764.
24. Véniant M, Clozel JP, Heudes D, Banken L, Menard J. Effect of Ro 40-5967, a new calcium antagonist, and enalapril on cardiac remodeling in renal hypertensive rats. *J Cardiovasc Pharmacol* 1993; **21**: 544–551.
25. Clozel JP, Osterrieder W, Kleinbloem CH, Welker HA, Schläppli B, Tudor R, et al. Ro 490-5967: A new nondihydropyridine calcium antagonist. *Cardiovasc Drug Rev* 1991; **9**: 4–17.
26. Colzel JP, Ertel EA, Ertel SI. Voltage-gated T-type Ca²⁺ channel and heart failure. *Proc Assoc Am Physicians* 1999; **111**: 429–437.
27. Po AL, Zhang WY. What lessons can be learnt from withdrawal of mibefradil from the market? *Lancet* 1998; **351**: 1829–1830.