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Novel effect of carvedilol on Ca^{2+} movement in renal tubular cells

Chun-Peng Liu[a,b,c], Hung-Ting Chiang[a,b,c], Chung-Ren Jan[d,*]

[a]*Department of Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, ROC*
[b]*School of Medicine, National Yang-Ming University, Taipei, Taiwan, ROC*
[c]*School of Medicine, National Defense Medical Center, Taipei, Taiwan, ROC*
[d]*Department of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, ROC*

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Abstract

The effect of carvedilol on intracellular free Ca^{2+} levels ($[Ca^{2+}]_i$) has not been explored previously. This study was aimed to examine the effect of carvedilol on Ca^{2+} handling in renal tubular cells. Madin–Darby canine kidney cells were used as a model for renal tubular cells and fura-2 was used as a fluorescent Ca^{2+} probe. Carvedilol increased $[Ca^{2+}]_i$ in a concentration-dependent manner with an EC_{50} value of 5 μM. Extracellular Ca^{2+} removal partly inhibited the $[Ca^{2+}]_i$ signals. Carvedilol-induced Ca^{2+} influx was verified by measuring Mn^{2+}-induced quench of fura-2 fluorescence. Carvedilol-induced store Ca^{2+} release was reduced by pretreatment with 1 μM thapsigargin (an endoplasmic reticulum Ca^{2+} pump inhibitor) but not with 5 μM ryanodine or 2 μM carbonylcyanide *m*-chlorophenylhydrazone (a mitochondrial uncoupler). Carvedilol (30 μM)-induced Ca^{2+} release was not affected by inhibiting phospholipase C with 1-(6-((17β-3-methoxyestra-1,3,5(10)-trien-17-l)amino)hexyl)-1*H*-pyrrole-2,5-dione (U73122; 2 μM), but was potentiated by increasing cAMP levels or inhibiting protein kinase C. The carvedilol-induced Ca^{2+} mobilization was not significantly sequestered by the endoplasmic reticulum or mitochondria. This study shows that carvedilol increased $[Ca^{2+}]_i$ in renal tubular cells by causing Ca^{2+} release from the endoplasmic reticulum and other unknown stores in an inositol-1,4,5-trisphosphate-independent manner, and by inducing Ca^{2+} influx. The Ca^{2+} release was modulated by cAMP and protein kinase C.

Keywords: Calcium; Carvedilol; Fura-2; MDCK cells; Renal; Thapsigargin

1. Introduction

Current knowledge of the mechanisms contributing to progression of heart failure suggests that therapies that limit or interfere with the consequences of neurohormonal activation and improve myocardial energetics appear to be most beneficial [1]. Carvedilol, a drug with vasodilating properties [2–4], reduces mortality, slows disease progression, and improves life quality in patients with heart failure when added to standard therapy [5]. When administered according to recommended guidelines, carvedilol is well-tolerated [6,7].

In vitro, carvedilol inhibits human ether-a-go-go related gene (HERG) K^+ channels which play a critical role in the pathogenesis of cardiac arrhythmias and sudden cardiac death [8]. Furthermore, carvedilol can inhibit reactive oxygen species generation [9–11]. Carvedilol does not display toxic effects on mitochondria up to the concentration showing maximal antioxidant effects (40 μM). At concentrations higher than 40 μM, however, the phosphorylation efficiency of mitochondria is depressed by carvedilol as deduced from a decrease in respiratory control and in the ADP/oxygen ratio [11]. Carvedilol is thought to stimulate nitric oxide synthesis in rat cardiac myocytes by a β-adrenoceptor-independent mechanism [12].

This study was aimed to investigate the effect of carvedilol on signal transduction in renal tubular cells, in light of the evidence that carvedilol exerts a nephroprotective effect on renal function in animals and in human. In dogs and rats, intrarenal infusion of carvedilol results in a renal vasodilator response with preservation of renal blood flow without inducing sodium retention [13]. Carvedilol has

* Corresponding author. Tel.: +886-734-22121x1509; fax: +886-734-68056.
E-mail address: crjan@isca.vghks.gov.tw (C.-R. Jan).

Abbreviations: ATP, adenosine 5′-triphosphate; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; fura-2/AM, (1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methylphenoxy)-ethane-*N*,*N*,*N*,*N*-tetraacetic acid pentaacetoxymethyl ester); MDCK, Madin–Darby canine kidney.

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been shown to exert nephroprotective effects in hypertensive-stroke prone rats *via* a mechanism involving inhibition of transforming growth factor β expression [14], to prevent severe nephrosclerosis [15], glomerulosclerosis [16], and gentamicin-induced nephrotoxicity in rats [17], to enhance renal function in patients with cirrhosis [18], and to reduce microalbuminuria in human [19]. How carvedilol exerts these actions is unclear.

Among various cellular second messengers, a transient change in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) is a pivotal signal for many biological phenomena, including contraction, secretion, fertilization, cell proliferation, and enzyme activation [20,21]. In renal tubular cells, a Ca^{2+} signal is a trigger for chloride secretion [22]. Our data suggest that carvedilol caused an increase in $[Ca^{2+}]_i$ in Madin–Darby canine kidney (MDCK) cells, by using fura-2 as a Ca^{2+} dye. The concentration–response relationships and the mechanisms underlying the $[Ca^{2+}]_i$ increase have been investigated.

2. Materials and methods

2.1. Cell culture

MDCK cells obtained from American Type Culture Collection (CRL-6253) were cultured in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum at 37° in 5% CO_2-containing humidified air.

2.2. Solutions used in $[Ca^{2+}]_i$ measurements

Ca^{2+}-containing medium had 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, 5 mM glucose, pH 7.4. In Ca^{2+}-free medium, Ca^{2+} was substituted with 1 mM EGTA. Carvedilol was dissolved in 100% ethanol and kept at −20° as a 0.1 M stock, and was diluted to the final concentration before experiments. Other drugs were dissolved in water, ethanol, or dimethyl sulfoxide as stock solutions. The concentration of organic solvents in the $[Ca^{2+}]_i$ measurements did not exceed 0.1% and did not alter basal $[Ca^{2+}]_i$ (N = 4).

2.3. $[Ca^{2+}]_i$ measurements

Trypsinized cells (10^6 per milliliter) were allowed to recover in serum-free culture medium for 1 hr before loading with 2 μM fura-2/AM for 30 min at 25° in the same medium. Cells were washed and resuspended in Ca^{2+}-containing medium. Fura-2 fluorescence measurements were performed in a water-jacketed cuvette (25°) with continuous stirring; the cuvet contained 1 mL of medium and 0.5 million cells. Fluorescence was monitored with a Shimadzu RF-5301PC spectrofluorophotometer (Shimadzu Corp.) by recording excitation signals at 340 and 380 nm and emission signal at 510 nm at 1-s intervals. Maximum and minimum fluorescence values were obtained by adding 0.1% Triton X-100 (plus 5 mM $CaCl_2$) and 10 mM EGTA sequentially at the end of each experiment. $[Ca^{2+}]_i$ was calculated as described previously [23–25] assuming a K_d of 155 nM [26].

2.4. Chemicals

Carvedilol in pure form was kindly supplied as a gift by Dr. Sven Hauptmann at ROCHE DIAGNOSTICS GMBH. All chemicals used in culture were obtained from Gibco. The other drugs were obtained from Sigma.

2.5. Statistics

The data were mean ± SEM of three to six separate experiments. Statistical analysis was made by Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

The basal $[Ca^{2+}]_i$ was 50 ± 2 nM (N = 6). At concentrations above 0.5 μM, carvedilol increased $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+} (Fig. 1A). The $[Ca^{2+}]_i$ signal comprised an immediate rise and a sustained phase within 250 s. At a concentration of 0.01 μM, carvedilol had no effect. The response saturated at 30 μM of carvedilol. At a concentration of 30 μM, carvedilol induced a $[Ca^{2+}]_i$ increase which reached a net (baseline subtracted) maximum value of 142 ± 2 nM (trace a; N = 4) followed by a sustained phase that last for the rest of the recording. The concentration-dependent plot shown in Fig. 1C indicates an EC_{50} of 5 μM.

Effort was made to determine the relative contribution of extracellular Ca^{2+} influx and store Ca^{2+} release in the carvedilol-induced Ca^{2+} signal. Acutely removing extracellular Ca^{2+} (experiments were started by adding 0.1 mL cell suspension into 0.9 mL Ca^{2+}-free medium in a cuvet) significantly decreased carvedilol-induced $[Ca^{2+}]_i$ increases without changing basal $[Ca^{2+}]_i$. Fig. 1B shows the response induced by 30 μM carvedilol (trace a). The drug induced an immediate increase in $[Ca^{2+}]_i$ that had a net maximum of 999 ± 2 nM (N = 5). The maximum phase was followed by a slow decay and a sustained phase. The concentration–response curve of carvedilol-induced $[Ca^{2+}]_i$ increases in Ca^{2+}-free medium is shown in Fig. 1C (open circles). The two curves in Fig. 1C suggest that removing extracellular Ca^{2+} partly reduced carvedilol-induced $[Ca^{2+}]_i$ increases (N = 3–5; $P < 0.05$).

These experiments were performed to confirm that the reduced carvedilol-induced $[Ca^{2+}]_i$ increase by Ca^{2+} removal was not due to Ca^{2+} removal-induced store Ca^{2+} depletion. Mn^{2+} enters cells through similar pathways as Ca^{2+} but quenches fura-2 fluorescence at all

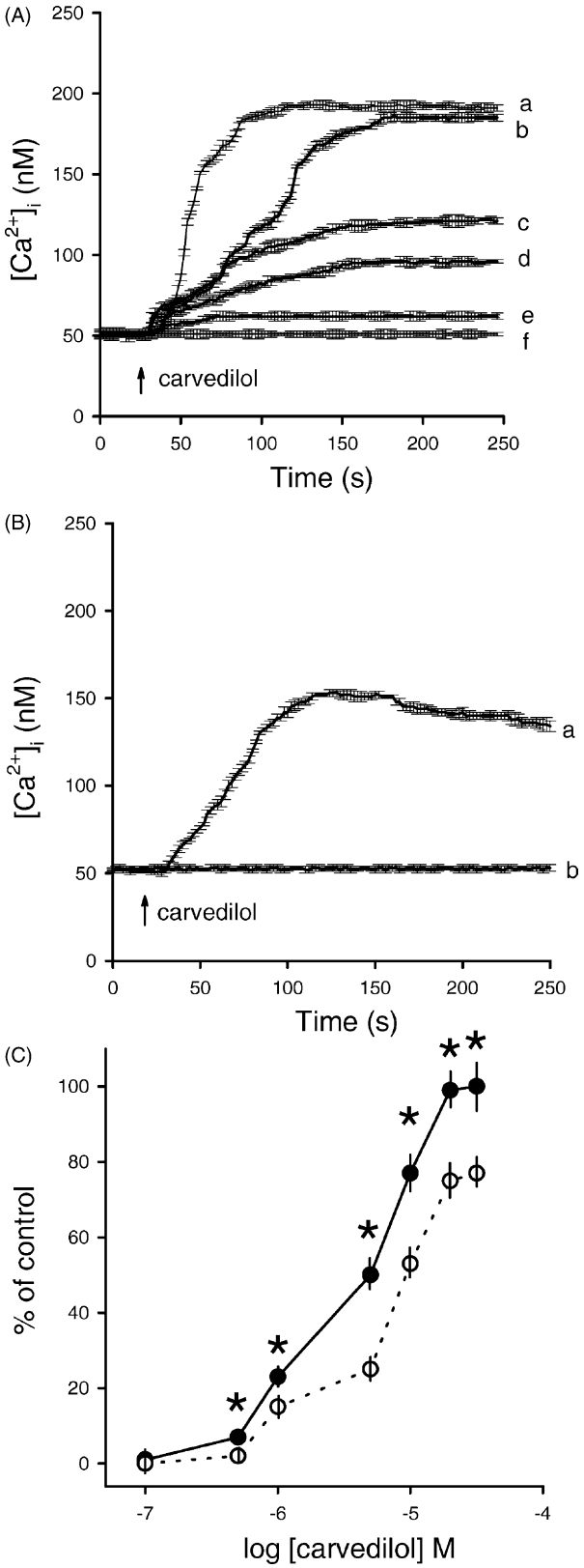

Fig. 1. (A) Concentration-dependent effects of carvedilol on $[Ca^{2+}]_i$ in MDCK cells. Carvedilol was added at 30 s. The concentration of carvedilol was 30 μM in trace a, 20 μM in trace b, 10 μM in trace c, 5 μM in trace d, 1 μM in trace e,and zero in trace f. The experiments were performed in Ca^{2+}-containing medium. (B) Effect of removing extracellular Ca^{2+} on carvedilol-induced $[Ca^{2+}]_i$ increases. Carvedilol (30 μM) was added at 30 s in Ca^{2+}-free medium (trace a). Trace b: control baseline without addition of carvedilol. The experiments were performed in Ca^{2+}-free medium. (C) Concentration–response plots of carvedilol-induced Ca^{2+} signals both in Ca^{2+}-containing medium (filled circles) and Ca^{2+}-free medium (open circles). The *y* axis is percentage of control which was the net maximum $[Ca^{2+}]_i$ induced by 30 μM carvedilol in Ca^{2+}-containing medium (142 ± 3 nM). The data were mean ± SEM of three to five replicates. $^*P < 0.05$ compared between filled circles and open circles at each carvedilol concentration.

excitation wavelengths [27]. Thus, quench of fura-2 fluorescence excited at the Ca^{2+}-insensitive excitation wavelength of 360 nm by Mn^{2+} indicates Ca^{2+} influx. Fig. 2 shows that 30 μM carvedilol induced an immediate decrease in the 360 nm excitation signal by 32 ± 3 (N = 6) arbitrary units (trace a), which was significantly lower than control (trace a, no carvedilol was added; $P < 0.05$). The carvedilol-induced Mn^{2+} influx sustained for several minutes.

Experiments were conducted to investigate the role of the endoplasmic reticulum Ca^{2+} store, the major Ca^{2+} store in MDCK cells [23–25], in carvedilol-induced Ca^{2+} release. Fig. 3A shows that in Ca^{2+}-free medium, addition of 1 μM thapsigargin, an endoplasmic reticulum Ca^{2+} pump inhibitor that increases $[Ca^{2+}]_i$ by passively depleting store Ca^{2+} [28], induced a $[Ca^{2+}]_i$ transient with a net maximum $[Ca^{2+}]_i$ value of 65 ± 3 nM (N = 4), implicating depletion of the endoplasmic reticulum Ca^{2+}. Carvedilol (30 μM) was subsequently added at the time point of 850 s and was found to induce a $[Ca^{2+}]_i$ increase with a net maximum value of 26 ± 2 nM (N = 4) which was smaller than the control carvedilol-induced $[Ca^{2+}]_i$ increase (Fig. 3C; 101 ± 2 nM; N = 4) by 75 ± 1% ($P < 0.05$). Effort was exerted to see if Ca^{2+} stored in mitochondria contributed to carvedilol-induced Ca^{2+} release. Fig. 3B shows that addition of carbonylcyanide *m*-chlorophenylhydrazone (CCCP; 2 μM), a mitochondrila uncoupler that has been shown to deplete mitochondrial Ca^{2+} in MDCK cells previously [23–25], induced a $[Ca^{2+}]_i$ increase with a net maximum value of 26 ± 2 nM (N = 4). Thapsigargin (1 μM) was added subsequently to deplete the endoplasmic reticulum Ca^{2+} store. Carvedilol (30 μM) added afterwards induced a $[Ca^{2+}]_i$ increase indistinguishable from the carvedilol-induced response seen in Fig. 3A where Ca^{2+} stores were depleted with thapsigargin alone. Conversely, pretreatment with carvedilol for 800 s almost abolished thapsigargin-induced $[Ca^{2+}]_i$ increases (Fig. 3C; N = 6).

Because in epithelial cells ryanodine receptor-coupled store Ca^{2+} may contribute to a $[Ca^{2+}]_i$ increase [20,21], experiments were performed to see whether ryanodine-sensitive Ca^{2+} stores participate in carvedilol-induced Ca^{2+} release. In Ca^{2+}-free medium, preincubation with ryanodine (5 μM) for 800 s did not alter basal $[Ca^{2+}]_i$ or 30 μM carvedilol-induced $[Ca^{2+}]_i$ increases (not shown; N = 3; $P > 0.05$).

The role of inositol 1,4,5-trisphosphate (IP_3) in carvedilol-induced Ca^{2+} release was explored. Fig. 4A shows

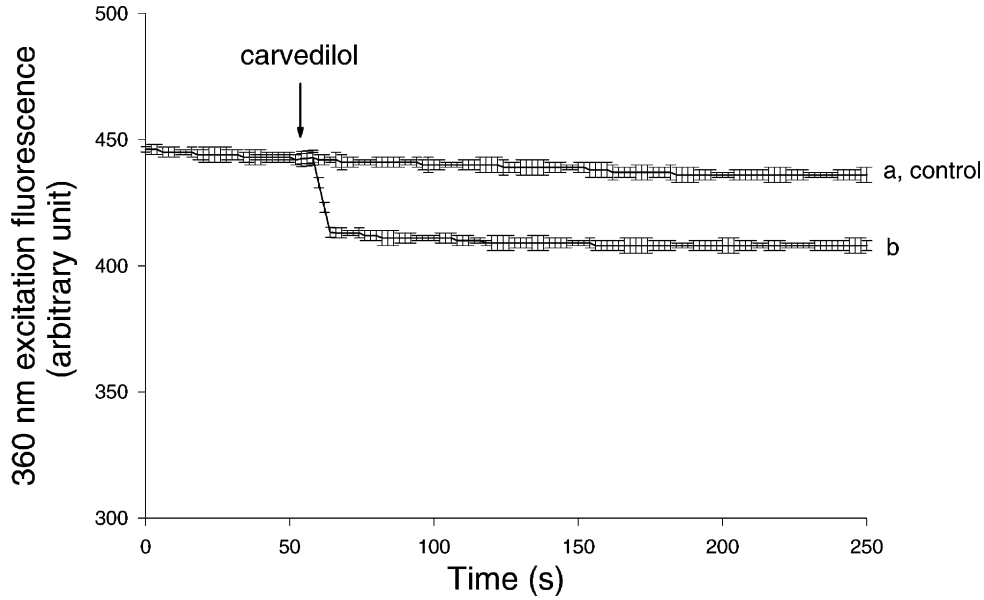

Fig. 2. Effect of carvedilol on Ca^{2+} influx by measuring Mn^{2+} quench of fura-2 fluorescence. Experiments were performed in Ca^{2+}-containing medium. $MnCl_2$ (50 μM) was added to cells before fluorescence measurements. Trace a: control (in the absence of carvedilol). Trace b: carvedilol (30 μM) was added at time indicated by the arrow. Data were mean ± SEM of three replicates.

that in Ca^{2+}-free medium, addition of ATP (10 μM), an IP_3-dependent Ca^{2+} mobilizer, induced a $[Ca^{2+}]_i$ increase with a net maximum value of 201 ± 3 nM (N = 4). Fig. 4B shows that after pretreatment with 2 μM U73122, a phospholipase C inhibitor [29], for 150 s abolished ATP-induced $[Ca^{2+}]_i$ increases without altering basal $[Ca^{2+}]_i$. Conversely, U73343, an inactive U73122 analogue [29], did not alter ATP-induced $[Ca^{2+}]_i$ increases (N = 3; $P > 0.05$; not shown). This suggests that U73122 effectively inhibited phospholipase C activity. Fig. 4B further shows that carvedilol (30 μM) added after ATP induced a $[Ca^{2+}]_i$ increase indistinguishable from control in magnitude and shape (Fig. 1B; N = 3; $P > 0.05$).

In order to explore the mechanism underlying carvedilol-induced Ca^{2+} release, the effect of several agents that alter cAMP levels or protein kinase C activity was tested. Fig. 5 shows that pretreatment with 100 μM Br-cAMP (trace a) for 3 min to increase cAMP levels potentiated 30 μM carvedilol-induced $[Ca^{2+}]_i$ increases by 2.1 ± 0.2 fold (N = 6; $P < 0.05$ compared with trace c) in the net maximum value. Forskolin (10 μM) produced a similar effect (N = 3; not shown). Pretreatment with 2 μM GF 109203X to inhibit protein kinase C (trace b) increased the carvedilol response by 1.3 ± 0.1 fold (N = 6; $P < 0.05$ compared with trace c). Activation of protein kinase C with 1 nM phorbol 12-myristate 13-acetate did not alter the carvedilol response (N = 4; $P > 0.05$; not shown).

In MDCK cells, mitochondria have been shown to play a role in sequestering cytosolic Ca^{2+} during drug stimulation [23]. Thus, experiments were performed to explore the role of mitochondria in sequestering carvedilol-induced Ca^{2+} release. Fig. 6A shows that in Ca^{2+}-free medium, depletion of mitochondrial Ca^{2+} with CCCP (2 μM) induced an immediate and sustained $[Ca^{2+}]_i$ increase with a net maximum value of 15 ± 2 nM (N = 5). Subsequently added carvedilol (30 μM) induced a $[Ca^{2+}]_i$ increase with a net maximum value of 111 ± 2 nM (N = 5) which was similar to the control carvedilol response shown in Fig. 6B ($P > 0.05$). Conversely, Fig. 6B shows that addition of CCCP (2 μM) after carvedilol had depleted store Ca^{2+} for 8 min induced a $[Ca^{2+}]_i$ increase indistinguishable from the control CCCP-induced response shown in Fig. 6A (N = 6; $P > 0.05$).

4. Discussion

The novel finding of the present study is that the cardioprotective drug carvedilol can induce significant increases in $[Ca^{2+}]_i$ in renal tubular cells at a concentration above 0.5 μM. The results may be interesting because although 95% of carvedilol is metabolized in liver, 5% of carvedilol is excreted in urine unchanged [16–19]. In order to explain the clinical effect of carvedilol, previous efforts have been focused on the drug's inhibitory effects on adrenergic receptors and channel currents and its anti-oxidative action [2,8–11]. Because an increase in $[Ca^{2+}]_i$ can alter many aspects of physiology in all cell types [20], caution must be exercised in explaining the clinical action of carvedilol. The therapeutic plasma concentration of carvedilol in human is 100 ng/mL (0.24 μM) [30], and the plasma level of carvedilol may reach beyond 1.5 μM if liver or renal function is compromised [31].

Our data suggest that carvedilol induced immediate and sustained $[Ca^{2+}]_i$ increases at concentrations normally used by researchers to examine its effect on other cellular

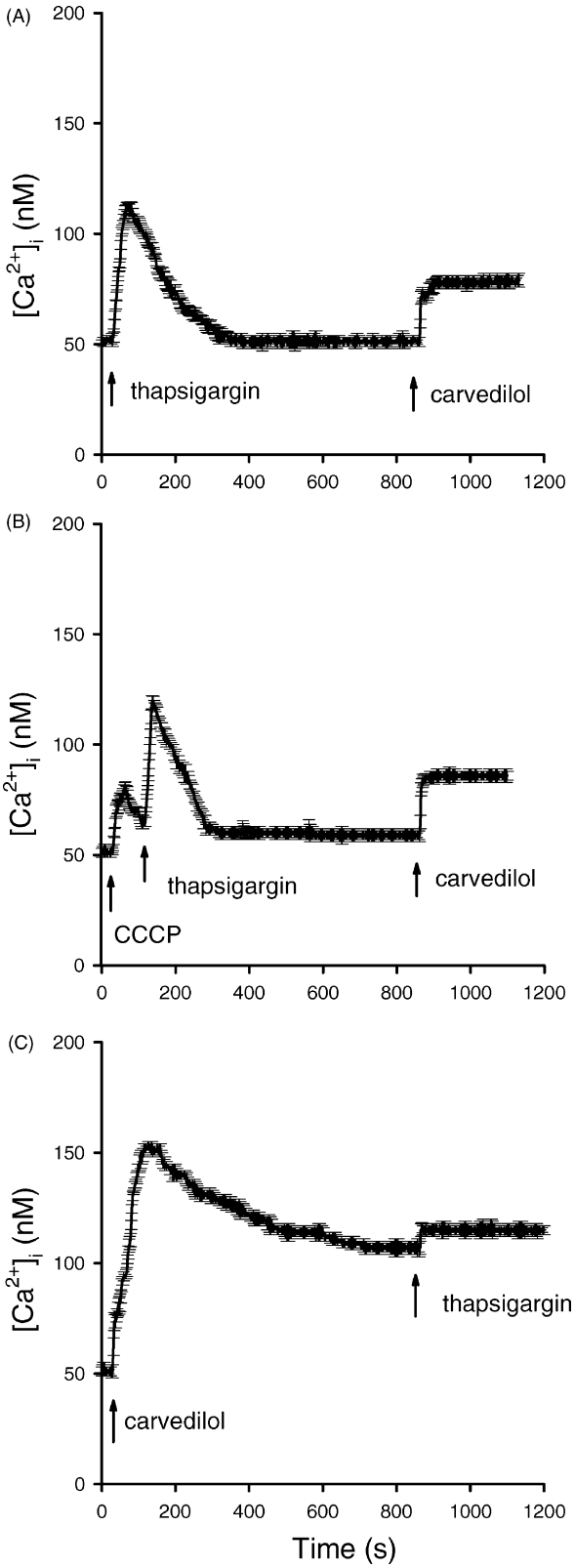

Fig. 3. Intracellular Ca^{2+} stores of carvedilol-induced $[Ca^{2+}]_i$ increases. The experiments were performed in Ca^{2+}-free medium. (A) Thapsigargin (1 μM) was added at 30 s followed by carvedilol (30 μM) added at 850 s. (B) CCCP (2 μM) was added at 30 s followed by thapsigargin (1 μM) and carvedilol added at 150 and 850 s, respectively. (C) Carvedilol (30 μM) was added at 30 s followed by thapsigargin (1 μM) added at 850 s. Data were mean ± SEM of three to five replicates.

processes. In ventricular myocytes, carvedilol blocks the delayed rectifier K^+ current and L-type Ca^{2+} currents, with IC_{50} values of 3–12 μM [8,32]. It seems that carvedilol increased $[Ca^{2+}]_i$ mainly by causing store Ca^{2+} release because the response was only reduced by 25% by removing extracellular Ca^{2+}. The carvedilol-induced Ca^{2+} influx was independently verified by carvedilol-induced Mn^{2+} quench of fura-2 fluorescence at the Ca^{2+}-insensitive 360 nm excitation wavelength. Carvedilol may induce Ca^{2+} influx *via* store-operated Ca^{2+} entry, a process triggered by store Ca^{2+} depletion [33], which has been previously shown to play a dominant role in Ca^{2+} influx in MDCK cells [23–25]. In Ca^{2+}-containing medium, 30 μM carvedilol-induced $[Ca^{2+}]_i$ increases display a peak followed by a stable, flat plateau (Fig. 1A, trace a). In contrast, trace a in Fig. 1B shows that in Ca^{2+}-free medium, 30 μM carvedilol induced a $[Ca^{2+}]_i$ increase followed by a clear decrease. This suggests that extracellular Ca^{2+} influx contributes not only to the initial increase, but also to the prolonged phase of carvedilol-induced $[Ca^{2+}]_i$ response in Ca^{2+}-containing medium.

Regarding the Ca^{2+} stores of the carvedilol response, the thapsigargin-sensitive endoplasmic reticulum store appears to play a key role because the carvedilol-induced Ca^{2+} release was significantly reduced by depletion of the endoplasmic reticulum Ca^{2+} store with thapsigargin. The store Ca^{2+} in mitochondria did not appear to play a role since depletion of mitochondrial Ca^{2+} with CCCP did not augment thapsigargin-induced reduction of the carvedilol-induced Ca^{2+} release. How carvedilol releases store Ca^{2+} is unclear, but the process may be independent of IP_3 or ryanodine receptors because suppression of phospholipase C activity or addition of ryanodine did not affect carvedilol-induced Ca^{2+} release. Notably, the carvedilol-induced $[Ca^{2+}]_i$ increase can be regulated by other key cellular effectors such as cAMP and protein kinase C because the response was considerably augmented by elevating cAMP levels (with Br-cAMP or forskolin) or inhibiting protein kinase C activity (with GF109203 X). This is consistent with previous findings that in MDCK cells, cAMP, protein kinase C, and other second messengers can modulate a $[Ca^{2+}]_i$ increase [34]. The result that the carvedilol-induced Ca^{2+} response was increased by inhibiting protein kinase C suggests that resting levels of protein kinase C activity may play an inhibitory role in carvedilol-induced $[Ca^{2+}]_i$ increase. The lack of effect of potentiating protein kinase C on carvedilol-induced response implies that resting levels of protein kinase C may have exerted a full inhibitory effect.

Transient increases of $[Ca^{2+}]_i$ drive many cellular processes, ranging from membrane channel kinetics to transcriptional regulation, and links of Ca^{2+} to other second messengers should activate signaling networks. In embryonic spinal neurons, it has been shown that blocking the production of cAMP transients decreases the intrinsic frequency of spontaneous Ca^{2+} spikes, whereas inducing cAMP increases causes spike frequency to increase.

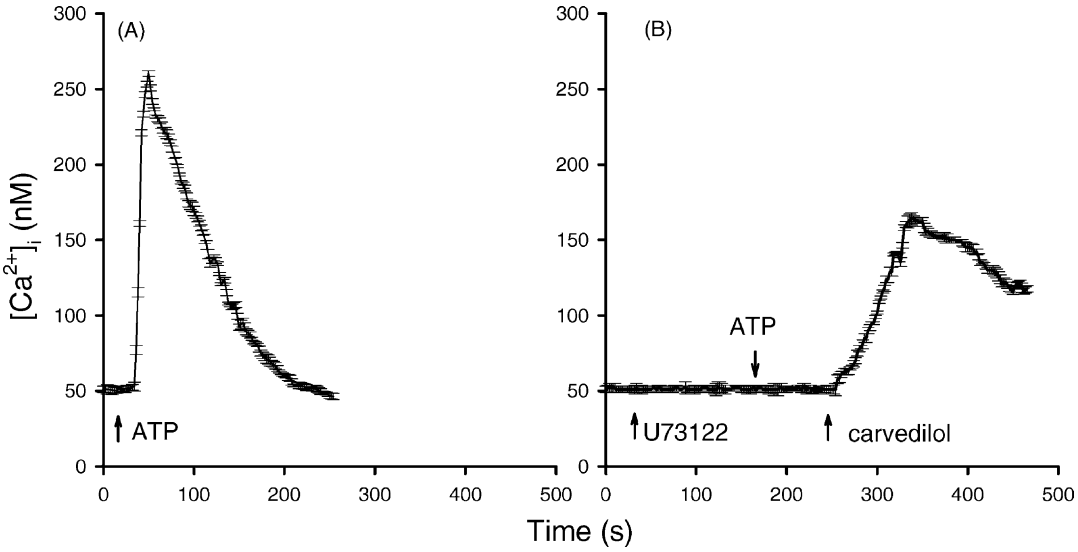

Fig. 4. Lack of involvement of phospholipase C in carvedilol-induced Ca^{2+} release. In Ca^{2+}-free medium, (A) ATP (10 μM) was added at 20 s, (B) U73122 (2 μM) was added at 20 s followed by ATP (10 μM), and carvedilol (30 μM) added at 175 and 250 s, respectively. Data were mean ± SEM of three to five replicates.

Moreover, simultaneous stimulation of Ca^{2+} and cAMP systems produces distinct temporal patterns of oscillations of both messengers [35]. In nonexcitable cells, agents that increase cAMP levels were shown to modulate mitochondrial Ca^{2+} stores [36]. Modulation of protein kinase C activation on $[Ca^{2+}]_i$ has been reported in various cell types [37,38]. It is not clear whether the greater carvedilol responses seen in MDCK cells with elevated cAMP levels or decreased protein kinase C activity were due to increased amount of stored Ca^{2+} or carvedilol became more effective. However, evidence from human embryonic kidney (HEK) cells shows that protein kinase C activation have multiple actions on the Ca^{2+} storage and signaling function of the endoplasmic reticulum, including decrease of intracellular Ca^{2+} storage capacity [39].

Several mechanisms may play a role in lowering a $[Ca^{2+}]_i$ increase in MDCK cells: Ca^{2+} efflux *via* Ca^{2+} pump or Na^+/Ca^{2+} exchange and Ca^{2+} sequestration into the endoplasmic reticulum or mitochondria [34,40]. The data in Fig. 3C suggest that carvedilol-released Ca^{2+} did not significantly return to the endoplasmic reticulum because thapsigargin added afterwards released little Ca^{2+}. Mitochondria also did not appear to play a role in sequestering carvedilol-released Ca^{2+} since carvedilol-

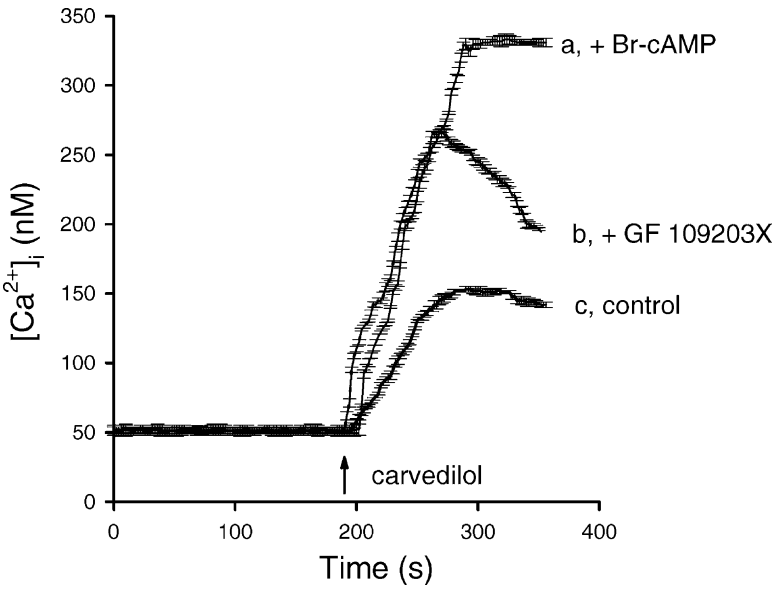

Fig. 5. Role of cAMP and protein kinase C in carvedilol-induced Ca^{2+} release. The experiments were performed in Ca^{2+}-free medium. Trace a: Br-cAMP (100 μM) was added at 20 s followed by carvedilol (30 μM) added at 190 s. Trace b: GF 109203X (2 μM) was added at 20 s followed by carvedilol (30 μM) added at 190 s. Trace c: control; carvedilol was added at 190 s. Data were mean ± SEM of three to five replicates.

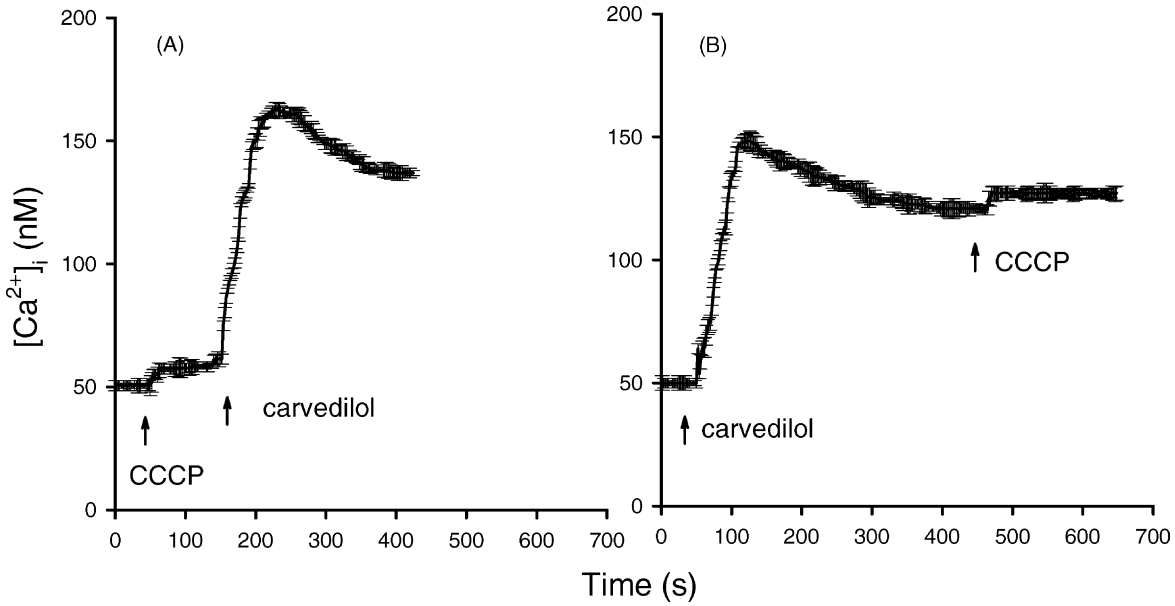

Fig. 6. Effect of CCCP on carvedilol-induced Ca^{2+} release. The experiments were performed in Ca^{2+}-free medium. (A) CCCP (2 μM) was added at 30 s followed by carvedilol (30 μM) added at 160 s. (B) Carvedilol (30 μM) was added at 30 s followed by CCCP (2 μM) added at 460 s. Data were mean ± SEM of five replicates.

induced Ca^{2+} release was not augmented by uncoupling mitochondria with CCCP.

Among its many effects, blockade of adrenergic receptors has long been thought to play a main role in the cardioprotective action of carvedilol. Compared with other commonly used adrenergic drugs, carvedilol is unique in its Ca^{2+} mobilizing effect. Five other adrenergic drugs (phenoxybenzamine, 200 μM; timolol, 130 μM; isoproterenol, 5.5 μM; naftopidil, 100 μM; norepinephrine, 100 μM) tested failed to alter basal $[Ca^{2+}]_i$ ($N = 3$; $P > 0.05$; data not shown). The only exception was propranolol which was able to increase $[Ca^{2+}]_i$ but only at concentrations above 100 μM as we previously described [40]. Conversely, carvedilol at a concentration of 0.5 μM could increase $[Ca^{2+}]_i$. Thus, it is unlikely that carvedilol caused $[Ca^{2+}]_i$ increases through interaction with adrenergic receptors.

Together, this study shows for the first time that carvedilol could induce $[Ca^{2+}]_i$ increases in renal tubular cells. These data are important because although carvedilol has been shown to exert nephroprotective effects in rats and in patients with cirrhosis and microalbuminuria [19], the underlying mechanism is unclear. An increase in $[Ca^{2+}]_i$ has been shown to be a key message for normal renal function. For example, the balance of a high extracellular osmolarity in the kidney medulla is mainly based on an accumulation of organic osmolytes in the cells. The regulation of cell volume during hypotonic conditions results in a release of organic osmolytes- a process that is partly Ca^{2+}-dependent [41]. Moreover, many endogenous agonists, such as ATP and bradykinin, activate renal cells *via* causing a well-tuned increase in $[Ca^{2+}]_i$ [34,40]. Conversely, an uncontrolled $[Ca^{2+}]_i$ increase may lead to tubular injury resulting in a profound fall in glomerular filtration rate, including increased tubuloglomerular feedback and distal tubular obstruction, in ischemic acute renal failure [42]. In light of the importance of a $[Ca^{2+}]_i$ increase in renal tubular cells for pathophysiological events, our findings may contribute to the unveiling of the mechanism underlying the nephroprotectivity of carvedilol.

Acknowledgments

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