Chinese Journal of Physiology 41(4): 181-188, 1998

Nifedipine, Verapamil and Diltiazem Block Shock-wave-induced Rises in Cytosolic Calcium in MDCK Cells

Chung-Ren Jan[1], Wei-Chuan Chen[2], Sheng-Nan Wu[1] and Ching-Jiunn Tseng[1]

[1]*Department of Medical Education and Research*
Veterans General Hospital-Kaohsiung
Kaohsiung, Taiwan 813
[2]*Division of Urology*
Ping Tung Christian Hospital
Ping Tung, Taiwan 813, ROC

Abstract

Nifedipine and verapamil have been shown previously to protect against renal function alterations induced by shock wave lithotripsy (SWL) in humans and rats; however, the mechanism is unclear. This study was aimed to examine whether these drugs could protect cultured kidney cells following shock wave exposure (SWE). The effect of nifedipine, verapamil and diltiazem on Madin Darby canine kidney (MDCK) cells following SWE was examined by determining the release of glutamate oxalactate transferase (GOT) and lactate dehydrogenasse (LDH) in cell suspensions; and also cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). Immediately after SWE, there was a transient release of GOT and LDH (16% and 4 fold, respectively). In contrast, $[Ca^{2+}]_i$ measured within 1-6 hr after SWE gradually increased by 15-156%. The Ca^{2+} entry blockers (1 or 10 μM) failed to inhibit the enzyme release; however, they abolished the progressive rises in $[Ca^{2+}]_i$. The Ca^{2+} entry blockers may protect the cells from damage of SWE via maintaining a low resting $[Ca^{2+}]_i$.

Key Words: MDCK cells, shock waves, Ca^{2+} entry blocker, fura-2, calcium imaging

Introduction

Shock wave lithotripsy (SWL) has become a modality for disintegrating kidney stones in treatment of urolithiasis since 1980 (3); however, a number of possible side effects exist (8, 18). In addition to local hematomas and edema (2) and a risk of hypertension (22), SWL causes renal function alterations, including a transient decrease in filtration rate (6); an increased excretion of α_1- and β_2-microglobulin and N-acetyl-β-glucosaminidase; and a decreased excretion of Tamm-Horsfall protein (1).

MDCK cells, a cell line derived from canine renal tubular cells (7), have been chosen as a model to investigate the effects of shock wave exposure (SWE) on renal cells (16, 17, 27, 28). Previously, SWE has been reported to result in an increase in the resting $[Ca^{2+}]_i$ (16, 17) and the release of cellular enzymes such as glutamate oxalactate transferase (GOT) and lactate dehydrogenase (LDH) (16, 17, 27, 28).

It has been reported that patients taking nifedipine or verapamil showed less excretion of α_1-microglobulin and N-acetyl-β-glucosaminidase and more excretion of Tamm-Horsfall protein following SWL (25, 26). In rats, verapamil was shown to lessen damage to the kidney during SWE (24). Similar protective effect of nifedipine in patients after SWL has also been reported by Li et al (21). However, how nifedipine and verapamil worked is unclear. As part of an effort to understand the mechanism underlying the *in vivo* protective action of Ca^{2+} entry blockers on SWL-induced side effects, we have examined whether nifedipine, verapamil and another Ca^{2+} entry blocker, diltiazem, could protect cultured renal cells against shock-wave-induced enzyme release and elevations in the resting $[Ca^{2+}]_i$.

Corresponding author: Dr. Chung-Ren Jan, Department of Medical Education and Research, Veterans General Hospital-Kaohsiung, 386 Ta Chung 1st Rd, Kaohsiung, Taiwan 813. Fax: 886-7-3468056, E-mail: crjan@isca.vghks.gov.tw

Materials and Methods

Chemical Reagents

The reagents for cell culture were from Gibco (Grand Island, N.Y , USA). All other reagents were from Sigma (St. Louis, Mo., USA) unless otherwise stated.

Cell Culture

As described previously (11, 12). MDCK cells obtained from American Type Culture Collection (CRL-6253, Rockville, Md., USA) were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in 5% CO_2-containing humidified air. Only passages 70-80 were used.

Shock Wave Exposure of Cell Suspensions

Confluent cells in flasks were trypsinized, centrifuged and suspended in DMEM (10^7/ml). For shock wave exposure, a 15-ml polyethylene tube with 1 ml of cell suspension was immersed in a water-filled plastic bag by a custom-made holder. A cork was squeezed into the tube to completely cover the suspension to minimize the possibility that air-liquid interface effects might be responsible for membrane damage (27). Shock waves were generated by a Lithostar II (SIEMENS Inc., Germany) lithotripter with an energy level set at 18 kV and a frequency set at 1 Hz. The cell-containing tube was adjusted to the second focus of the ellipsoid by adjusting the holder. Cells received 64 shock wave impulses which took about 64 seconds.

Enzyme Assay

After SWE, the cell suspensions were centrifuged and the supernatant was assayed for GOT and LDH with a kit from HUMAN (Cat-No: 12011, Taunusstein, Germany). A mixture of 10 μl of supernatant and 50 μl of assay solution was added to a cuvette and the absorbance at 340 nm was detected by a Beckman DU640 spectrophotometer. The absorbance was calculated according to the equation: U/l = Δ Absorbance/min × 952 (GOT) or 8095 (LDH).

Cell Viability Assay

Cell viability was determined by trypan blue exclusion several minutes after SWE. 50 μl of cell suspension was mixed with 50 μl of trypan blue isotonic solution (0.2% w/v) for 15 min. Cell viability was determined on a hemocytometer under a microscope.

Optical Measurements of $[Ca^{2+}]_i$ in Single Cells

Trypsinized cells were centrifuged and suspended in DMEM. The cells were seeded on poly-lysine-coated glass coverslips at an appropriate density allowing imaging of 30-40 single cells. Polylysine coating made cells attach to the coverslips within 30 min. The attached cells were loaded with 2 μM fura-2/AM (Molecular Probes, Eugene, Oreg., USA) for 30 min at 25°C. The coverslip was transferred to a chamber (25°C) on the stage of a Nikon Diaphot microscope and viewed under bright light and UV illumination via a 40 × (1.3 NA) oil immersion fluorescence objective (Nikon Fluor). Solutions were applied as a 3.5-ml bolus to the chamber (containing 0.5 ml of solution). This method allowed rapid and complete change of solution (removed by continued aspiration as the volume of the chamber solution exceeded 0.5 ml temporarily for the new solution).

The MiraCal imaging system (Life Science Resources Ltd., Cambridge, UK) in conjunction with a slow scan cooled CCD camera (CMCA Nikon DF/SB 0.45 x WIDEFIELD) was used for digital video imaging of the spatiotemporal changes of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was calculated to 8-bit accuracy (256 grey levels) every 2 s. Data were analyzed for $[Ca^{2+}]_i$ changes by measurement of the 340 nm (F_{340}) and 380 nm (F_{380}) of excitation signals and emission signal at 510 nm. Ratio values were converted to an estimate of $[Ca^{2+}]_i$ using the formula:

$$[Ca^{2+}]_i = K_d\ \beta\ (R-R_{min})/(R_{max}-R),$$

where R is the ratio F_{340}/F_{380}, R_{min} and R_{max} are the minimum and maximum values of the ratio, attained at zero and saturated Ca^{2+} concentrations, respectively. F_{340} is the fluorescence emitted by the dye when excited at 340 nm and F_{380} is the fluorescence emitted by the dye when excited at 380 nm. β is the ratio of fluorescence intensities for Ca^{2+}-free and Ca^{2+}-bound indicator measured with 380 nm excitation. These constants were obtained by addition of 10 μM ionomycin in solutions containing 2 mM Ca^{2+} (R_{max}=2.1), and no added Ca^{2+} plus 10 mM EGTA (R_{min}=0.2). The value of β is 3.13 and a Kd of 155 nM was assumed [19].

All experiments in this study were performed at 23-25°C.

Statistical Analysis

Values were reported as mean±S.E.. Statistical

comparisons were done by utilizing the Student's two-tailed *t* test or ANOVA with Bonferoni's correction (20). A significant difference was accepted when $p<0.05$.

Results

Ca^{2+} Entry Blockers do not Inhibit the SWE-Induced Enzyme Release

We and others have reported that SWE evoked a transient release of GOT and LDH in MDCK cells (16, 17, 27, 28). In the present study, we first examined the effect of SWE on GOT and LDH release from MDCK cell suspensions. Upon exposure to 64 impulses of shock waves, cells immediately increased release of GOT (Figure 1) by 16% (n=10; $p<0.01$) and of LDH (Figure 2) by 400% (n=10; $p<0.01$). This release was transient because the net release (control subtracted) did not significantly increase in the following 6 hr. Trypan blue assay performed several minutes after SWE demonstrated that both the percentage (>95%) and absolute number of live cell in SWE-treated cells were similar to that of non-SWE-treated cells. Thus, the SWE-induced GOT and LDH release was transient and that cell permeability recovered to normal level within several minutes after SWE. Although there appears to be a slight tendency of increasing in the control release of GOT and LDH at 3 hr and 6 hr, the diffendeces were not statistically significant ($p>0.05$). As shown in Figures 1 and 2, none of the three Ca^{2+} entry blockers (1 or 10 μM) significantly inhibited the evoked enzyme release within 6 hr after SWE ($p>0.05$); except that the GOT release immediately after SWE (0 h) was inhibited by verapamil ($p<0.01$). In Figure 2B, verapamil appears to increase LDH release at time 0 hr and 3 hr; however this was not statistically significant ($p>0.05$).

Because the integrity of cell membrane was perturbed by SWE, implicated by increased enzyme release, it is possible that there might be a rise in $[Ca^{2+}]_i$. Since elevated $[Ca^{2+}]_i$ often associates with cell injury, if SWE causes a rise in $[Ca^{2+}]_i$ in the cells, it is possible that Ca^{2+} entry blockers might protect the cells following SWE by inhibiting extracellular Ca^{2+} influx. Hence, the following experiments were performed to test this hypothesis.

Ca^{2+} Entry Blockers Abolish SWE-Induced Rises in $[Ca^{2+}]_i$

We have recently found that SWE evoked progressive rises in $[Ca^{2+}]_i$ in MDCK cells (9, 10). SWE-treated cells showed no differences in their ability to attach to coverslips and to sequester and hydrolyze fura-2/AM than non-SWE-treated control

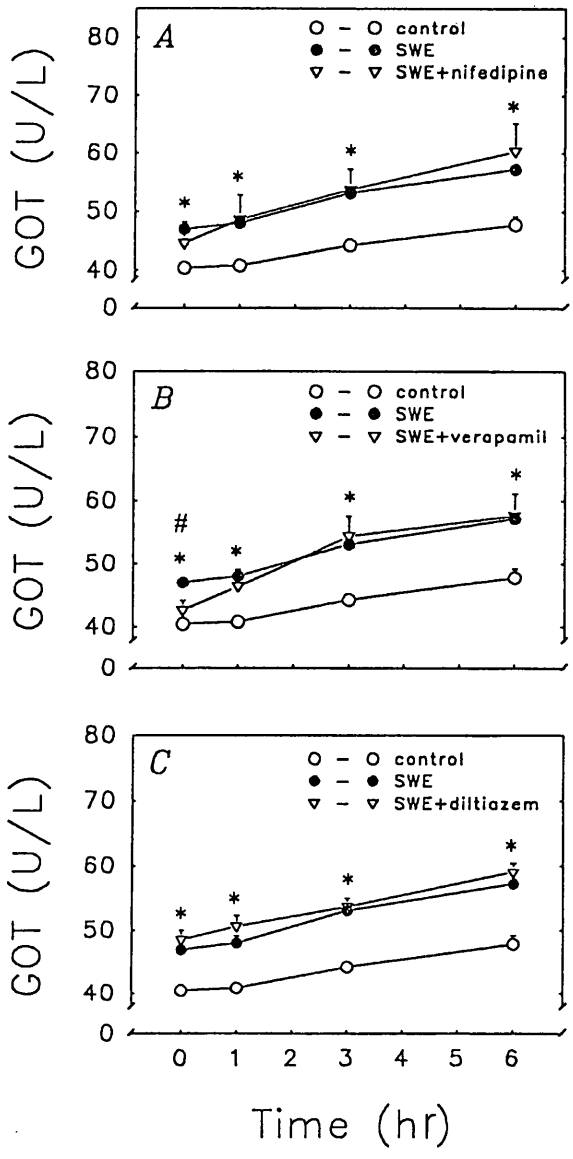

Fig. 1. Effects of nifedipine, verapamil and diltiazem on SWE-induced GOT release in MDCK cells. GOT release was determined in suspended cells within 6 hr after SWE. In blocker-treated group (SWE+blocker; ▽-▽), blocker (1 or 10 μM) was added to cell suspensions before and during SWE and throughout the GOT assay. Data are mean±S.E. of ten (control, ○-○; and SWE; ●-●) or four (SWE+blocker) experiments. *$p<0.01$ between SWE group and control. #$p<0.01$ between SWE+verapamil group and SWE group.

cells judging from cell number and brightness of intracellular fura-2 fluorescence under microscope. Results in Figure 3 shows that $[Ca^{2+}]_i$ in control cells was 68±1 nM (n=178 cells) at 1 hr and 63±2 nM (n=82 cells) at 6 hr. The $[Ca^{2+}]_i$ ranged from 61±2 nM (n=77 cells; 5 hr) to 83±3 nM (n=95 cells; 4 hr).

However, in SWE-treated cells, $[Ca^{2+}]_i$ was significantly ($p<0.01$) elevated above control 1-6 hr after SWE. At 1 hr, $[Ca^{2+}]_i$ was 78±2 nM (n=122 cells; 15% above control); while at 6 hr, $[Ca^{2+}]_i$ was

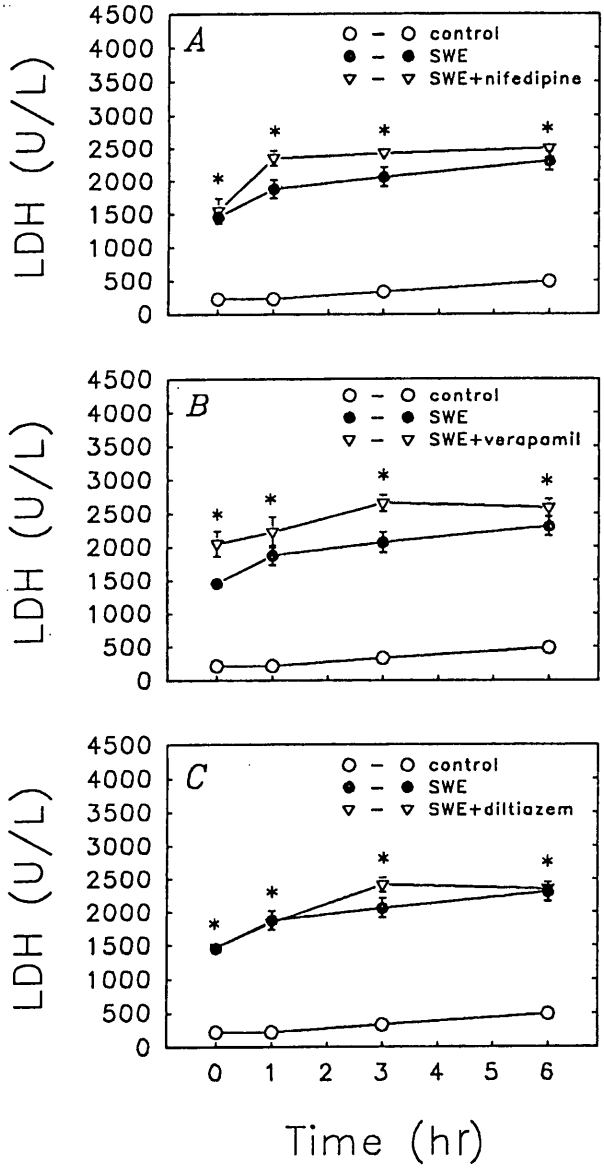

Fig. 2. Effects of nifedipine, verapamil and diltiazem on SWE-induced LDH release in MDCK cells. LDH release was determined in suspended cells within 6 h after SWE. In blocker-treated group (SWE+blocker; ▽-▽), blocker (1 or 10 μM) was added to cell suspensions before and during SWE and throughout the LDH assay. Data are mean±S.E. of ten (control, ○-○; and SWE, ●-●) or four (SWE+blocker) experiments. *p<0.01 between SWE group and control.

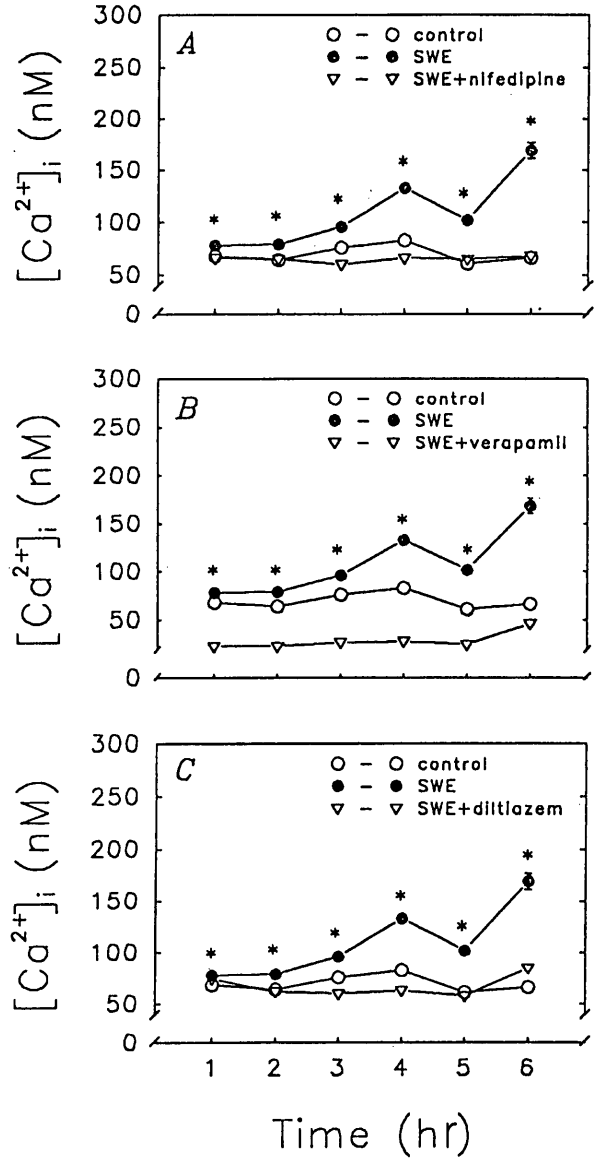

Fig. 3. Inhibition of SWE-evoked $[Ca^{2+}]_i$ rises of single MDCK cells by nifedipine, verapamil and diltiazem. $[Ca^{2+}]_i$ was measured in control (○-○), SWE-treated (SWE; ●-●) and SWE- plus blocker-treated cells (SWE+blocker; ▽-▽) within 6 h after SWE. In SWE+blocker group, blocker (10 μM) was added to cells before and during SWE and throughout the $[Ca^{2+}]_i$ measurements. Data are mean±S.E. of $[Ca^{2+}]_i$ values from 100-400 cells recorded from 10 experiments. *p<0.01 between SWE group and control.

169±8 nM (n=67 cells; 156% above control). In summary, in keeping with our previous findings, we found in this study that SWE induced a gradual rise in $[Ca^{2+}]_i$ within 1-6 hr after SWE.

We next examined the effects of the Ca^{2+} entry blockers on the SWE-evoked $[Ca^{2+}]_i$ rises. Cells were treated with the blocker (1 or 10 μM) before and during SWE and throughout the measurements. Figure 3 shows that in cells treated with nifedipine or diltiazem, $[Ca^{2+}]_i$ within 6 hr after SWE was maintained at 60-70 nM, a level indistinguishable from control. With verapamil-treated cells, $[Ca^{2+}]_i$ within 6 hr after SWE was about 30 nM, which was 50% lower than control. Thus, the results suggest that the Ca^{2+} entry blockers could prevent the SWE-evoked rises in the resting $[Ca^{2+}]_i$ in MDCK cells.

Ca^{2+} Entry Blockers have Minor Effects on Agonists-evoked $[Ca^{2+}]_i$ Changes

Given that the Ca^{2+} entry blockers abolished the SWE-evoked $[Ca^{2+}]_i$ rises, it was critical to determine

Table 1. Effects of Nifedipine, Verapamil and Diltiazem on Peak Amplitude of $[Ca^{2+}]_i$ Changes Evoked by ATP (1 μM), Bradykinin (1 μM) and Thapsigargin (1 μM) in Single MDCK Cells 2-6 hr after SWE.

		ATP	bradykinin	thapsigargin
2 hr	*Control*	673±16 (117)	–	–
	Verapamil	478±30 (7)*	–	–
	Diltiazem	851±17 (21)*	–	–
3 hr	*Control*	–	535±20 (24)	488±15 (46)
	Verapamil	–	512±68 (11)	342±23 (21)*
5 hr	*Control*	–	529±15 (20)	503±18 (48)
	Nifedipine	–	–	541±36 (17)
	Diltiazem	–	754±15 (21)*	566±26 (21)
6 hr	*Control*	623±32 (18)	535±20 (24)	–
	Nifedipine	841±20 (17)*	789±37 (15)*	–
	Verapamil	–	417±17 (19)*	–
	Diltizem	–	635±31 (20)*	–

Control: non-SWE-treated cells. *Nifedipine*, *Verapamil* or *Diltiazem*: cells treated with the blocker (10 μM) which was present during SWE and throughout the $[Ca^{2+}]_i$ measurements. Data are mean±S.E. (nM) of cells from 3-4 separate tubes of cell suspension with the number of cells (n) indicated in parentheses. *$p<0.01$ between blocker group and control. Similar results were obtained by using 1 μM blockers

whether the $[Ca^{2+}]_i$ changes evoked by physiological Ca^{2+}-mobilizing transmitters were altered. We measured the $[Ca^{2+}]_i$ changes evoked by two physiological agonists, ATP and bradykinin, which interact with the plasma membrane receptors. Thapsigargin, which inhibits the endoplasmic reticulum (ER) Ca^{2+}-ATP pump and depletes the ER Ca^{2+} store (30), was used to examine the effects of the blockers on the membrane integrity of the intracellular organelles. We have recently shown that ATP (13), bradykinin (14) and thapsigargin (15) evoke robust $[Ca^{2+}]_i$ increases in MDCK cells. The effects of the Ca^{2+} entry blockers (1 or 10 μM) on the peak amplitude of the evoked $[Ca^{2+}]_i$ changes in SWE-treated cells are summarized in Table 1. The peak of the $[Ca^{2+}]_i$ changes evoked by ATP was increased by 35% in nifedipine-treated cells and by 20-26% (for different time points) in diltiazem-treated cells. The peak of bradykinin-evoked $[Ca^{2+}]_i$ changes was increased by 47% in nifedipine-treated cells and by 18-43% (for different time points) in diltiazem-treated cells. However, in verapamil-treated cells, the peak of ATP- and bradykinin-evoked $[Ca^{2+}]_i$ changes was comparable to control with a maximal difference less than 28%. Verapamil reduced the peak of the thapsigargin-evoked $[Ca^{2+}]_i$ changes by 28%; while nifedipine and diltiazem had no effect. Figure 4 illustrates representative recordings showing that in cells 2 h after SWE, ATP (1 μM), bradykinin (1 μM) or thapsigargin (1 μM) evoked robust $[Ca^{2+}]_i$ changes (Fig. 4A-4C; left-hand panels). The peak amplitude and kinetics of these $[Ca^{2+}]_i$ changes were largely similar to those from non-SWE-treated cells (16). When verapamil was present, the net peak amplitude of the $[Ca^{2+}]_i$ changes evoked by ATP was reduced by 27% (Fig. 4A; 478±30; n=7 vs. 673±16; n=117); those evoked by thapsigargin was reduced by 28% (Fig. 4C; 342±23; n=21 vs. 488±15; n=46); while the bradykinin-evoked response was unaltered (Fig. 4B; 512±68; n=11 vs. 535±20; n=24). Verapamil (or nifedipine and diltiazem; not shown) did not affect the kinetics of the $[Ca^{2+}]_i$ changes. Collectively, the data suggest that the Ca^{2+} entry blockers abolished the SWE-induced rises in the resting $[Ca^{2+}]_i$ without dramatic effects (less than 50%) on the $[Ca^{2+}]_i$ changes evoked by ATP, bradykinin or thapsigargin.

Discussion

In this study we have examined the protective effect of nifedipine, verapamil and diltiazem on the SWE-induced injury in MDCK cells. We have found that the net release of GOT and LDH in suspended cells was increased immediately but transiently after SWE; and the resting $[Ca^{2+}]_i$ was gradually elevated within 1-6 hr afterward as we have reported previously (16, 17). Specifically, the bockers abolished the SWE-induced long-term rises in the resting $[Ca^{2+}]_i$ without dramatic effects on the $[Ca^{2+}]_i$ changes evoked by Ca^{2+} mobilizing agents. The blockers did not inhibit the transient SWE-induced enzyme release.

The underlying cause of the possible protection of Ca^{2+} entry blockers on the kidney cells appears to be a normal resting $[Ca^{2+}]_i$. Fura-2 measurements revealed a significant rise in $[Ca^{2+}]_i$ in MDCK cells between 1 to 6 hr following SWE. We must emphasize

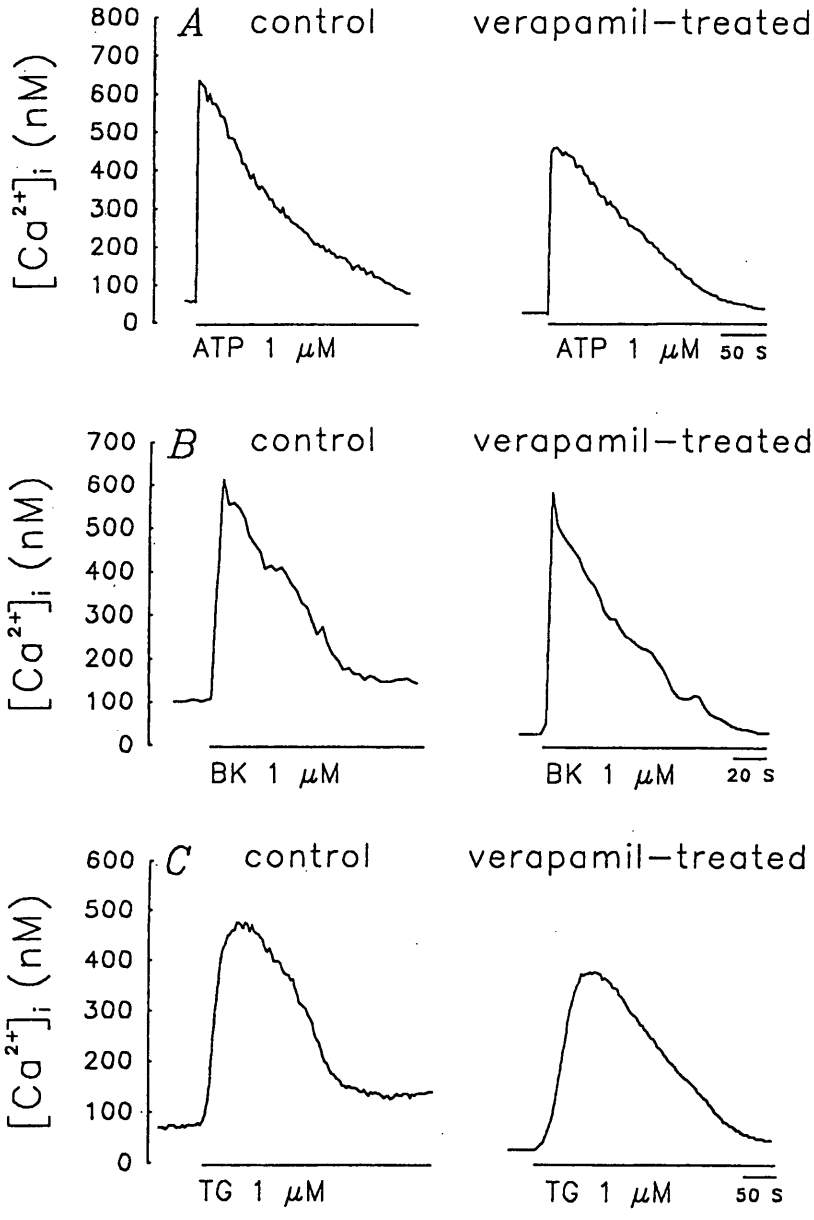

Fig. 4. Representative recordings of $[Ca^{2+}]_i$ in single MDCK cells 2 h after SWE. Addition of 1 μM ATP (A), 1 μM bradykinin (BK; B) or 1 μM thapsigargin (TG; C) evoked $[Ca^{2+}]_i$ changes in verapamil-treated and non-verapamil-treated (control) cells. The long bars indicate both the reagent that was used and the time during which the reagent was applied. Verapamil (10 μM) was added to cells before and during SWE and throughout the $[Ca^{2+}]_i$ measurements. The short bars indicate time scale in seconds (s). Each recording is representative of 7-117 cells with the n shown in Table 1.

here that because the measurements were made 1 hr after SWE (due to the time needed for cell attachment and fura-2/AM loading), the results did not reflect the transient $[Ca^{2+}]_i$ changes occurring during or immediately after SWE. As we have discussed previously (16, 17), during SWE, since intracellular enzymes had leaked out, extracellular Ca^{2+} would have flowed into the cell due to the 20,000-fold Ca^{2+} gradient across the plasma membrane. Interestingly, this expected rise in $[Ca^{2+}]_i$ returned to physiological levels (<100 nM) within 1 h after SWE was removed (Fig. 3; 1 hr); but to measure the instantaneous $[Ca^{2+}]_i$ changes was beyond the capacity of our experimental protocol.

However, the impairment that SWE inflicted upon the cells rendered $[Ca^{2+}]_i$ to rise gradually in the following 5 h. The long-term $[Ca^{2+}]_i$ rise may represent increased extracellular Ca^{2+} influx and/or internal Ca^{2+} release (16, 17). Although cell membrane permeability recovered rapidly after SWE, the impairment caused by SWE might have weakened the cells and hindered them from long-term regulation of

normal resting $[Ca^{2+}]_i$. This increased Ca^{2+} influx during the long-term phase after the transient shock wave insult is likely because shock waves are known to enhance production of free radicals (29). Because the organelle membrane could be damaged, as indicated by the reduced thapsigargin-sensitive endoplasmic reticulum Ca^{2+} stores and release of enzymes, $[Ca^{2+}]_i$ might rise due to Ca^{2+} release from damaged organelles and/or impaired ability of Ca^{2+} pumps to pump cytosolic Ca^{2+} into organelles. $[Ca^{2+}]_i$ levels in many tissues, including the kidney, often rise when cell injury develops in the presence of extracellular Ca^{2+}. In LLC-PK1 cells, another kidney cell line, ischemia was found to increase $[Ca^{2+}]_i$ (4).

SWE-evoked enzyme release occurred immediately after the insult and stopped once SWE was removed. This suggests that cell permeability to GOT and LDH recovered quickly to the extent that large molecules stopped leaking out (this study; 16, 17). Despite the damage of the cell membrane integrity induced by SWE, ATP and bradykinin receptors and the endoplasmic reticulum Ca^{2+} pump, and also the effector molecules involved in Ca^{2+} mobilization were largely intact, because all three reagents evoked robust $[Ca^{2+}]_i$ changes which were only slightly different from control (16).

Note that the $[Ca^{2+}]_i$ changes we measured were that within 1-6 hr after SWE when the enzyme release had stopped, not the instantaneous changes during the short insult of shock waves when the enzymes were transiently leaking out. This may explain why in SWE-treated cells, despite the Ca^{2+} entry blockers blocked the long-term rises in the resting $[Ca^{2+}]_i$ 1-6 hr after SWE, they did not inhibit the transient release of enzymes occurring during SWE. It is interesting to note that despite the transient damage done by SWE, the cells regained transient normal membrane permeability quickly within 1 hr after SWE, and retained responsiveness to Ca^{2+}-mobilizing reagents.

The fact that in SWE-treated cells, Ca^{2+} entry blockers kept the resting $[Ca^{2+}]_i$ at a level similar to or lower than that in non-SWE-treated cells while did not markedly inhibit the $[Ca^{2+}]_i$ changes evoked by agonists supports the hypothesis that Ca^{2+} entry blockers might directly protect the kidney cells in vivo against SWL-induced damage and, in turn, result in a protection of renal function.

In cells within 1-6 hr after SWE, the peak amplitude and kinetics of the $[Ca^{2+}]_i$ changes evoked by ATP, bradykinin and thapsigargin were not much altered by the blockers. This is critical because even these Ca^{2+} entry blockers maintained a normal resting $[Ca^{2+}]_i$ in SWE-treated cells, they would not protect the cells if they markedly affect the $[Ca^{2+}]_i$ changes evoked by physiological Ca^{2+}-mobilizing transmitters.

While the cause of the rise in $[Ca^{2+}]_i$ induced by SWE is not known, there are at least two mechanisms by which nifedipine, verapamil and diltiazem could act: 1) a direct block of Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels; 2) a direct block of Ca^{2+} influx through voltage-insensitive Ca^{2+} channels. The first possibility is unlikely because MDCK cells do not possess voltage-gated Ca^{2+} channels (20). The second possibility is plausible because it was reported that nifedipine or verapamil inhibited basal $^{45}Ca^{2+}$ uptake in MDCK cells (5), even though a voltage-insensitive Ca^{2+} current has not been identified by electrophysiology. The fact that in SWE-treated cells verapamil lowered $[Ca^{2+}]_i$ to 50% of control suggests that verapamil could inhibit extracellular Ca^{2+} influx through voltage-insensitive Ca^{2+} channels. In hepatocytes which also lack voltage-gated Ca^{2+} channels (10), $[Ca^{2+}]_i$ has been shown to increase after ischemia; and this increase was blocked by diltiazem (19).

Alterations of renal function after SWL are not only a question of direct effects on renal tubular cells, but also on intracellular hemodynamic processes, and of course, cannot be assessed with cell cultures. Nevertheless, our results might still provide useful clues for future investigations of the mechanisms underlying the protective effects of Ca^{2+} entry blockers on patients receiving SWL.

Acknowledgments

This work was supported by NSC 87-2314-B-075B-014, VGHKS87-53 to CRJ. We thank Dr. L.P. Ger for performing the ANOVA.

References

1. Assimos, D.G., W.H. Boyce, E.G. Furr, N.A. Espeland, R.P. Holmes, L.H. Harrison, R.L. Kroovand, and D.L. McCullough. Selective elevation of urinary enzyme levels after extracorporeal shock wave lithotripsy. *J. Urol.* 142: 687-690, 1989.
2. Baumgartner, B.R., K.W. Dickey, and S.S. Ambrose. Kidney changes after extracorporeal shock wave lithotripsy. *Radiology* 163: 531-534, 1987.
3. Caussy, C., W. Brendel and E. Schmiedt. Extracorporeally-induced destruction of kidney stones by shock waves. *Lancet* 2: 1265-1268, 1990.
4. Cheung, J.Y., J.M. Constantine, and J.V. Bonventre. Regulation of cytosolic free calcium concentration in cultured renal epithelial cells. *Am. J. Physiol.* 251: F690-F701, 1986.
5. Contreras, R.G., J.H. Miller, M. Zamora, L. Gonzalez-Mariscal, M. Cereijido. Interaction of calcium with plasma membrane during junction formation. *Am. J. Physiol.* 263: C313-318, 1992.
6. Corbally, M.T., J. Ryan, J. Fitzpatrick, and R.J. Fitzgerald. Renal function following extracorporeal lithotripsy in children. *J. Ped. Surg.* 26: 539-540, 1990.
7. Gaush, C.R., W.L. Hard, and T.F. Smith. Characterization of an established line of canine kidney cells. *Proc. Soc. Exp. Biol. Med.* 122: 931-935, 1996.
8. Gilbert, B.R., R.A. Riehle, and E.D. Jr. Vaughan. Extracorporeal shock wave lithotripsy and its effects on renal function. *J. Urol.* 139:

482-485, 1998.
9. Grynkiewicz, G., M. Poenie, and R.Y. Tsien. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260: 3440-3450, 1985.
10. Hughes, B.P., K, Both, L. Harland, J. Hunt, K.M. Hurst, M. Lewis, and G.J. Barritt. Identification of an mRNA species which encodes a voltage-operated Ca^{2+} channel in rat liver mRNA. *Biochem. Mol. Biol. Int.* 31: 193-200, 1993.
11. Jan, C.R., C.M. Ho, S.N. Wu, and C.J. Tseng. La^{3+} inhibits the UTP-induced Ca mobilization in MDCK cells. *Chin. J. Physiol.* 41: 59-64, 1998.
12. Jan, C.R., C.M. Ho, S.N. Wu, and C.J. Tseng. ADP-evoked calcium signals in MDCK cells. *Chin. J. Physiol.* 41: 67-73, 1998.
13. Jan, C.R., C.M. Ho, S.N. Wu, J.K. Huang, and C.J. Tseng. Mechanism of lanthanum inhibition of extracellular ATP-evoked calcium mobilization in MDCK cells. *Life Sci.* 62: 533-540, 1998.
14. Jan, C.R., C.M. Ho, S.N. Wu, and C.J. Tseng. Bradykinin-evoked Ca mobilization in MDCK cells. *Eur. J. Pharmacol.* 355: 219-233. 1998.
15. Jan, C.R., C.M. Ho, S.N. Wu, and Tseng, C.J. The phospholipase C inhibitor U73122 elevates cytoplasmic calcium levels in Madin Darby canine kidney cells by activating calcium influx and releasing stored calcium. *Life Sci.* 63: 885-898, 1998.
16. Jan, C.R., J.K. Huang, and C.J. Tseng. High energy shock waves alter cytosolic calcium mobilization in single MDCK cells. *Nephron* 78: 187-194, 1998.
17. Jan, C.R., W.C. Chen, Y.H. Lee, J.K. Huang, H.C. Ou, and C.J. Tseng. Allopurinol blocks shock-wave-induced rises in cytosolic calcium levels in MDCK cells. *Urol. Res.* 25: 427-432, 1997.
18. Kishimoto, T., K. Yamamoto, T. Sugimoto, H. Yashihara, and M. Maekawa. Side effects of extracorporeal shock-wave exposure in patients treated by extracorporeal shock-wave lithotripsy for upper urinary tract stones. *Eur. Urol.* 12: 308-313, 1986.
19. Kurita, K., G. Tanabe, T. Aikou, and H. Shimazu. Inhibition of the increase of intrahepatic Ca^{2+} by diltiazem in rats with liver ischemia. *J. Hepatol.* 21: 567-571, 1994.
20. Lang, F. and M. Paulmichl. Properties and regulation of ion channels in MDCK cells. *Kidney Int.* 48: 1200-1205, 1995.
21. Li, B., W. Zhou, and P. Li. Protective effects of nifedipine and allopurinol on high energy shock wave induced acute changes of renal function. *J. Urol.* 153: 596-598, 1993.
22. Lingeman, J.E., J.R. Woods, and P.D. Toto. Blood pressure changes following extracorporeal shock wave lithotripsy and other forms of treatment for nephrolithiasis. *JAMA* 263: 1789-1794, 1990.
23. Scheffe, H.A.. The analysis of variance. New York, John Wiley & Sons, 1959. 21. Humes, H.D.. Role of calcium in pathogenesis of acute renal failure. *Am. J. Physiol.* 250: F579-F589, 1986.
24. Strohmaier, W.L., A. Abelius, I. Billes, T. Grossmann, D.M. Wilbert, and K.H. Bichler. Verapamil limits shock wave-induced renal tubular damage in vivo. *J. Endourol.* 8: 260-273, 1994.
25. Strohmaier, W.L., J.K. Bichler, J. Koch, N. Balk, and D.M. Wilbert. Protective effect of verapamil on shock wave induced renal tubular dysfunction. *J. Urol.* 150: 27-29, 1993.
26. Strohmaier, W.L., J. Koch, N. Balk, D.M. Wilbert, and K.H. Bichler. Limitation of shock-wave-induced renal tubular dysfunction by nifedipine. *Eur. Urol.* 25: 99-104, 1994.
27. Strohmaier, W.L., K.H. Bichler, S. Kleinknecht, M. Pedro, and D.M. Wilber. Damaging effects of high energy shock waves on cultured Madin Darby canine kidney (MDCK) cells. *Urol. Res.* 18: 255-258, 1990.
28. Strohmaier, W.L., M. Pedro, D.M. Wilbert, and K.H. Bichler. Reduction of shock wave-induced tubular alteration by fosfomycin. *J. Endourol.* 5: 57-60, 1991.
29. Suhr, D., Brummer, F., and Hulser, D.F. Cavitation-generated free radicals during shock wave exposure: investigations with cell-free solutions and suspended cells. *Ultrasound. Med. Biol.* 17: 761-768, 1991.
30. Thastrup, O., P.T. Cullen, B.K. Drobak, M.R. Hanley, and A.P. Dawson. Thapsigargin, a tumor promotor, discharges intracellular calcium stores by specific inhibition of the endoplasmic reticulum calcium ATPase. *Proc. Natl. Acad. Sci.* USA 87: 2466-2470, 1990.