Original Paper

NEPHRON

Nephron 1998;78:187–194

Accepted: July 29, 1997

Chung-Ren Jan
Jong-Khing Huang
Ching-Jiunn Tseng

Department of Medical Education and Research, Veterans General Hospital-Kaohsiung, Taiwan, ROC

High-Energy Shock Waves Alter Cytosolic Calcium Mobilization in Single MDCK Cells

Key Words
Shock waves
MDCK cells
Calcium imaging
Fura-2
Calcium signaling

Abstract
The damaging effect of shock waves on intracellular calcium homeostasis in renal tubular cells has not been investigated previously. We have examined the effects of shock waves on Madin Darby canine kidney (MDCK) cells by determining the release of two cellular enzymes: glutamate oxalactate transferase (GOT) and lactate dehydrogenase (LDH); and both the basal cytosolic calcium concentration ($[Ca^{2+}]_i$) and the $[Ca^{2+}]_i$ rises evoked by Ca^{2+}-mobilizing agonists after shock wave exposure (SWE). Immediately but transiently after SWE, the release of GOT and LDH increased by 15% and 5-fold, respectively, implicating cell membrane damage; and within 1–7 h after SWE, basal $[Ca^{2+}]_i$ was elevated by 15–141%. In SWE-treated cells, the peak amplitude of the $[Ca^{2+}]_i$ transients evoked by ATP, bradykinin, thapsigargin and 2,5-Di-(t-butyl)-1,4-hydroquinone (BHQ) was slightly altered, and the plateau amplitude was markedly elevated. The ATP and bradykinin receptors, the Ca^{2+} pump on the endoplasmic reticulum membrane, and the effector molecules involved in regulating the $[Ca^{2+}]_i$ transients were slightly affected. Shock waves appeared to cause a mild impairment in the mechanisms responsible for the decay of the evoked $[Ca^{2+}]_i$ rises.

Introduction

The MDCK cell line has been used as a model for renal cells for over three decades [1]. Ca^{2+} homeostasis is important in many aspects of the MDCK cell function. Previous studies have shown that several hormones could elevate $[Ca^{2+}]_i$ in MDCK cells. These hormones include bradykinin [2–4], ATP [5], carbachol [4], epinephrine [6] and prostaglandins [7]. The Ca^{2+} signal could activate Ca^{2+}-dependent K^+ currents [5] leading to cell hyperpolarization [8] and chloride secretion [9].

It has been reported that shock wave exposure (SWE) induced an increase in GOT and LDH release from MDCK cell suspensions, which might indicate cell membrane damage [10, 11].

The present study was aimed at investigating the effects of SWE on Ca^{2+} homeostasis in cultured cells. We have examined the effects of SWE on MDCK cells by measuring two fine-tuned cellular phenomena: basal $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ rises evoked by agonists. In order to choose an appropriate intensity of SWE for the Ca^{2+} work, we have also examined the effects of SWE on the release of GOT and LDH from cell suspensions.

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 1998 S. Karger AG, Basel
0028–2766/98/0782–0187$15.00/0

This article is also accessible online at:
http://BioMedNet.com/karger

C.R. Jan
Department of Medical Education and Research
Veterans General Hospital-Kaohsiung
386 Ta Chung 1st Rd, Kaohsiung, Taiwan 813 (ROC)
Tel. 886-7-3422121 x 1509, Fax 886-7-3468056, E-Mail crjan@isca.vghks.gov.tw

Downloaded by:
Univ. of California San Diego
132.239.1.231 - 5/10/2017 10:17:31 AM

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

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 of 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 in order to minimize the possibility that air-liquid interface effects might be responsible for membrane damage [10]. Shock waves were generated by a Lithostar II (Siemens, Germany) lithotripter with an energy level set at 18 kV. The cell-containing tube was adjusted to the second focus of the ellipsoid by adjusting the holder.

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 concentrations of GOT and LDH were calculated from the absorbance 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. 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 Individual Cells

Trypsinized cells were centrifuged and suspended in DMEM. Cells were seeded on polylysine-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 μ*M* 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, Mass., UK) in conjunction with a slow scan cooled CCD camera (CMCA Nikon DF/SB 0.45× Widefield) was used for digital video imaging of the changes of $[Ca^{2+}]_i$ in individual cells. $[Ca^{2+}]_i$ 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 μ*M* ionomycin in solutions containing 2 m*M* Ca^{2+} (R_{max} = 2.1), and no added Ca^{2+} plus 10 m*M* EGTA (R_{min} = 0.2). The value of β is 3.13 and a K_d of 155 n*M* was assumed [12].

All experiments in this study were done at 23–25°C.

Statistical Analysis

Values are reported as mean ± SE. Statistical comparisons were done by utilizing the Student's two-tailed t test or ANOVA with Bonferroni's correction. A significant difference was accepted when $p < 0.05$.

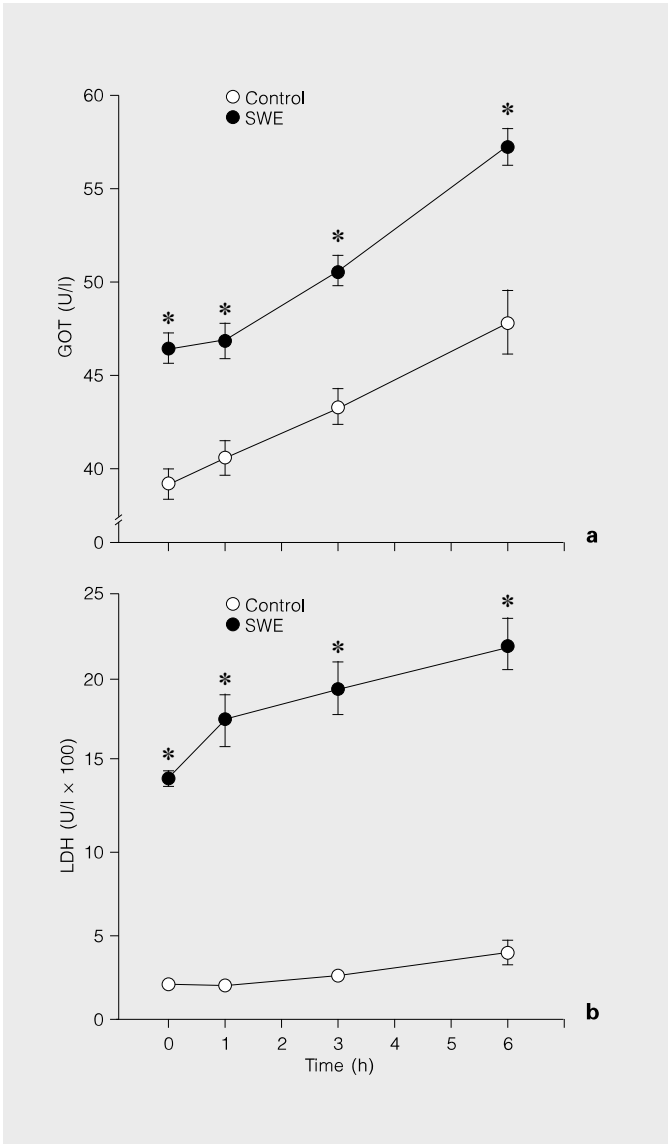
Results

Increased Release of GOT and LDH from MDCK Cells after SWE

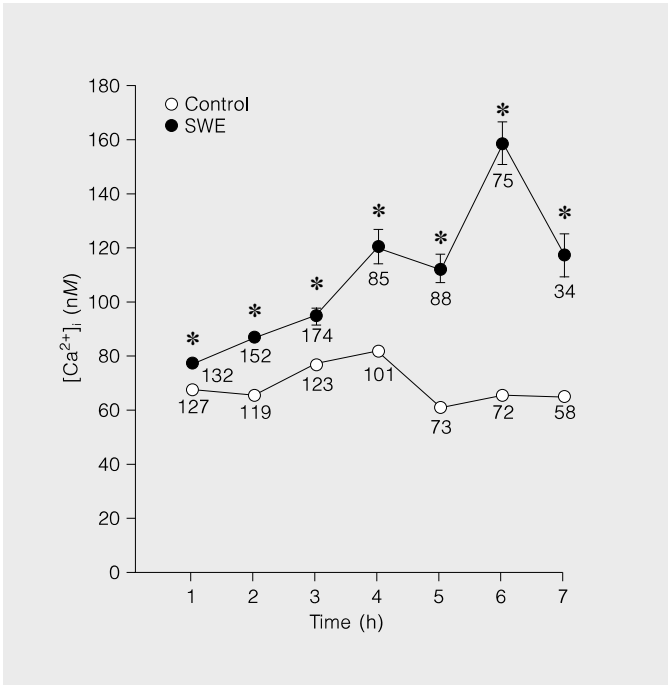
To choose an appropriate shock wave intensity for determining the effects of shock waves on $[Ca^{2+}]_i$ in MDCK cells, we first examined the effects of shock waves of several impulse rates on enzyme release from MDCK cells. An immediate increase in GOT and LDH release was found in cells exposed to 16, 32, 64 and 128 impulses of shock waves with 16–32 impulses giving a minimal effect, 64 impulses a moderate effect, and 128 impulses a dramatic effect (not shown), consistent with a previous report [10]. The enzyme release appeared to be transient because it did not increase within 7 h after SWE of different impulse rates. Trypan blue exclusion assay done several minutes after SWE showed that the viability of cells exposed to 64 impulses was similar to control (>95%). Based on the results from these experiments and a previous report [10], an intensity of 64 impulses was chosen for the following experiments to produce moderate damage to the cells.

Upon exposure to 64 impulses of shock waves, MDCK cells significantly increased release of GOT by 15% (fig. 1 top; n = 8; $p < 0.01$) and of LDH by 5-fold (fig. 1 bottom; n = 8; $p < 0.01$) above control within 6 h after SWE, implicating that the cell membrane integrity was perturbed.

Downloaded by:
Univ. of California San Diego
132.239.1.231 - 5/10/2017 10:17:31 AM



1



2

Fig. 1. Effect of SWE on GOT and LDH release in MDCK cells. Enzyme release was determined in suspended MDCK cells within 6 h after SWE. Data are mean ± SE of 8 separate experiments. * $p < 0.01$ between SWE group and control.

Fig. 2. Effect of SWE on basal $[Ca^{2+}]_i$ of single MDCK cells. Basal $[Ca^{2+}]_i$ was measured in control and SWE-treated cells within 7 h after SWE. Data are mean ± SE with n (number of cell examined) indicated under each data point. * $p < 0.01$ between SWE group and control.

The SWE-induced net release of enzymes did not significantly increase ($p > 0.05$) within 6 h after SWE.

SWE-Induced Rises in Basal $[Ca^{2+}]_i$

Because the MDCK cell membrane was perturbed by SWE, it was conceivable that a rise in basal $[Ca^{2+}]_i$ could occur due to either Ca^{2+} influx from extracellular fluid and/or Ca^{2+} release from intracellular Ca^{2+} stores. Thus, we examined basal (i.e. unstimulated) $[Ca^{2+}]_i$ in non-SWE-treated and SWE-treated cells. For these experiments, suspended cells which had been exposed to 64 impulses of shock waves were plated on coverslips 1 h before the $[Ca^{2+}]_i$ measurements. Non-SWE-treated cells were plated as control. Most of the cells attached to the coverslips firmly in 30 min. The attached cells were loaded with the membrane-permeant, Ca^{2+} indicator dye, fura-2-AM [12] for 30 min to monitor $[Ca^{2+}]_i$. We must emphasize here that enzyme assay was done immediately after SWE, while due to the time needed for cell attachment and dye-loading, the $[Ca^{2+}]_i$ measurements could only be performed 1 h after SWE, and thus only reflected the long-term effect of SWE 1–7 h after SWE was removed. SWE-treated cells showed no differences in their ability to attach to coverslips and to sequester and hydrolyze fura-2-AM from non-SWE-treated controls. Figure 2 shows that basal $[Ca^{2+}]_i$ in controls was 67 ± 1 n*M* (n =

Downloaded by:
Univ. of California San Diego
132.239.1.231 - 5/10/2017 10:17:31 AM

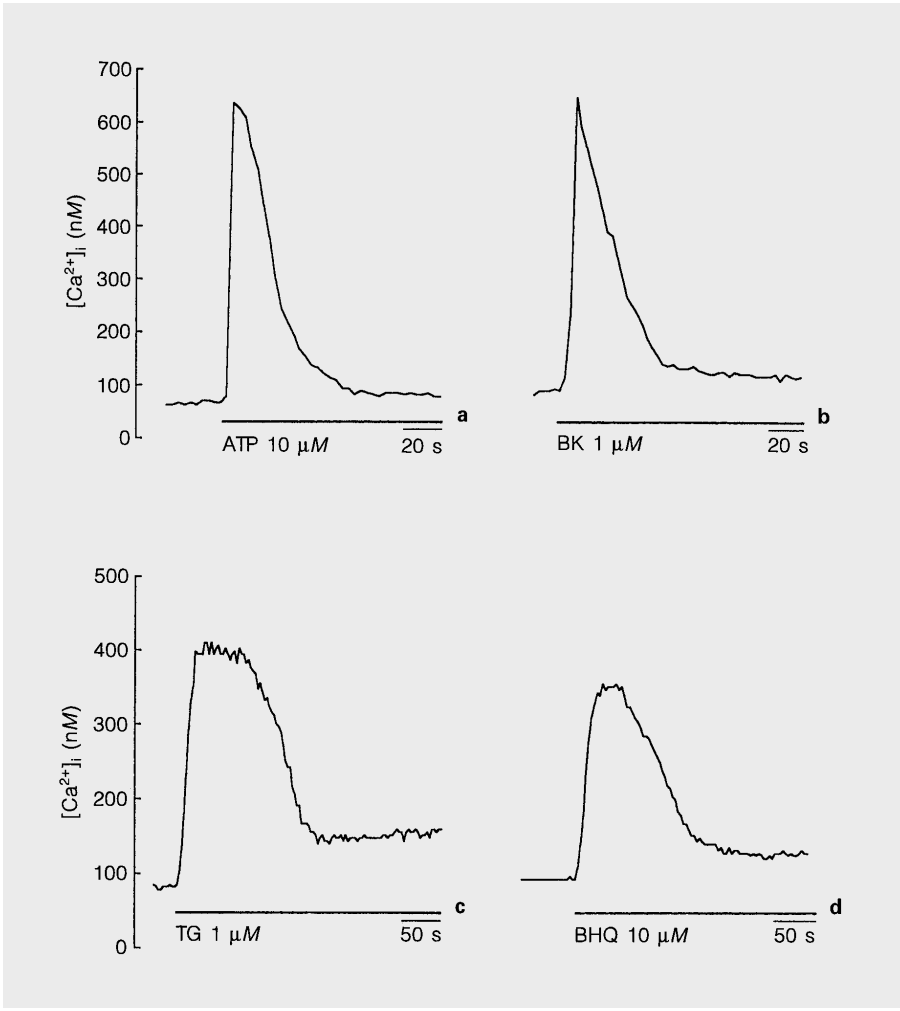


Fig. 3. Representative recordings of evoked $[Ca^{2+}]_i$ rises in single MDCK cells 2 h after SWE. Addition of 10 μ*M* ATP (**a**), 1 μ*M* bradykinin (BK; **b**), 1 μ*M* thapsigargin (TG; **c**) or 10 μ*M* BHQ (**d**) to the cells evoked $[Ca^{2+}]_i$ rises. The long bars indicate both the reagent that was used and the time during which the reagent was applied. The short bars indicate time scale in seconds. Each recording is typical of similar responses from 50–70 cells.

127 cells) at 1 h and 65 ± 2 n*M* (n = 58 cells) at 7 h. The $[Ca^{2+}]_i$ ranged from 61 ± 2 n*M* (n = 73 cells; 5 h) to 82 ± 2 n*M* (n = 101 cells; 4 h). Thus it appears that in control cells within 7 h after plating, $[Ca^{2+}]_i$ was maintained within physiological resting ranges, i.e. ~100 n*M*.

However, $[Ca^{2+}]_i$ in unstimulated SWE-treated cells was significantly ($p < 0.01$) elevated above control within 1–7 h after SWE. At 1 h, the $[Ca^{2+}]_i$ was 77 ± 2 n*M* (n = 132 cells; 15% above control), while at 6 h, it was 159 ± 8 n*M* (n = 75 cells), which was 141% above control (66 ± 1 n*M*; n = 72 cells). At 7 h, $[Ca^{2+}]_i$ declined to 118 ± 8 n*M* (n = 34 cells), which was still 82% above control. Collectively, the data suggest that within 1–7 h after SWE there was a significant rise in $[Ca^{2+}]_i$ in unstimulated MDCK cells.

Effects of Shock Waves on $[Ca^{2+}]_i$ Rises Evoked by Agonists

We next examined the effects of shock waves on the $[Ca^{2+}]_i$ rises evoked by potent Ca^{2+}-mobilizing agents. To determine the effects of SWE on both the plasma membrane level and the organelle membrane level, we measured the $[Ca^{2+}]_i$ rises evoked by two physiological agonists, ATP [5] and bradykinin [2–4], which activate the plasma membrane receptors; and two agents, thapsigargin [14] and BHQ [15], which elevate $[Ca^{2+}]_i$ by inhibiting endoplasmic reticulum (ER) Ca^{2+} pumps and deplete ER Ca^{2+} stores. In previous studies, ATP [5], bradykinin [2–4] and thapsigargin [16] have been shown to evoke robust $[Ca^{2+}]_i$ rises in MDCK cells. Figure 3 shows examples of experiments illustrating recordings of the $[Ca^{2+}]_i$ rises evoked by these agents. All of the $[Ca^{2+}]_i$ rises consist of a promptly peak, a decaying slope and a lasting plateau. In

Downloaded by:
Univ. of California San Diego
132.239.1.231 - 5/10/2017 10:17:31 AM

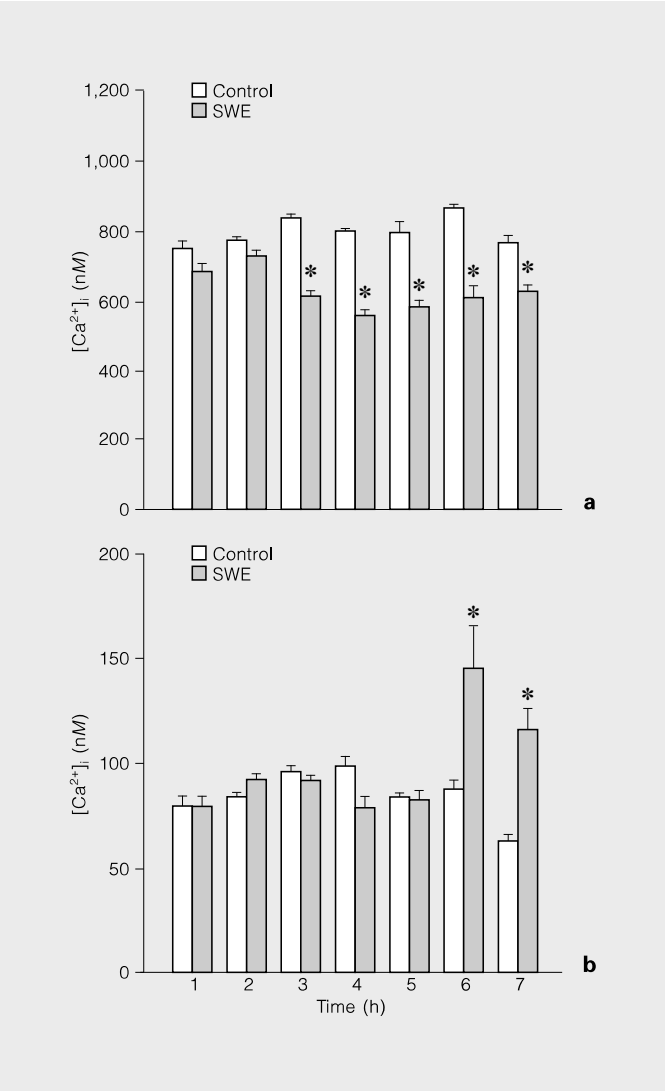


Fig. 4. Amplitude of peak (**a**) and plateau (**b**) of ATP-evoked $[Ca^{2+}]_i$ rises in single non-SWE-treated cells (control) and SWE-treated cells within 7 h after SWE. Concentration of ATP: 10 μ*M*. Data are mean ± SE of 40–45 cells. * $p < 0.01$ between SWE group and control.

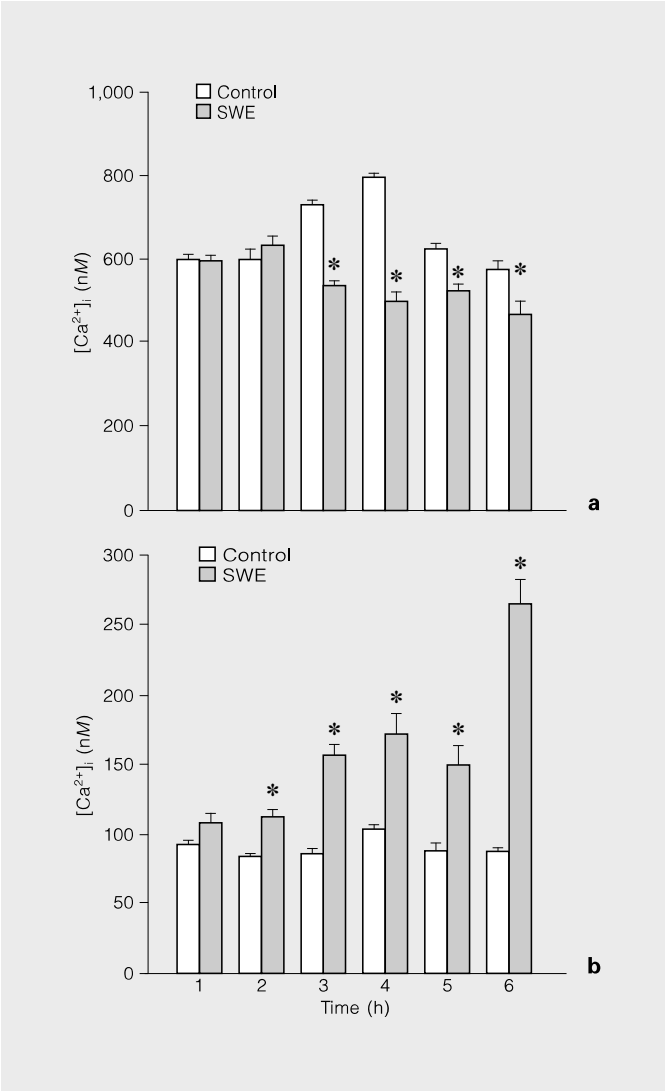


Fig. 5. Amplitude of peak (**a**) and plateau (**b**) of bradykinin-evoked $[Ca^{2+}]_i$ rises in single non-SWE-treated cells (control) and SWE-treated cells within 7 h after SWE. Concentration of bradykinin: 1 μ*M*. Data are mean ± SE of 45–55 cells. * $p < 0.01$ between SWE group and control.

this set of experiments (1–3 h after SWE), the kinetics of the $[Ca^{2+}]_i$ rises in SWE-treated cells were indistinguishable from that from non-SWE-treated controls (not shown) except a higher plateau in thapsigargin- and BHQ-evoked $[Ca^{2+}]_i$ transients. The amplitude of the peak and the plateau of the $[Ca^{2+}]_i$ transients evoked by ATP, bradykinin and thapsigargin in controls and SWE-treated cells (within 1–7 h after SWE) is shown in figures 4–6 (peak: A; plateau: B). In the $[Ca^{2+}]_i$ rises evoked by all three agents, there appears to be a small but significant reduction ($p < 0.01$) in the peak amplitude in SWE-treated cells compared to non-SWE-treated cells; especially within 3–6 h after SWE with a mean maximum reduction of 37% (n = 50 cells) found in the bradykinin-evoked rises 4 h after SWE. In contrast, the plateau amplitude of the evoked $[Ca^{2+}]_i$ rises was remarkably elevated more than 50% above control in most cases with a mean maximum elevation as high as 202% (n = 48 cells) observed in bradykinin-evoked rises 6 h after SWE (fig. 5). In cells 3 h after SWE, the mean peak amplitude of BHQ-evoked

Downloaded by:
Univ. of California San Diego
132.239.1.231 - 5/10/2017 10:17:31 AM

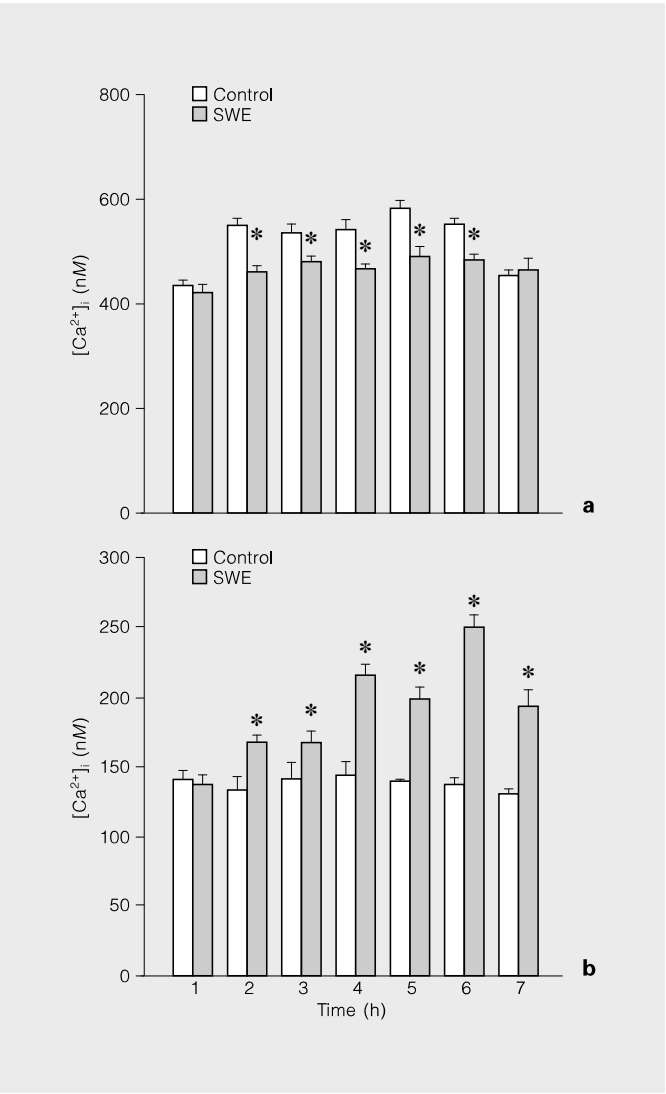


Fig. 6. Amplitude of peak (**a**) and plateau (**b**) of thapsigargin-evoked $[Ca^{2+}]_i$ rises in single non-SWE-treated cells (control) and SWE-treated cells within 7 h after SWE. Concentration of thapsigargin: 1 μ*M*. Data are mean ± SE of 48–52 cells. * $p < 0.01$ between SWE group and control.

$[Ca^{2+}]_i$ rises was reduced to 69% of control, while the mean plateau amplitude was elevated by 81% above control (n = 45 cells; not shown).

Discussion

In this study we examined the damaging effects of shock waves on MDCK cells. We found that the GOT and LDH release in suspended cells increased immediately but transiently after SWE, and the basal $[Ca^{2+}]_i$ in unstimulated cells measured within 1–7 h after SWE was markedly elevated; both implicating that cell membranes were damaged. The $[Ca^{2+}]_i$ rises evoked by agonists in SWE-treated cells showed a slightly altered peak amplitude but a markedly elevated plateau amplitude. We conclude that the plasma membrane receptors, the Ca^{2+} pump on the endoplasmic reticulum membrane, and the effector molecules responsible for the evoked $[Ca^{2+}]_i$ rises were only slightly altered after SWE, despite a transient release of cellular enzymes and an elevated basal $[Ca^{2+}]_i$. The mechanisms involved in lowering the evoked $[Ca^{2+}]_i$ rises to the normal plateau appear to be slightly impaired.

SWE has been reported previously to induce GOT and LDH release from MDCK cell suspensions [10, 11]. It is thought that GOT exists in the cytosol and mitochondria, LDH exists on the plasma membrane and in the cytosol, and increases in the release of GOT and LDH are signs of cell injury, with GOT level indicating more severe membrane damage [11]. In this study, we found that SWE increased GOT release by 15% and LDH by 5-fold above control immediately. The fact that LDH release was increased more than GOT could be explained by the different cellular location of these enzymes. This evoked GOT and LDH release apparently was a transient phenomenon because the net increase did not significantly progress within 6 h. It appears that, upon SWE, the cell membrane was transiently damaged, and then rapidly return to close to normal to stop enzyme release. The fact that the viability of SWE-treated cells was similar to that of non-SWE-treated cells (>95%) by trypan blue exclusion assay provides independent evidence for this interpretation. Another line of strong evidence supporting this view comes from the fact that SWE-treated cells were loaded with fura-2, a small molecular (MW = 636), just as well as non-SWE-treated cells, inasmuch as that cells with high permeability could not trap fura-2. Nevertheless, the integrity of the cell membrane did not completely recover because basal $[Ca^{2+}]_i$ was elevated within 1–7 h after SWE and the plateau amplitude of the evoked $[Ca^{2+}]_i$ transients was distinctively elevated.

Because cellular enzymes had leaked out during SWE, there should be an extracellular Ca^{2+} entry due to the huge Ca^{2+} gradient across the plasma membrane (2 m*M* in extracellular fluid: ~100 n*M* in cytoplasm). Interestingly, this expected rise in $[Ca^{2+}]_i$ returned to only 15% above control within 1 h after SWE was removed (fig. 2), which was still within the physiological ranges (~100 n*M*); but to measure these instantaneous $[Ca^{2+}]_i$ changes was beyond the capacity of our experimental protocol due to

Downloaded by:
Univ. of California San Diego
132.239.1.231 - 5/10/2017 10:17:31 AM

the time needed for cell attachment (30 min) and fura-2 loading (30 min).

Despite the damage of the cell membrane induced by SWE, the ATP and bradykinin receptors and the endoplasmic reticulum Ca^{2+} pump, and also the effector molecules responsible for the $[Ca^{2+}]_i$ rises triggered by ATP, bradykinin, thapsigargin and BHQ were only slightly altered, because all these agents evoked robust $[Ca^{2+}]_i$ rises which were not markedly different from that from non-SWE-treated cells in peak amplitude.

In order to examine the damaging effects of shock waves on the evoked $[Ca^{2+}]_i$ rises, potent Ca^{2+}-mobilizing agents were preferred. Thus ATP, bradykinin, thapsigargin and BHQ were used in this study because these agents had been shown to evoke robust $[Ca^{2+}]_i$ rises in MDCK cells and other cells [2–5, 14–16].

There are at least four mechanisms which by independent or combined action could contribute to the rises in basal $[Ca^{2+}]_i$ in SWE-treated cells: (1) Ca^{2+} influx through plasmalemmal Ca^{2+} channels; (2) Ca^{2+} influx through the plasma membrane via non-specific pathways due to increased cell permeability; (3) Ca^{2+} release from intracellular stores; (4) impaired ability of cells to lower the elevated $[Ca^{2+}]_i$ to the normal plateau.

The first possibility is likely because although MDCK cells lack voltage-gated Ca^{2+} channels [17], other mechanisms that allow Ca^{2+} influx exist, e.g. Na^+-Ca^{2+} exchange [18], and mechanisms that are responsible for basal Ca^{2+} uptake and are sensitive to nifedipine and verapamil block [19]. In fact, we have found that nifedipine, verapamil, and diltiazem were able to completely abolish the SWE-induced basal $[Ca^{2+}]_i$ rises [Jan, unpubl. data].

Secondly, while the evidence in shock wave exposure is absent, free radicals are thought to play a key role in perpetuating ischemia-induced cell damage by increasing cell permeability [20]. Because shock waves have been shown to produce free radicals in isolated cells [21–23], it is possible that the elevated basal $[Ca^{2+}]_i$ in SWE-treated cells was due to increased cell permeability induced by free radicals. The fact that allopurinol, which reduces free radical formation by inhibiting xanthine oxidase [24], blocked rises in basal $[Ca^{2+}]_i$ in SWE-treated MDCK cells [Jan, unpubl. data] gives support for this hypothesis.

The third possibility is that basal $[Ca^{2+}]_i$ could rise from Ca^{2+} release from intracellular Ca^{2+} stores, e.g. the endoplasmic reticulum, mitochondria and lysosomes. One line of evidence that the organelle membranes could be damaged by SWE comes from the fact that the release of GOT (an enzyme existing in mitochondria and cytosol [11]) was increased. Another line of evidence comes from the data in figures 4–6. In the $[Ca^{2+}]_i$ rises evoked by ATP, bradykinin and thapsigargin, the peak amplitude was slightly but significantly reduced (particularly within 3–6 h after SWE) with a mean maximum reduction of 37% of control. Because the peak amplitude of the $[Ca^{2+}]_i$ rises evoked by ATP and bradykinin depends substantially on the release of Ca^{2+} from inositol-1,4,5-trisphosphate (IP_3)-sensitive endoplasmic reticulum Ca^{2+} stores [Jan, unpubl. data], it appears that the endoplasmic reticulum Ca^{2+} stores could be impaired by SWE. The fact that the $[Ca^{2+}]_i$ rises evoked by thapsigargin and BHQ, two agents that act by depleting the endoplasmic reticulum Ca^{2+} stores also showed a reduced peak amplitude argues for this hypothesis.

Lastly, the SWE-induced rises in basal $[Ca^{2+}]_i$ could be due to impaired ability of cells to lower the $[Ca^{2+}]_i$ rises to the normal plateau. Figures 4–6 show that the mean plateau amplitude of the $[Ca^{2+}]_i$ rises evoked by ATP, bradykinin and thapsigargin was elevated more than 50% above control in most cases with a remarkable mean maximum elevation as high as 202% observed in bradykinin-evoked $[Ca^{2+}]_i$ rises 6 h after SWE. Although it is not completely clear how MDCK cells maintain the plateau phase of the evoked $[Ca^{2+}]_i$ rises, the results suggest that SWE impaired the ability of cells to fully recover from stimulation of the Ca^{2+}-mobilizing agents. This impaired ability of cells to maintain the normal plateau after the evoked $[Ca^{2+}]_i$ rises could involve leakage of cytosolic Ca^{2+} binding proteins (evidenced by leakage of GOT and LDH), damage of the Ca^{2+} pump and Na^+/Ca^{2+} exchangers on the plasma membrane, and impairment of the Ca^{2+} pump on organelles, e.g. the endoplasmic reticulum, mitochondria and lysosomes.

In conclusion, to our knowledge, this study is the first to examine the effects of shock waves on Ca^{2+} homeostasis in cultured cells. We found that after transient shock wave exposure MDCK cells exhibited a higher basal $[Ca^{2+}]_i$, while maintained the ability to respond to stimulation of Ca^{2+}-mobilizing agents quite normally although the peak $[Ca^{2+}]_i$ was slightly altered and the mechanisms responsible for the decay of the $[Ca^{2+}]_i$ rise suffered from a mild impairment.

Acknowledgements

This work was supported by grants from National Science Council in Taiwan (NSC 85-2331-B-075B-007 and NSC 86-2314-B-075B-010), Veterans General Hospital-Kaohsiung (85-58) and Veterans General Hospital Yang-Ming (S85-16) to C.R.J. We thank the Department of Urology in Veterans General Hospital-Kaohsiung for using the lithotriptor.

Downloaded by:
Univ. of California San Diego
132.239.1.231 - 5/10/2017 10:17:31 AM

References

1 Madin SH, Darby NB: American Type Culture Collection Catalogue of Strains, 1958, vol 2, pp 574–576.
2 Pidikiti N, Gamero D, Gamero J, Hassid A: Bradykinin-evoked modulation of cytosolic Ca concentrations in cultured renal epithelial (MDCK) cells. Biochem Biophys Res Commun 1985;130:807–813.
3 Lang F, Paulmichl M, Pfeilschifter J, Friedrich F, Woll E, Waldegger S, Ritter M, Tschernko E: Cellular mechanisms of bradykinin-induced hyperpolarization in renal epithelioid MDCK cells. Biochim Biophys Acta 1991;1073:600–608.
4 Lou H, Lindeman RP, Chase JR: Participation of protein kinase C in desensitization to bradykinin and to carbachol in MDCK cells. Am J Physiol 1992;262:F499–F506.
5 Friedrich F, Weiss H, Paulmichl M, Lang F: Activation of potassium channels in renal epithelioid cells (MDCK) by extracellular ATP. Am J Physiol 1989;256:C1016–C1021.
6 Ritter M, Lang F: Effect of bradykinin, ATP and adrenaline on cell membrane resistances of Madin-Darby canine kidney cells. J Physiol 1991;443:45–54.
7 Aboolian A, Molen MV, Nord EP: Differential effects of phorbol esters on PGE_2 and bradykinin-induced elevation of $[Ca^{2+}]_i$ in MDCK cells. Am J Physiol 1989;256:F1135–F1143.
8 Jungwirth A, Lang F, Paulmichl M: Effect of extracellular adenosine triphosphate on electrical properties of subconfluent Madin-Darby canine kidney cells. J Physiol (Lond) 1989;408:333–343.
9 Simmons NL: Acetylcholine and kinin augmentation of Cl^- secretion stimulated by prostaglandins in a canine renal epithelial cell line. J Physiol 1992;447:1–15.
10 Strohmaier WL, Bichler KH, Kleinknecht S, Pedro M, Wilber DM: Damaging effects of high energy shock waves on cultured Madin Darby canine kidney (MDCK) cells. Urol Res 1990;18:255–258.
11 Strohmaier WL, Pedro M, Wilbert DM, Bichler KH: Reduction of shock wave-induced tubular alteration by fosfomycin. J Endourol 1991;5:57–60.
12 Grynkiewicz G, Poenie M, Tsien RY: A new generation of Ca indicators with greatly improved fluorescence properties. J Biol Chem 1985;260:2440–3450.
13 Ritter M, Lang F: Effect of bradykinin, ATP and adrenaline on cell membrane resistance of Madin-Darby canine kidney cells. J Physiol (Lond) 1991;443:45–54.
14 Thastrup O, Cullen PT, Drobak BK, Hanley MR, Dawson AP: Thapsigargin, a tumor promotor, discharges intracellular calcium stores by specific inhibition of the endoplasmic reticulum calcium ATPase. Proc Natl Acad Sci USA 1990;87:2466–2470.
15 Demaurex N, Lew DP, Krause KH: Cyclopiazonic acid depletes intracellular Ca^{2+} stores and activates an influx pathway for divalent cations in HL-60 cells. J Biol Chem 1992;267:2318–2324.
16 Lien YH, Wang X, Gillies RJ, Martinez-Zaguilan R: Modulation of intracellular Ca by glucose in MDCK cells: Role of endoplasmic reticulum Ca-ATPase. Am J Pysiol 1995;268:F671–F679.
17 Lang F, Paulmichl M: Properties and regulation of ion channels in MDCK cells. Kidney Int 1995;48:1200–1205.
18 Snowdowne KW, Borle AB: Effects of low extracellular sodium on cytosolic ionized calcium: Na-Ca exchange as a major calcium influx pathway in kidney cells. J Biol Chem 1985;260:14998–15007.
19 Contreras RG, Miller JH, Zamora M, Gonzalez-Mariscal L, Cereijido M: Interaction of calcium with plasma membrane of epithelial (MDCK) cells during junction formation. Am J Physiol 1992;263:C313–C318.
20 Mccord JM: Oxygen-derived free radicals in post-ischemic tissue injury. N Engl Med 1985;312:159–163.
21 Suhr D, Brummer F, Hulser DF: Cavitation-generated free radicals during shock wave exposure: Investigations with cell-free solutions and suspended cells. Ultrasound Med Biol 1991;17:761–768.
22 Morgan TR, Laudone VP, Heston WD, Zeitz L, Fair WR: Free radical production by high energy shock waves: Comparison with ionizing irradiation. J Urol 1988;139:186–189.
23 Suhr D, Brummer F, Irmer U, Schlachter M, Hulser DF: Reduced cavitation-induced cellular damage by the antioxidative effect of vitamin E. Ultrasonics 1994;32:301–307.
24 Emmerson BT: Therapeutics of hyperuricemia and gout. Med J Aust 1984;141:31–36.

Downloaded by:
Univ. of California San Diego
132.239.1.231 - 5/10/2017 10:17:31 AM