Molecular and Cellular Biochemistry 271: 51–59, 2005. © Springer 2005

# External bioenergy-induced increases in intracellular free calcium concentrations are mediated by $Na^+/Ca^{2+}$ exchanger and L-type calcium channel

Juliann G. Kiang,[1,2,3] John A. Ives[4] and Wayne B. Jonas[4]

[1]Department of Cellular Injury, Walter Reed Army Institute of Research, Silver Spring; [2]Department of Medicine; [3]Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [4]Samueli Institute for Information Biology, Alexandria, VA, USA

Received 12 July 2004; accepted 21 September 2004

## Abstract

External bioenergy (EBE, energy emitted from a human body) has been shown to increase intracellular calcium concentration ($[Ca^{2+}]_i$, an important factor in signal transduction) and regulate the cellular response to heat stress in cultured human lymphoid Jurkat T cells. In this study, we wanted to elucidate the underlying mechanisms. A bioenergy specialist emitted bioenergy sequentially toward tubes of cultured Jurkat T cells for one 15-minute period in buffers containing different ion compositions or different concentrations of inhibitors. $[Ca^{2+}]_i$ was measured spectrofluorometrically using the fluorescent probe fura-2. The resting $[Ca^{2+}]_i$ in Jurkat T cells was $70 \pm 3$ nM ($n = 130$) in the normal buffer. Removal of external calcium decreased the resting $[Ca^{2+}]_i$ to $52 \pm 2$ nM ($n = 23$), indicating that $Ca^{2+}$ entry from the external source is important for maintaining the basal level of $[Ca^{2+}]_i$. Treatment of Jurkat T cells with EBE for 15 min increased $[Ca^{2+}]_i$ by $30 \pm 5\%$ ($P < 0.05$, Student $t$-test). The distance between the bioenergy specialist and Jurkat T cells and repetitive treatments of EBE did not attenuate $[Ca^{2+}]_i$ responsiveness to EBE. Removal of external $Ca^{2+}$ or $Na^+$, but not $Mg^{2+}$, inhibited the EBE-induced increase in $[Ca^{2+}]_i$. Dichlorobenzamil, an inhibitor of $Na^+/Ca^{2+}$ exchangers, also inhibited the EBE-induced increase in $[Ca^{2+}]_i$ in a concentration-dependent manner with an IC50 of $0.11 \pm 0.02$ nM. When external $[K^+]$ was increased from 4.5 mM to 25 mM, EBE decreased $[Ca^{2+}]_i$. The EBE-induced increase was also blocked by verapamil, an L-type voltage-gated $Ca^{2+}$ channel blocker. These results suggest that the EBE-induced $[Ca^{2+}]_i$ increase may serve as an objective means for assessing and validating bioenergy effects and those specialists claiming bioenergy capability. The increase in $[Ca^{2+}]_i$ is mediated by activation of $Na^+/Ca^{2+}$ exchangers and opening of L-type voltage-gated $Ca^{2+}$ channels. (Mol Cell Biochem **271:** 51–59, 2005)

*Key words*: lymphoid cells, intracellular calcium, intracellular signal, calcium channel, $Na^+/Ca^{2+}$ exchanger, bioenergy

## Introduction

Intracellular free calcium has long been recognized as a ubiquitous second messenger in various physiological systems. Increases in resting intracellular free calcium concentration ($[Ca^{2+}]_i$) trigger a variety of cell functions including metabolism, growth, differentiation, hormonal secretion, gene expression, protein synthesis, and cell movement

*Address for offprints*: Dr Juliann G. Kiang, Assistant Chief, Department of Cellular Injury, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Room 1N07, Silver Spring, MD 20910-7500, U.S.A. (E-mail: Juliann.Kiang@na.amedd.army.mil)

[1, 2]. It is known that $[Ca^{2+}]_i$ is maintained by three main mechanisms: the influx of extracellular $Ca^{2+}$, $Ca^{2+}$-binding proteins in the cytoplasm, such as calmodulin, and $Ca^{2+}$ release from intracellular pools, such as the endoplasmic reticulum, mitochondria, and Golgi apparatus. The endoplasmic reticulum involves inositol 1,4,5-trisphosphate that is generated by a membrane transduction process comprising a receptor, a coupling G protein, and phospholipase C, whereas influx of extracellular $Ca^{2+}$ is through voltage-gated, 2nd messenger-mediated, or receptor-mediated $Ca^{2+}$ channels [3].

Bionergy (BE) such as therapeutic touch, distant healing, and qigong has been used for healing and self-healing as part of traditional medical practices for centuries. It has different names in different cultures (Table 1). It is defined as energy generated by a biological system (e.g., electrical, acoustic, thermal, chemical) [4]. When BE is emitted by a bioenergy specialist and applied to another person, it is called external bioenergy (EBE). On the other hand, when BE is emitted within a person and applied to adjust one's own biological system, then it is called internal bioenergy (IBE). There have been a number of reports indicating that BE can influence a variety of biological activities. It has been reported to enhance immunity, promote normal cell proliferation, increase tumor cell death, accelerate bone fracture recovery [5], and prevent oxidative stress-induced apoptosis [6]. We have used an objective and easily monitored cellular outcome such as $[Ca^{2+}]_i$ to investigate the effect of EBE on $[Ca^{2+}]_i$ in human lymphoid Jurkat T cells. We were the first to show that a single treatment with EBE increases $[Ca^{2+}]_i$ but not $Ca^{2+}$-dependent heat shock protein 72 kDa (HSP-72). However, the EBE-treated cells can diminish their response to heat stress [7].

*Table 1.* Nomenclatures of bioenergy in Eastern and Western cultures

| |
|---|
| Eastern culture |
| Vital energy (China) |
| Qi (China) |
| Chi (Taiwan) |
| Ki (Japan) |
| Prana (India) |
| Mana (Hawaii and Philippine) |
| Western Culture (mainly U.S.A.) |
| Bioenergy |
| Biofields |
| Bioelectromagnetics |
| Subtle energy |
| Vital force |
| Life energy |

The biological and therapeutic effects of EBE have been extensively described in the literature [5, 6]. However, the mechanisms that underlie its activity are not clear. $Ca^{2+}$ is an important transducing signal in the cell. The effects of $[Ca^{2+}]_i$ on eukaryotic cell responses are excitatory, including inducing muscle contraction, increasing hormonal secretion, and activating metabolic systems [2]. Analogous responses would be expected as a result of any EBE-induced increases in $[Ca^{2+}]_i$ in cultured human T cells.

In this study, we explored whether the distance between the source of EBE and the location of cells alters the sensitivity of cells responding to EBE and whether repetitive treatment of cells with EBE desensitizes cellular $[Ca^{2+}]_i$ responses. The mechanism underlying the EBE-induced increase in $[Ca^{2+}]_i$ was also elucidated. We report that the distance between the source of EBE and the location of cells did not reduce the $[Ca^{2+}]_i$ response to EBE. Cells treated repetitively with EBE preserved their sensitivity to EBE. The EBE-induced increase in $[Ca^{2+}]_i$ was likely mediated by activation of $Na^+/Ca^{2+}$ exchanger and L-type voltage-gated $Ca^{2+}$ channels. The EBE-induced increase in $[Ca^{2+}]_i$ may serve as an objective means for assessing and validating bioenergy effects and those specialists claiming bioenergy capability [7].

## Materials and methods

### *Cell culture*

Cells from the Jurkat cell line (a human leukemic T cell clone from American Types Cell Culture, Rockville, MD) were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 $\mu$g/mL streptomycin, and 100 U/ml penicillin, and 25 mM HEPES, pH 7.4 (Gibco BRL, Gaithersburg, MD), in a humidified incubator with a 5% $CO_2$ atmosphere. Cells were fed every 3–4 days.

### *Intracellular $Ca^{2+}$concentration measurements*

Jurkat T cells were washed and then loaded in suspension with 5 $\mu$M fura-2AM (Molecular Probes, Inc., Eugene, OR) at 37 °C for 60 min. The procedure to measure $[Ca^{2+}]_i$ has been described elsewhere [7]. Briefly, the suspended cells were placed in a cuvette, and the fluorescence signal was measured with a PTI DeltaScan spectrofluorometer (Photon Technology International, Inc., South Brunswick, NJ) with dual emission at 340 and 380 nm and single excitation at 510 nm (slit width 4 nm). To minimize any contribution to the fluorescence signal resulting from dye in the medium, cells were washed thoroughly in Hanks' solution before measurement of $[Ca^{2+}]$. Cuvettes with cells were randomly assigned to each experiment. To perform experiments in the absence of

extracellular $Ca^{2+}$, $Na^{+}$, $Mg^{2+}$, or in the presence of a high concentration of $K^{+}$, cells were incubated in the specified buffer for 1 min prior to a 15-min EBE treatment. Sham-operated cells without exposure to a 15-min EBE treatment were also conducted.

*Measurements of cell viability*

Cell viability was determined by the trypan blue exclusion assay. Twenty microliters of cell suspension were mixed with 20 $\mu$L of 0.4% trypan blue solution (Sigma Chemical Co., St. Louis, MO). The viability was calculated accordingly [8].

*Solutions*

Hanks' solution contained in mM: 145 NaCl, 4.5 KCl, 1.3 $MgCl_2$, 1.6 $CaCl_2$, and 10 HEPES (pH 7.40 at 24 °C). High $K^{+}$ Hanks' solution contained in mM: 124.5 NaCl, 25 KCl, 1.3 $MgCl_2$, 1.6 $CaCl_2$, and 10 HEPES (pH 7.40 at 24 °C). $Ca^{2+}$-free Hanks' solution was prepared by adding 10 mM EGTA to nominally $Ca^{2+}$-free Hanks' solution. To remove external $Na^{+}$, an equal molar of *N*-methyl-D-glucamine was used to replace $Na^{+}$.

*External bioenergy treatment*

In survey experiments, we observed that three reputable bioenergy specialists (recommended by the Samueli Institute of Information Biology) were capable of stimulating an increase in $[Ca^{2+}]_i$ in our Jurkat cell system, whereas five randomly picked persons (non-bioenergy specialists) were not. In this study, EBE was administered only by Mietek Wirkus, a bioenergy specialist with a long history in practice and research.

After Jurkat T cells were loaded with the fura-2 $Ca^{2+}$ probe, they were resuspended in a cuvette with fura-2 free buffer. The cuvettes were randomly assigned [4, 9] for either EBE or sham treatment. Initial studies showed that standard spectrofluorometer procedures required modification, since cells showed no $[Ca^{2+}]_i$ changes when the bioenergy specialist attempted to treat the cells with the metal spectrofluorometer sample chamber lid in place (15-min treatment with palms 1 inch above the chamber lid). The following procedure was used instead. The initial basal level of $[Ca^{2+}]_i$ was measured. Cells in cuvettes then received EBE treatment outside of the spectrofluorometer with the bioenergy specialist using both palms facing the cuvette from a distance of 3 inches for a single 15 min application. A basal $[Ca^{2+}]_i$ level was first established by monitoring for 1 min. After EBE application, the $[Ca^{2+}]_i$ level was then monitored for another 4 min, with a second measurement then taken immediately at the end of EBE treatment for purposes of statistical analysis [7].

Each EBE experiment was performed along with a sham treatment that omitted the EBE treatment. Cell samples were blind-coded [9]. The bioenergy specialist was unaware of which tests were being performed on the cells and was not involved in any cell processing, data collections and analysis, or result interpretation. Technicians processing the cells were also blinded to the experimental conditions. The project coordinator who originally assigned the codes performed the evaluation of data.

*Statistical analysis*

All data are expressed as the mean ± S.E.M. Student's paired *t*-test, one-way ANOVA, two-way ANOVA, and Bonferroni's inequality were used for comparison of $[Ca^{2+}]$ levels in the same cells just immediately before and after EBE treatment.

*Chemicals*

Chemicals used in this study were *N*-methyl-D-glucamine, EGTA, $CoCl_2$, $CdSO_4$, $LaCl_3$ (Sigma-Aldrich, St. Louis, MO), verapamil, nifedipine, dichlorobenzamil (CalBiochem, Torrance, CA), and fura-2AM (Molecular Probes, Eugene, OR).

## Results

*External bioenergy-induced increases in $[Ca^{2+}]_i$ are not affected by the distance between the source of external bioenergy and the cells*

The resting $[Ca^{2+}]_i$ in Jurkat T cells was $70 \pm 3$ nM ($n = 130$) in the presence of external calcium. Removal of external calcium decreased the resting $[Ca^{2+}]_i$ to $52 \pm 2$ nM ($n = 23$), indicating that $Ca^{2+}$ entry from the external source is important to maintain the basal level of $[Ca^{2+}]_i$.

Treatment of Jurkat T cells with a single treatment of external bioenergy (EBE) for 15 min increased $[Ca^{2+}]_i$ by $30 \pm 5\%$ ($n = 4$; $p < 0.05$ versus Sham, Student's *t*-test) when the distance between the source of EBE and the location of cells was 3 inches (Fig. 1A). Sham treatment failed to increase $[Ca^{2+}]_i$ (Fig. 1A). When the distance was increased to 10 inches, the increase in $[Ca^{2+}]_i$ was greater. However, 30 inches did not result in an additional increase in $[Ca^{2+}]_i$ (Fig. 1B).

Previously, we observed that $[Ca^{2+}]_i$ increased if cells were simply placed for 15 min at the site where EBE had been performed [5]. In this study, the lingering effect of EBE placed at the site where EBE had been performed was again observed,

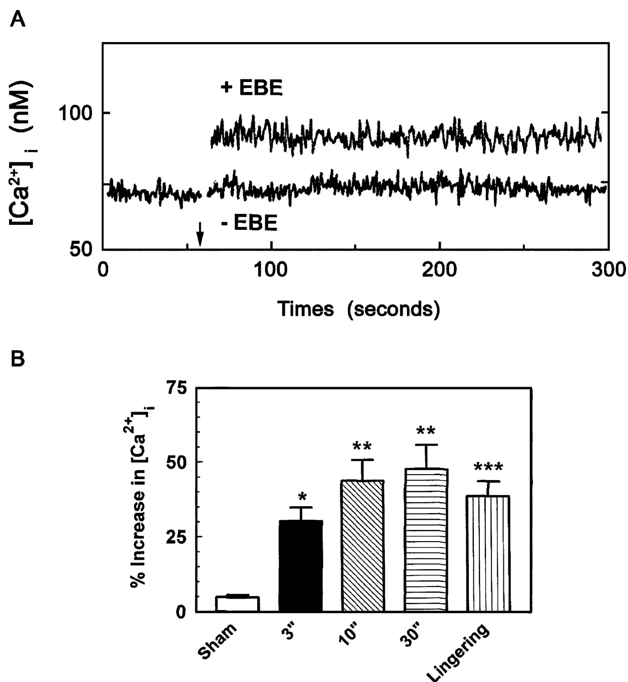


*Fig. 1.* External bioenergy-induced increases in $[Ca^{2+}]_i$ is not affected by distance between source of bioenergy and cells. Jukat T cells in suspension were treated with single exposure to external bioenergy (EBE) for 15 min prior to $[Ca^{2+}]_i$ measurement. (A) Representative fluorometer tracings after treatment with EBE 3 inches from cells (+EBE; $n = 130$ total from six different experiments) and sham-treated cells (−EBE; $n = 20$ from six different experiments). Initial tracing is basal level of $[Ca^{2+}]_i$. Arrow indicates EBE treatment. (B) Cells were treated with single treatment of EBE at distance of 3, 10, or 30 inches between source of EBE and cells ($n = 4$–8). After bioenergy specialist left site, cells were placed at same site for 15 min, which showed lingering effect. $^*p < 0.05$ vs. Sham, 10″, and 30″; $^{**}p < 0.5$ versus Sham and 3″; $^{***}$p<0.05 vs. Sham, determined by one-way ANOVA and Bonferroni's inequality.

resulting in an increase in $[Ca^{2+}]_i$ of 39 ± 5% (n = 6; P < 0.05 vs. Sham, Student's *t*-test; Fig. 1B). Cells remained viable after treatment with EBE (data not shown).

### *Repetitive treatments of external bioenergy do not reduce $[Ca^{2+}]_i$ response*

In many cases, repetitive treatments with drugs or medicines such as painkillers have been found to reduce their potency, a process called tolerance or cross-tolerance [10]. To determine whether repetitive treatments of EBE would lead to tolerance, Jurkat T cells were treated with multiple applications of EBE with an interval of 1 h. Figure 2 shows that two applications of EBE indeed induced a greater increase in $[Ca^{2+}]_i$ than one application. Three applications did not induce an additional increase.

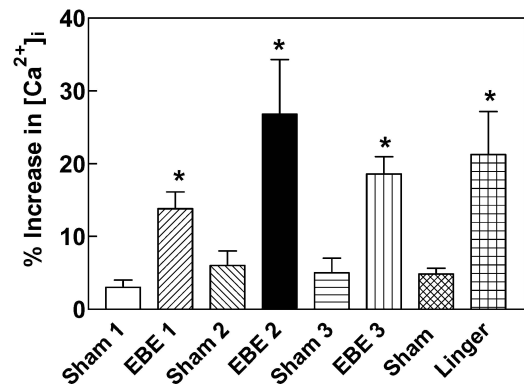


*Fig. 2.* Repetitive treatments of external bioenergy does not reduce $[Ca^{2+}]_i$ response. Jurkat T cells in suspension were treated with external bioenergy (EBE) for 15 min with interval of 1 h ($n = 6$–7). Each scenario was accompanied by its own sham-operated group (Sham, $n = 6$–7). EBE1, 2, and 3 represent 1, 2, and 3 treatments with EBE, respectively. Lingering effect was also observed in this experiment. $^*p < 0.05$ versus respective own sham-operated group, determined by Student's *t*-test.

The lingering effect was observed when untreated cells in a cuvette was placed for 15 min at the site where the bioenergy specialist emitted his BE. The effect was similar to that with one application of EBE (Fig. 2).

### *External bioenergy-induced $[Ca^{2+}]_i$ increase is $Ca^{2+}$-dependent*

The increase in $[Ca^{2+}]_i$ is normally contributed by either $Ca^{2+}$ influx or $Ca^{2+}$ mobilization from the intracellular $Ca^{2+}$ pools such as mitochondria, golgi apparatus, and endoplasmic reticulum [2]. To determine where the EBE-induced $[Ca^{2+}]_i$ increase originated, cells were incubated in $Ca^{2+}$-free buffer containing 100 $\mu$M EGTA for 1 min before treatment with EBE. In the absence of external $Ca^{2+}$, EBE failed to increase $[Ca^{2+}]_i$ (Fig. 3), suggesting that the EBE-induced $[Ca^{2+}]_i$ increase was extracellular.

### *External bioenergy-induced $[Ca^{2+}]_i$ increase is blocked by $Ca^{2+}$ channel blockers*

The result from experiments conducted in the absence of external $Ca^{2+}$ suggests that the EBE-induced $[Ca^{2+}]_i$ increase is due primarily to $Ca^{2+}$ influx. The entry is known to be blocked by $Co^{2+}$, $Cd^{2+}$, or $La^{3+}$ (inorganic $Ca^{2+}$ channel blockers) present in the external medium [11, 12]. In our study, bathing cells in a solution containing 2 mM $CdSO_4$ alone increased the basal $[Ca^{2+}]_i$. EBE increased $[Ca^{2+}]_i$, further, but the increase was attenuated (Fig. 4B and Table 2). Likewise, cells in a solution containing $LaCl_3$ alone also demonstrated a higher basal $[Ca^{2+}]_i$, but subsequent treatment with EBE failed to increase $[Ca^{2+}]_i$, leading to a decrease instead (Fig. 4C and

*Table 2.* Effects of $Ca^{2+}$-channel blockers on EBE-induced increase in $[Ca^{2+}]_i$

| Blocker | Concentration (mM) | $[Ca^{2+}]_i$ (nM) | | Changes (%) |
|---|---|---|---|---|
| | | −EBE | +EBE | |
| Control | – | 70 ± 2 | 97 ± 2* | 39 ± 6 |
| $CdSO_4$ | 2 | 113 ± 4$^+$ | 130 ± 5* | 14 ± 4 |
| $LaCl_3$ | 2 | 98 ± 8$^+$ | 65 ± 6* | −34 ± 3 |
| $CoCl_2$ | 2 | 72 ± 7 | 110 ± 2* | 53 ± 6 |
| Verapamil | 1 | 97 ± 7$^+$ | 85 ± 4* | −12 ± 4 |
| KCl | 25 | 105 ± 16$^+$ | 97 ± 16* | −8 ± 3 |

Jurkat T cells were treated with EBE (15 min) in presence of indicated blocker, and $[Ca^{2+}]_i$ was measured before and after EBE treatment. Results are means ± S.E. ($n$ = 3–5). $^*p < 0.05$ versus blocker-treated cells alone; $^+p < 0.05$ versus control cells.

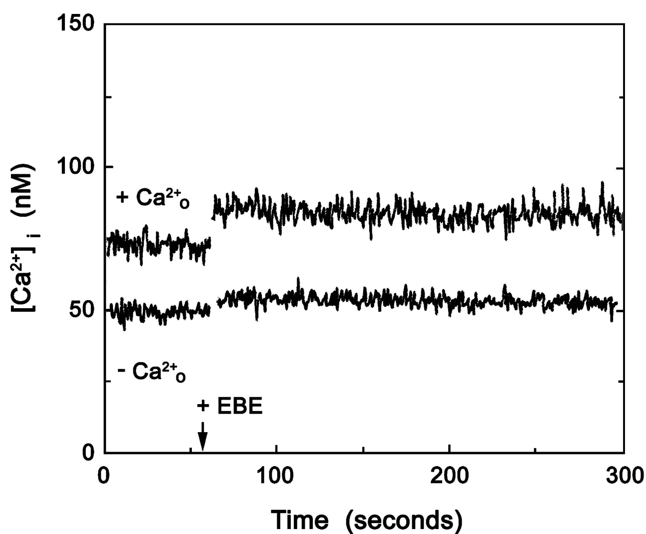


*Fig. 3.* External bioenergy-induced $[Ca^{2+}]_i$ increase is $Ca^{2+}$-dependent. Jurkat T cells in suspension were treated with external bioenergy (+EBE) for 15 min in presence of external $Ca^{2+}$ ($+Ca^{2+}{}_o$) or absence of external $Ca^{2+}$ ($-Ca^{2+}{}_o$). Representative fluorometer tracings with treatment with EBE in presence of external $Ca^{2+}$ ($n = 4$) or absence of external $Ca^{2+}$ ($n = 6$). Initial tracing is basal level of $[Ca^{2+}]_i$. Arrow indicates EBE treatment.

Table 2). $CoCl_2$ did not block the EBE-induced $[Ca^{2+}]_i$ increase (Fig. 4D and Table 2).

The organic $Ca^{2+}$ channel blocker [12] verapamil (1 mM) increased the basal $[Ca^{2+}]_i$ and not only blocked the EBE-induced $[Ca^{2+}]_i$ increase but induced a decrease in $[Ca^{2+}]_i$ (Fig. 4E and Table 2), indicating L-type voltage-gated $Ca^{2+}$ channels are present and associated with the EBE effect. Nifedipine at 1 mM was toxic to the cells; therefore, no further studies with nifedipine were conducted.

Since verapamil significantly inhibited the EBE-induced $[Ca^{2+}]_i$ increase, we determined the effect of bathing cells in a solution containing a high $[K^+]$. We found that 25 mM $K^+$ elevated the basal $[Ca^{2+}]_i$ but significantly abolished the EBE-induced $[Ca^{2+}]_i$ increase (Fig. 4F and Table 2).

### *External bioenergy-induced $[Ca^{2+}]_i$ increase is $Na^+$-dependent*

The results indicate that the EBE-induced $[Ca^{2+}]_i$ increase was related to external $Ca^{2+}$ and $K^+$. We also determined whether other ions affect the EBE-induced $[Ca^{2+}]_i$ increase. We removed $Na^+$ from the medium and replaced it with 145 mM *N*-methyl-D-glucamine. The absence of external $Na^+$ slightly but significantly elevated the basal $[Ca^{2+}]_i$ and totally inhibited the EBE-induced $[Ca^{2+}]_i$ increase (Fig. 5A and C). In contrast, the absence of external $Mg^+$ did not alter the basal $[Ca^{2+}]_i$, and treatment of cells with EBE still induced a significant increase in $[Ca^{2+}]_i$ (Fig. 5B and C). These results, taken together with data from experiments in the absence of external $Ca^{2+}$, suggest that external $Na^+$ and $Ca^{2+}$ are associated with the EBE-induced increase in $[Ca^{2+}]_i$.

### *External bioenergy-induced $[Ca^{2+}]_i$ increase is mediated by $Na^+/Ca^{2+}$ exchanger*

Since removal of either external $Ca^{2+}$ or $Na^+$ significantly blocked the EBE-induced $[Ca^{2+}]_i$ increase, these data suggested that the $Na^+/Ca^{2+}$ exchange system was involved. To test this, cells were treated with dichlorobenzamil (a known inhibitor of $Na^+/Ca^{2+}$ exchanger) at different concentrations before treatment with EBE. Dichlorobenzamil inhibited the EBE-induced $[Ca^{2+}]_i$ increase in a concentration-dependent manner with a median inhibitory concentration (IC50) of 0.11 ± 0.02 nM (Fig. 6).

## Discussion

This study demonstrates that the resting $[Ca^{2+}]_i$ in suspended Jurkat T cells was 70 ± 2 nM as determined by fura-2. The reduction in $[Ca^{2+}]_i$ measured in cells incubated in the absence of external $Ca^{2+}$ indicated that there is a $Ca^{2+}$ influx that maintains the resting $[Ca^{2+}]_i$. A single treatment of EBE applied 3 inches away from the cells significantly increased $[Ca^{2+}]_i$. The increase was enhanced when the distance was extended to 10 inches, although EBE applied 30 inches away did not show an additional increase from the 10-inches measurements. We also attempted experiments at a distance greater than 30 inches; however, the effort fatigued the bioenergy specialist, and only a small increase in $[Ca^{2+}]_i$ was measured [7]. There are several reports on EBE emitted over long distances to experimental subjects [5], but these observations await verification by other laboratories.

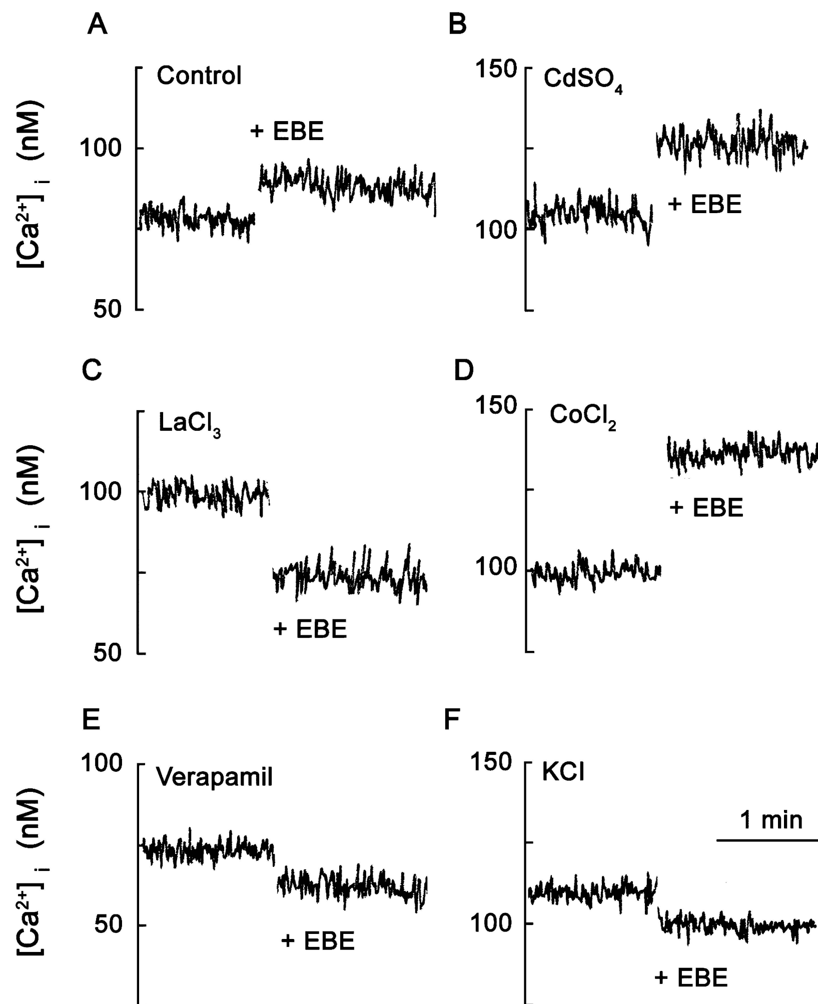


*Fig. 4.* External bioenergy-induced $[Ca^{2+}]_i$ increase is blocked by $Ca^{2+}$ channel blockers. Jurkat T cells in suspension were treated with external bioenergy (+EBE) for 15 min in presence of various $Ca^{2+}$ channel blockers ($n = 3$–5). Initial tracing is basal level of $[Ca^{2+}]_i$ in presence of each $Ca^{2+}$ channel blocker.

Repetitive treatments with EBE did not diminish the sensitivity of the cells to EBE. We conducted the EBE treatment within a period of 1 h on one day due to the unavailability of the bioenergy specialist. Additional studies with longer intervals and different days should be carried out before the absence of a tolerance response can be verified. However, many experiments with EBE have been conducted in past two decades. While most of these studies were not well designed, many of them used multiple EBE treatments. It has been reported that repetitive treatments with EBE displayed a reduced interleukin 2 level and an increased interferon activity in Con A-treated spleen cells [13, 14] and an increased phagocytotic function, activity of acid phosphatase, and amount of IgM antibodies [15]. In studies with cancer models, treatment with EBE markedly reduced the number of B16 melanoma pulmonary metastases nodules in the lungs and increased survival time of rats over untreated controls when C57BL/6 mice were inoculated with B16 melanoma tumor cells via the tail veins [16]. Similar results were reported with mice injected with MO4 cells [17, 18] or U27 cancer cells [18, 19]. Mice injected with ascitic cancer fluid followed by treatment with EBE had increased hemoglobin levels, numbers of red blood cells and white blood cells, and smaller tumor sizes [20]. Other studies reported that tumor formation was prevented in NC-Z strain mice inoculated with nasopharyngeal squamous carcinoma CNE-2 cells [21] or human hepatocarcinoma BEL-7420 cells [22] and weight reduction delayed after injection of sarcoma 180 tissue [23, 24]. It has also been shown that EBE reduced the size of G422 cell neurogliomas implanted in mice [25].

In *in vivo* experimental models, treatment with EBE has been shown to increase body weight gain, reduce blood glucose, increase in insulin activity [26], decrease MDA and lipid peroxides [27], and promote faster recovery of bone fracture

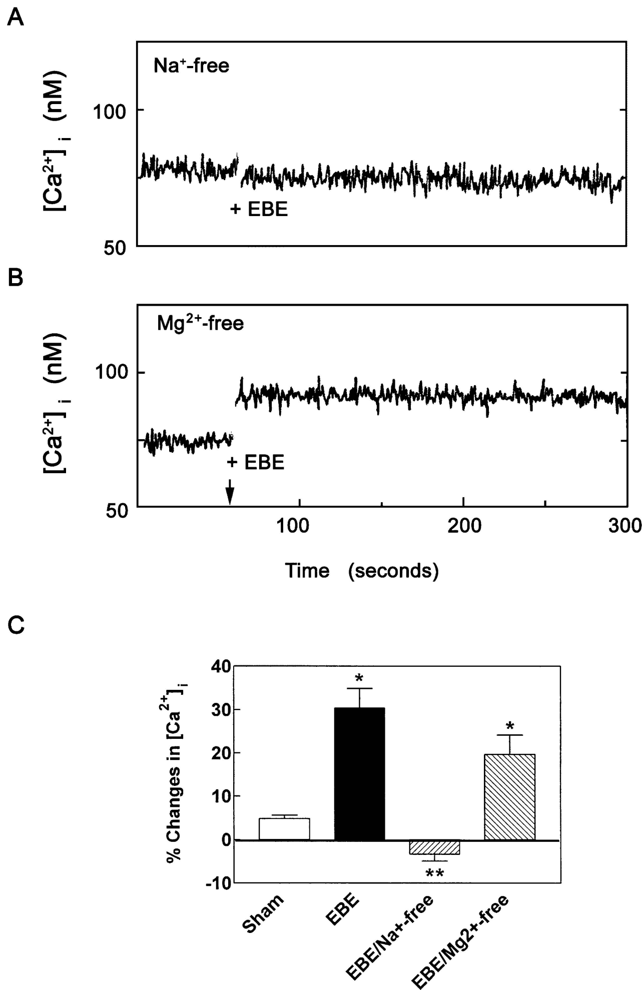


*Fig. 5.* External bioenergy-induced $[Ca^{2+}]_i$ increase is $Na^+$-dependent. Jurkat T cells in suspension were treated with external bioenergy (+EBE) for 15 min in absence of external $Na^+$ (A) or $Mg^{2+}$ (B). Initial tracings are basal level of $[Ca^{2+}]_i$ in absence of external $Na^+$ or $Mg^{2+}$, $79 \pm 3$ nM ($n = 8$) and $71 \pm 4$ nM ($n = 9$), respectively. (C) Quantitative analysis of EBE effects on $[Ca^{2+}]_i$ in absence of external $Na^+$ or $Mg^{2+}$. $^*p < 0.05$ versus Sham, EBE/$Na^+$-free, and EBE/$Mg^{2+}$; $^{**}P < 0.05$ vs. Sham and EBE, determined by one-way ANOVA and Bonferroni's inequality.

[28, 29]. It also has been observed to increase sedative and analgesic effects [30–32], elevate volume of blood flow [33], and restore T cell proliferation and activity of interleukin 2 [34]. In human patients treated with EBE, natural killer cell activity increased while the CD4/CD8 ratio remained unchanged [35]. Negative results with EBE studies have been reported as well [5].

The presence of the $Na^+/Ca^{2+}$ exchanger was detected in human Jurkat T cells used in this study [36], which is in agreement with reports by others [37]. The EBE-induced increase in $[Ca^{2+}]_i$ is mediated by the $Na^+/Ca^{2+}$ exchanger. The view is strongly supported by the following findings: (1) the EBE-induced increase in $[Ca^{2+}]_i$ did not occur in the absence of external $Ca^{2+}$; (2) the EBE-induced increase in $[Ca^{2+}]_i$ also did not occur in the absence of external $Na^+$; (3) an inhibitor of the $Na^+/Ca^{2+}$ exchanger blocked the EBE-induced increase in $[Ca^{2+}]_i$ in a concentration-dependent fashion, with an IC50 of $0.11 \pm 0.02$ nM; and (4) the EBE-induced increase in $[Ca^{2+}]_i$ remained regardless of the presence or absence of external $Mg^{2+}$, indicating that this observation is specific to external $Ca^{2+}$ and $Na^+$. Similar findings have been reported with cells exposed to heat stress [2, 7, 36, 38, 39], NaCN [40], or remedies to modulate the exchanger [36, 41, 42].

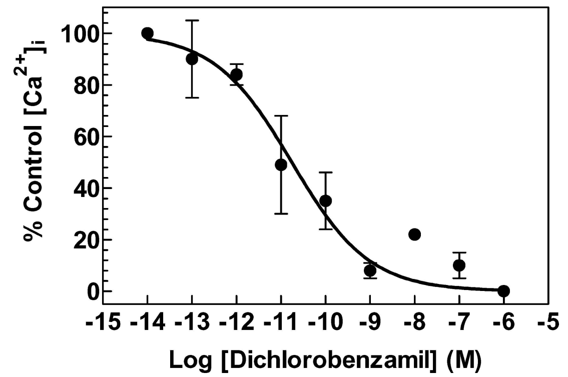


*Fig. 6.* External bioenergy-induced $[Ca^{2+}]_i$ increase is mediated by $Na^+/Ca^{2+}$ exchanger. Jurkat T cells in suspension were treated with dichlorobenzamil at various concentrations 1 min prior to treatment with external bioenergy (+EBE) for 15 min. Inhibitor was present during exposure of cells to EBE ($n = 3$–4 for each concentration). Median inhibitory concentration (IC50) was $0.11 + 0.02$ nM using Prism 3.0 program.

The $Na^+/Ca^{2+}$ exchanger in mammalian heart [43] is known to have 11 putative transmembrane segments and a large hydrophilic domain of 520 amino acids between the fifth and sixth transmembrane segments. The transmembrane segments are responsible for regulating $K_m$ and $V_{max}$. Iwamoto *et al.* [41, 44] reported that the second and third transmembrane segments (i.e., the $\alpha$-1 repeat) as well as the seventh transmembrane segment (i.e., the $\alpha$-2 repeat) contain amino acid residues that regulate the $K_m$ of the exchanger for extracellular $Ca^{2+}$. The hydrophilic domain also has regulatory potential, because the 219-RRLLFYKYVYKRAGKQRG region binds the exchanger inhibitory peptide (XIP) and the 446-DDDIFEEDE and 498-DDDHAGIFTFE regions represent the $Ca^{2+}$ binding domains for intracellular $Ca^{2+}$. Cytosolic $Na^+$ in the absence of ATP and absence of cytosolic $Ca^{2+}$ controls exchanger activity [41, 43, 45]. It is possible that EBE may act on the $\alpha$-1 repeat, $\alpha$-2 repeat, or the 446-DDDIFEEDE and 498-DDDHAGIFTFE regions, because our preliminary data indicated EBE also induces an increased cellular ATP level (Kiang, unpublished data) and because a close correlation between the $Na^+/Ca^{2+}$ exchanger expression and ATP has been detected [41].

It is known that the $Na^+/Ca^{2+}$ exchanger implements electrogenic exchange of $Na^+$ and $Ca^{2+}$ across the plasma membrane in either the $Ca^{2+}$-efflux (forward) mode or $Ca^{2+}$-influx (reverse) mode, depending on the electrochemical gradients of the substrate ions. Since the EBE-induced increases in $[Ca^{2+}]_i$ were not observed in the absence of external $Ca^{2+}$ or $Na^+$, the $Ca^{2+}$-influx mode is suggested. Although treatment with dichlorobenzamil significantly inhibited the EBE-induced increase in $[Ca^{2+}]_i$, its potential non-specific effect [37, 41, 42] cannot be ignored. Therefore, further studies with other inhibitors of $Na^+/Ca^{2+}$ exchanger [41, 42] are needed. Apparently, a better understanding on this regard may allow an efficient use of bioenergy for needed patients in the field of energy medicine.

The EBE-induced increase in $[Ca^{2+}]_i$ is also mediated by L-type voltage-gated $Ca^{2+}$ channel, because both external $K^+$ at a high concentration and the L-type voltage-gated $Ca^{2+}$ channel blocker verapamil significantly increased the basal $[Ca^{2+}]_i$ and abolished the EBE-induced increase in $[Ca^{2+}]_i$. This observation is unique to the EBE effects because exposure of cells to heat stress [12] or NaCN [40] activates only the $Na^+/Ca^{2+}$ exchanger. Activation of L-type voltage-gated $Ca^{2+}$ has been shown in dorsal root ganglion-neuroblastoma hybrid ND8-47 cells [46]. It is possible Jurkat T cells possess properties of both systemic cells and neuronal cells.

In summary, this study is the first to show that treatment with EBE significantly increased $[Ca^{2+}]_i$, due to activation of the $Na^+/Ca^{2+}$ exchanger and L-type voltage-gated $Ca^{2+}$ channels. The EBE-induced increase in $[Ca^{2+}]_i$ may serve an objective means for assessing and validating bioenergy effects and those specialists claiming bioenergy capability [7].

## Acknowledgments

The authors thank bioenergy specialists and sham imitators for their voluntary involvements and Mr R. Lee Collins for his graphic work. The authors also thank researchers in the field of energy medicine for their effort to investigate how energy medicine works scientifically. This work is supported by Samueli Institute for Information Biology and Rockefeller-Samueli Center for Research in Mind-Body Energy (G175JO-01). Views presented in this paper are those of authors; no official endorsement by the U.S. Department of the Army, Walter Reed Army Institute of Research, or Uniformed Services University of The Health Sciences has been given or should be inferred.

## References

1. Meldolesi J, Pozzan T: Pathways of $Ca^{2+}$ influx at the plasma membrane: Voltage-, receptor-, and second messenger-operated channels. Exp Cell Res 171: 271–278, 1987
2. Kiang JG, Tsokos GC: Heat shock protein 70 kDa: Molecular Biology, Biochemistry, and Physiology. Pharmacol Ther 80: 183–201, 1998
3. Berridge MJ, Irivine RF: Inositol phosphates and cell signaling. Nature 341: 197–200, 1989
4. Hintz KJ, Yount GL, Kadar I, Schwartz G, Hammerschlag R, Lin S: Bioenergy definitions and research guidelines. Altern Ther 9: A13-A30, 2003
5. Yan X, Lu PY, Kiang JG: Qigong: Basic science studies in biology. In: W.B. Jonas and C.C. Crawford (eds). Healing, Intention, and Energy Medicine, Churchill Livingstone, New York, pp 103–119, 2003
6. Yan X, Shen H, Zaharia M, Wang J, Wolf D, Li F, Lee GD, Cao W: Involvement of phosphatidylinositol 3-kinase and insulin-like growth factor-1 in YXLST-mediated neuroprotection. Brain Res 1006: 198–206, 2004
7. Kiang JG, Marotta D, Wirkus M, Wirkus M, Jonas W: External bioenergy increases intracellular free calcium concentrations and reduces cellular response to heat stress. J Investig Med 50: 38–45, 2002
8. Kiang JG, Gist ID, Tsokos GC: Regulation of heat shock protein 72 kDa and 90 kDa in human breast cancer MDA-MB-231 cells. Mol Cell Biochem 204: 169–178, 2000
9. Caspi O, Millen C, Sechrest L: Integrity and research: Introducing the concept of dual blindness, how blind are double-blind clinical trials in alternative medicine? Altern Complement Med 6: 493–498, 2000
10. Kiang JG, Dewey WL, Wei ET: Tolerance to morphine bradycardia in the rat. J Pharmacol Exp Ther 226: 187–192, 1983
11. Hagiwara S: Membrane potential-dependent ion channels in cell membrane. In: Phylogenic and Development Approaches, New York, Raven, 1983
12. Kiang JG, Koenig ML, Smallridge RC: Heat shock increases cytosolic free $Ca^{2+}$ concentration via $Na^+$-$Ca^{2+}$ exchange in human epidermoid A431 cells. Am J Physiol 263: C30-C38, 1992
13. Cao X, Ye T, Gao Y: Effect of emitted qi in enhancing the induction *in vitro* of lymphokines in relation to antitumor mechanisms, 1st World Conf Acad Exch Med Qigong, Beijing, China, p 51, 1988
14. Guan H, Yang J: Effect of external qi on IL-2 activity and multiplication action of spleen cells in mice, 2nd Int Conf on Qigong, Xian, China, p 92, 1989
15. Feng L, Wang Y, Chen S, Chen H: Effect of emitted qi on the immune functions of mice, 1st World Conf Acad Exch Med Qigong, Beijing, China, p 4, 1988
16. Cao X, Ye T, Gao Y: Antitumor metastases activity of emitted qi in tumor bearing mice, 1st World Conf Acad Exch Med Qigong, Beijing, China, p 50, 1988
17. Qian S, Shen H: Curative effect of emitted qi on mice with MO4 tumors, 2nd World Conf Acad Exch Med Qigong; Beijing, China, p 107, 1993
18. Qian S, Sun W, Liu Q, Wan Y, Shi X: Influence of emitted qi on cancer growth, metastasis and survival time of the host, 2nd World Conf Acad Exch Med Qigong; Beijing, China, p 106, 1993
19. Qian Z: Experimental research of influence of qigong waiqi on the cancer growth metastasis and survival time of host. Chin J Som Sci 4: 117–118, 1994
20. Zhao S, Mao X, Zhao B, Li Z, Zhou D: Preliminary observation of the inhibitory effect of emitted qi on transplanted tumors in mice, 1st World Conf Acad Exch Med Qigong, Beijing, China, p 46, 1988
21. Chen X, Yi Q, Liu K, Zhang J, Chen Y: Double-blind test of emitted qi on tumor formation of a nasopharyngeal carcinoma cell line in nude mice, 2nd World Conf Acad Exch Med Qigong, Beijing, China, p 105, 1993
22. Chen Y: Analysis of effect of emitted qi on human hepatocarcinoma cell (BEL-7402) by using flow cytometry, 2nd World Conf Acad Exch Med Qigong, Beijing, China, p 102, 1993

23. Liu T, Wan M, Lu O: Experiment of the emitted qi on animals, 1st World Conf Acad Exch Med Qigong, Beijing, China, p 60, 1988
24. Xu H, Xue H, Zhang C, Shao X, Liu G, Zhou Q, Yu F, Wu K: Study of the effects and mechanism of qigong waiqi (emitted qi) on implanted tumors in mice, 3rd Nat Acad Conf on Qigong Science, Guangzhou, China. p 82, 1990
25. Li C: Effect of qigong-waiqi on immune function of mice. Chin J Som Sci 2: 67–72, 1992
26. Feng L, Peng L, Qian J, Cheng S: Effect of qigong information energy on diabetes mellitus, 4th Intl Conf on Qigong, Vancouver, British Columbia, Canada, 1995: 17–19, 1995
27. Liu C, Sun C, Dong X: Study of the mechanism of the effect of qigong for diabetes, 3rd World Conf Acad Exch Med Qigong, Beijing, China, p 107, 1996
28. Jia L, Jia J: Effects of the emitted qi on healing of experimental fracture, 1st World Conf Acad Exch Med Qigong, Beijing, China, p 13, 1988
29. Jia L, Jia J, Lu D: Effects of emitted qi on ultrastructural changes of the overstrained muscle of rabbits, 1st World Conf Acad Exch Med Qigong, Beijing, China, p 14, 1988
30. Lin M, Zhang J, Hu D, Ye Z: Effect of qigong waiqi (emitted qi) on blood chemistry of mice radiated with X-rays, 3rd Nat Acad Conf on Qigong Science, Guangzhou, China, p 58, 1990
31. Yang K, Xu H, Guo Z, Zhao B, Li Z: Analgesic effect of emitted qi on white rats. 1st World Conf Acad Exch Med Qigong, Beijing, China, p 45, 1988
32. Yang K, Guo Z, Xu H, Lin H: Influence of electrical lesion of the periaqueductal gray (PAG) on the analgesic effect of emitted qi in rats,1st World Conf Acad Exch Med Qigong, Beijing, China, p 43, 1988
33. Zhang J: Influence of qigong waiqi (emitted qi) on volume of blood flow to visceral organs in rabbits under normal and hemorrhagic shock conditions, 3rd Nat Acad Conf on Qigong Science, Guangzhou, China, p 47, 1990
34. Zhang L, Yan X, Wang S, Tao J, Gu L, Xu Y, Zhou Y, Liu D: Immune regulation effect of emitted qi on immunosuppressed animal model, 1st World Conf Acad Exch Med Qigong, Beijing, China, p 27, 1988
35. Higuchi Y, Kotani Y, Higuuchi H, Yu Y, Chang YU: Immune changes during qigong therapy. J Intl Soc Life Info Sci 17: 297–300, 1999
36. Kiang JG, McClain DE, Warke VG, Krishnan S, Tsokos GC: Constitutive NO synthase regulates the $Na^{+}/Ca^{2+}$ exchanger in human Jurkat T cells: role of $[Ca^{2+}]_i$ and tyrosine phosphorylation. J Cell Biochem 89: 1030–1043, 2003
37. Blaustein MP, Lederer WJ: Sodium/calcium exchange: Its physiological implications. Physiol Rev 79: 763–854, 1999
38. Kiang JG, Tsokos GC: Signal transduction and heat shock protein expression. J Biomed Sci 3: 379–388, 1996
39. Kiang JG, McClain DE: Heat stress. In: G.C. Tsokos and J.L. Atkins (eds). *Combat Medicine* Basic and Clinical Research in Military, Trauma, and Emergency Medicine, Humana Press, New Jersey, pp 83–101, 2003
40. Kiang JG, Smallridge RC: Sodium cyanide increases cytosolic free calcium: Evidence of activation of the reversed mode of $Na^{+}/Ca^{2+}$ exchanger and $Ca^{2+}$ mobilization from inositol trisphosphate-insensitive pools. Toxicol Appl Pharmacol 127: 173–181, 1994
41. Shigekawa M, Iwamoto T: Cardiac $Na^{+}$-$Ca^{2+}$ exchange: Molecular and pharmacological aspects. Circ Res 88: 864–876, 2001
42. Iwamoto T, Satomi K: Development and application of $Na^{+}$/Ca$2^{+}$ exchange inhibitors. Mol Cell Biochem 259: 157–161, 2004
43. Reeves JP, Condrescu M, Chernaya G, Gardner JP: $Na^{+}/Ca^{2+}$ antiport in the mammalian heart. J Exp Biol 196: 375–388, 1994
44. Iwamoto T, Uehara A, Imanaga I, Shigekawa M: The $Na^{+}/Ca^{2+}$ exchanger NCX1 has oppositely oriented reentrant loop domains that contain conserved aspartic acids whose mutation alters its apparent $Ca^{2+}$affinity. J Biol Chem 275: 38571–38580, 2000
45. Ottolia M, John S, Qiu Z, Philipson KD: Split $Na^{+}$-$Ca^{2+}$ exchangers. Implications for function and expression. J Biol Chem 276: 19603–19609, 2000
46. Tang T, Kiang JG, Cox BM: Opioid acting through delta receptors elicit a transient increase in the intracellular free calcium concentration in dorsal root ganglion-neuroblastoma hybrid ND8–47 cells. J Pharmacol Exp Ther 279: 40–46, 1994