

# External Bioenergy Increases Intracellular Free Calcium Concentration and Reduces Cellular Response to Heat Stress

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## ABSTRACT

**Background:** External bioenergy (energy emitted from the body) can influence a variety of biological activities. It has been shown to enhance immunity, promote normal cell proliferation, increase tumor cell death, and accelerate bone fracture recovery. In this study, we investigated whether external bioenergy could alter 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.

**Methods:** A practitioner emitted bioenergy toward tubes of cultured Jurkat cells for one 15-minute period.  $[Ca^{2+}]_i$  was measured spectrofluorometrically using the fluorescent probe indo-1. The heat shock protein 72 kd was detected using  $^{35}S$ -methionine prepulse and Western blot analysis.

**Results:** The resting  $[Ca^{2+}]_i$  in Jurkat T cells was  $90 \pm 3$  nM in the presence of external calcium. The removal of external calcium decreased the resting  $[Ca^{2+}]_i$  to  $54 \pm 2$  nM, indicating that  $Ca^{2+}$  entry from the external source is im-

portant for maintaining the basal level of  $[Ca^{2+}]_i$ . In the presence of external  $Ca^{2+}$ , treatment of Jurkat T cells with external bioenergy for 15 minutes increased  $[Ca^{2+}]_i$  by  $22 \pm 3\%$ .  $[Ca^{2+}]_i$  remained elevated in these cells for 2 hours. Surprisingly, we also observed that  $[Ca^{2+}]_i$  increased by  $11 \pm 1\%$  if cells were simply placed in the area where external bioenergy had been used. This lingering effect of external bioenergy dissipated within 24 hours. Treatment with external bioenergy reduced cellular responses to heat stress and did not induce the production of heat shock protein 72 kd, which is known to provide cytoprotection.

**Conclusions:** These results suggest that externally applied bioenergy can upregulate  $[Ca^{2+}]_i$  and downregulate the cellular response to stress. The association between the external bioenergy and increases in  $[Ca^{2+}]_i$  may be a good index for detecting presence of bioenergy. (J Investig Med 2002;50:38–45) **Key Words:** lymphoid cells • intracellular calcium • intracellular signal • heat stress • bioenergy

## INTRODUCTION

Intracellular free calcium has been long recognized as a ubiquitous second messenger in various physiological sys-

tems. 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.<sup>1,2</sup> 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 transduc-

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Views presented in this paper are those of authors; no endorsement by the Department of the Army has been given or should be inferred. The raw data of this paper may be acquired from J.G.K.

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tion process comprising a receptor, a coupling G protein, and phospholipase C.<sup>3</sup>

Bioenergy such as therapeutic touch, distant healing, and qigong has been used for healing and self-healing as part of traditional medical practices for centuries. There have been a number of reports that bioenergy can influence a variety of biological activities.<sup>4</sup> It has been reported to enhance immunity, promote normal cell proliferation, increase tumor cell death, and accelerate bone fracture recovery.<sup>4</sup> We have reviewed the overall quality of these studies and found most of them marginal, with none measuring objective and continuous outcomes such as  $[Ca^{2+}]_i$ . The relationship between bioenergy and an important, objective, and easily monitored cellular outcome, such as  $[Ca^{2+}]_i$ , is not known and has never been investigated. In this study, we used human lymphoid Jurkat cells to explore  $[Ca^{2+}]_i$  before and after treatment with a single dose of external bioenergy. It is the first time that a single treatment with external bioenergy has been shown to increase  $[Ca^{2+}]_i$  but not  $Ca^{2+}$ -dependent heat shock protein 72 kd (HSP-72). The external bioenergy-treated cells in turn diminished their response to heat stress. The association between the external bioenergy and increases in  $[Ca^{2+}]_i$  may be a good index for detecting the presence of bioenergy.

## 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 fetal bovine serum, 2 mM glutamine, 100  $\mu$ g/mL of streptomycin, and 100 U/mL of 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 to 4 days.

### Intracellular $Ca^{2+}$ Measurements

Jurkat T cells were washed and then loaded in suspension with 5  $\mu$ M Indo-1AM (Molecular Probes, Inc, Eugene, Ore) at 37°C for 60 minutes. The suspended cells were placed in a cuvette, and the fluorescence signal was measured with a PTI Delta Scan spectrofluorometer<sup>TM</sup> (Photon Technology International, Inc, South Brunswick, NJ) with dual emission at 395 and 480 nm and single excitation at 350 nm (slit width, 4 nm). To minimize any contribution to the fluorescence signal resulting from dye in the medium, cells were washed thoroughly in  $Na^+$  Hanks' solution before measurements of  $[Ca^{2+}]_i$ . To perform experiments in the absence of extracellular  $Ca^{2+}$ , cells were incubated in the  $Ca^{2+}$ -free buffer containing 10

mM EGTA for 10 minutes before measurements of  $[Ca^{2+}]_i$ .

### Measurements of Cell Viability

Cell viability was determined by 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). A drop of the mixture was placed on the hemocytometer, and cells were counted under the microscope. Cells that turned blue represented the nonviable cells, whereas others represented the viable cells. The viability was calculated according to the following equation: viability (%) = [number of viable cells/(number of viable cells + number of nonviable cells)] $\times$ 100%.<sup>5</sup>

### [<sup>35</sup>S]methionine Prepulse and Electrophoresis

After the specified treatment, cells ( $5\times 10^6$ ) were returned to the incubator for 2 hours. Then, these cells were prepulsed with [<sup>35</sup>S]methionine (2  $\mu$ Ci/mL, 1.7 pmol/mL, New England Nuclear, Boston, Mass) and returned to the incubator for additional 2 hours. These cells were washed with phosphate-buffered saline to remove extra radioactivity and then resuspended in phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride and 1 mM dithiothreitol, 10  $\mu$ g/mL of leupeptin, and 5  $\mu$ g/mL of aprotinin, pH 7.4. The suspension was sonicated. The protein content was determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, South Richmond, Calif). Each sample, containing 10  $\mu$ g of protein, was loaded onto precasted 10% tris-glycine polyacrylamide gels (Novex, San Diego, Calif). After electrophoresis, the gel was air-dried and exposed to Kodak film (Eastman Kodak Co, Rochester, NY) to show the newly synthesized protein bands.<sup>6</sup>

### Western Blot Analysis

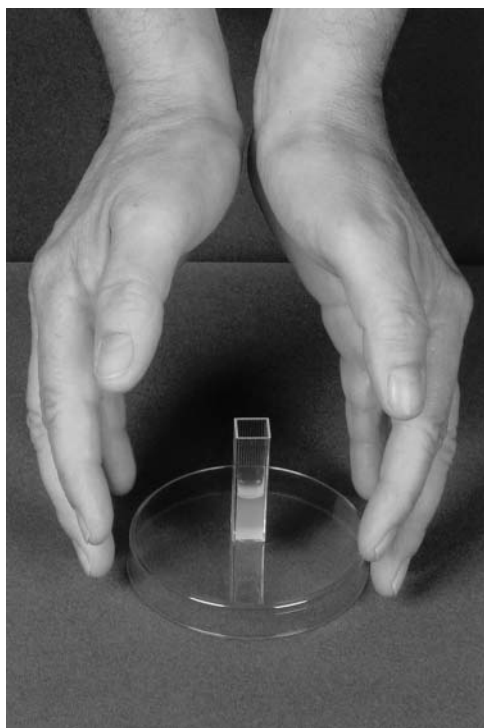
After electrophoresis, separated proteins were transferred to a nitrocellulose membrane (MSI Micron Separations, Inc, Westborough, Mass) using a Novex blotting module (Novex, San Diego, Calif). The blot was incubated with HSP-72 monoclonal antibody (1  $\mu$ g/mL, Santa Cruz Biotechnology, Santa Cruz, Calif) for 1 hour at room temperature. Then, the manufacturer's protocol with the enhanced chemiluminescence kit was used.<sup>6</sup>

### Solutions

Sodium Hanks' solution contained the following (in mM): 145 NaCl, 4.6 KCl, 1.2  $MgCl_2$ , 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.

### Application of Bioenergy

Bioenergy was administered by Mietek Wirkus, a bioenergy practitioner with a long history in practice and research. To administer external bioenergy, Jurkat T cells were suspended in a cuvette. During initial pilot tests, the cuvette remained in the sample chamber of the spectrofluorometer where the metal lid was in place. The bioenergy specialist placed his two palms facing the lid with 1 inch distance between the palm and the lid and emitted energy to cells for 15 minutes. This approach failed to increase  $[Ca^{2+}]_i$ . However, if the bioenergy specialist placed his two palms facing directly over the top and both sides of the cuvette containing cells, with 3 inches distance between the center of his palms and the cells (Figure 1), the application was successful in increasing  $[Ca^{2+}]_i$ , suggesting that the bioenergy cannot transmit through the metal of the spectrofluorometer chamber. Therefore, the emission of bioenergy herein was conducted in a specified way as shown in Figure 1 and directly facing the cells at 3 inches distance for a single 15-minute application. A baseline  $[Ca^{2+}]_i$  level was established by monitoring for 1 minute. Bioenergy was applied for 15 minutes as previously de-



**Figure 1.** Performance of external bioenergy. The bioenergy practitioner placed his two palms facing directly over the top and both sides of the cuvette containing human Jurkat T cells, with 3 inches distance between the center of his palms and the cells. The external energy was applied to cells for 15 minutes.

scribed, and then the  $[Ca^{2+}]_i$  levels were monitored for another 4 minutes. A series of sham bioenergy treatments were also performed, in which an identical procedure was performed by a nonbioenergy performer.

To study the lingering effect of external bioenergy at the performing site, the sham-operated control Jurkat T cells were placed at other laboratory where no external bioenergy was applied before. Similarly, the sham-operated control cells were also placed at the previously external bioenergy performed site after the external bioenergy had been dissipated 24 hours later. In either case, no statistically significant changes in  $[Ca^{2+}]_i$  were found.

### Statistical Analysis

Because each conducted experiment was lengthy and the basal level of  $[Ca^{2+}]_i$  fluctuated with the time, the percentage change to the basal level was presented throughout the study to accurately reflect the effect of external bioenergy on  $[Ca^{2+}]_i$ . All data are expressed as the mean  $\pm$  SEM. The paired *t* test was used for comparisons of  $[Ca^{2+}]_i$  levels in the same cells just immediately before and immediately after bioenergy treatment, whereas the unpaired *t* test was used to make comparisons between groups after heat stress in cells pretreated with or without external bioenergy. Because the focus of this study was to investigate  $[Ca^{2+}]_i$  before and after the bioenergy application on human Jurkat T cells, the data were compared only once. Therefore, the paired or unpaired *t* test was sufficient. No correction such as Bonferroni's inequality was needed. The level of statistical significance for comparisons was 0.05.

## RESULTS

### External Bioenergy Increases $[Ca^{2+}]_i$

The resting  $[Ca^{2+}]_i$  in Jurkat T cells was  $90 \pm 3$  nM ( $n=51$ , pooled from all different experiments) in the presence of external calcium. The removal of external calcium decreased the resting  $[Ca^{2+}]_i$  to  $54 \pm 2$  nM ( $n=17$ , from the actual experiment of interest), 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 a single dose of external bioenergy for 15 minutes increased  $[Ca^{2+}]_i$  by  $22 \pm 3\%$  ( $n=12$ ,  $t=2.1$ ,  $P<0.05$  vs baseline, paired *t* test). The Table lists the raw data for the reader's interest. The increase was long lasting (Figure 2A);  $[Ca^{2+}]_i$  remained elevated in these cells and returned to the baseline 2 hours later. The sham operation with a nonbioenergy performer displayed a slight but not statistically significant jump of  $[Ca^{2+}]_i$  (Figure 2B).

**Increases in  $[Ca^{2+}]_i$  by external bioenergy.**

Before (ie, baseline, nM)	After (nM)	% Increase
61	79	29.51
63	90	42.86
84	93	10.71
96	113	17.71
106	126	18.87
63	72	14.65
66	71	7.58
68	77	13.24
67	76	13.43
68	89	30.88
100	129	29.00
89	115	29.20

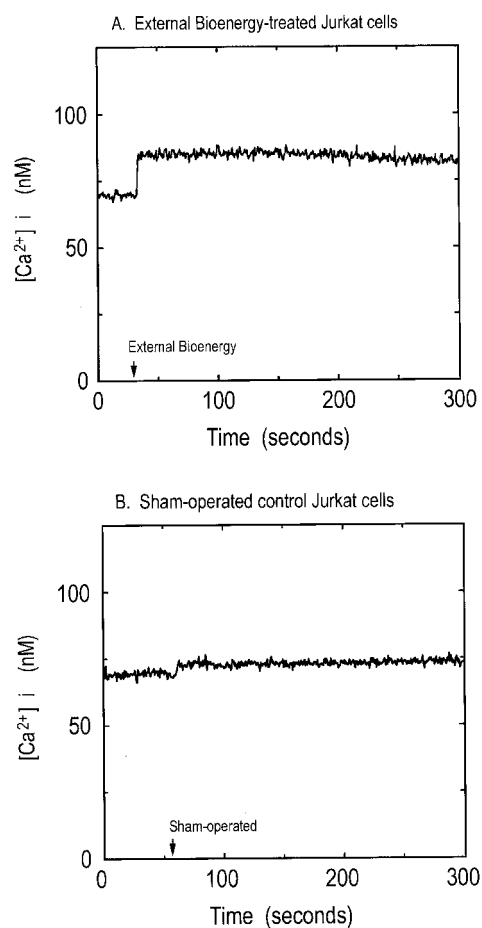
*NOTE.* Jurkat T cells were treated with external bioenergy for 15 minutes.  $[Ca^{2+}]_i$  was measured immediately before and immediately after the treatment with external bioenergy.

Surprisingly, we also observed that  $[Ca^{2+}]_i$  increased by  $11 \pm 1\%$  ( $n=10$ ,  $t=4.94$ ,  $P<0.001$  vs baseline, paired  $t$  test) if cells were simply placed in the area where external bioenergy had been performed (Figure 3A). This lingering effect of bioenergy was persistent for up to 8 hours and dissipated 24 hours later (data not shown). Likewise, the lingering effect was not observed in cells placed in areas without prior performance of external bioenergy (Figure 3B). Using trypan blue exclusion assay, cells remained viable after treatment with external bioenergy (data not shown).

To determine whether the increase in  $[Ca^{2+}]_i$  induced by external bioenergy is a result of the temperature change caused by body heat, temperatures in the buffer and laboratory when the experiment was conducted were measured. There were 1 to 1.5°C differences before and after treatment of bioenergy. However, exposing cells to buffer with temperature increased by 1 to 1.5°C for 15 minutes failed to increase  $[Ca^{2+}]_i$  (data not shown). Therefore, the effect of bioenergy on  $[Ca^{2+}]_i$  is not a result of temperature changes in the cells.

### External Bioenergy Does Not Induce HSP-72 Production

Because induction of HSP-72 is known to be  $Ca^{2+}$ -dependent,<sup>2,5,7-9</sup> we determined whether treatment with a single dose of external bioenergy induced the production of HSP-72, which is known to provide cytoprotection. Two hours after external bioenergy treatment, cells were prepulsed with [<sup>35</sup>S]methionine for 2 hours. No newly



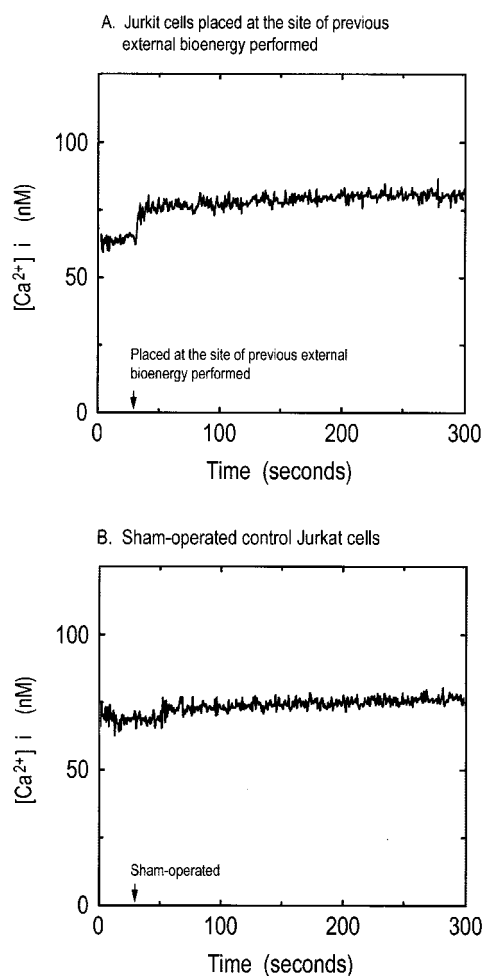
**Figure 2.** Increase in  $[Ca^{2+}]_i$  in Jurkat T cells by treatment of external bioenergy. Cells in suspension were treated with a single treatment of external bioenergy for 15 minutes before  $[Ca^{2+}]_i$  measurement. Representative fluorometer tracings with treatment with external bioenergy (A,  $n=12$ , total, from three independent experiments) and with a sham-operated nonbioenergy performance (B,  $n=15$  from three independent experiments) are presented. The initial tracing is the basal level of  $[Ca^{2+}]_i$ . The arrow indicates bioenergy treatment.

synthesized protein was found in these cells (Figure 4A), whereas cells exposed to 45°C for 10 minutes displayed a new protein band, with molecular weight approximately 70 kd (Figure 4B). Western blot analysis indicated that the new protein band was HSP-72 (Figure 4C). Using trypan blue exclusion assay, cells remained viable after exposure to heat stress or treatment with external bioenergy (data not shown).

### External Bioenergy Reduces the $Ca^{2+}$ Response to Heat Stress

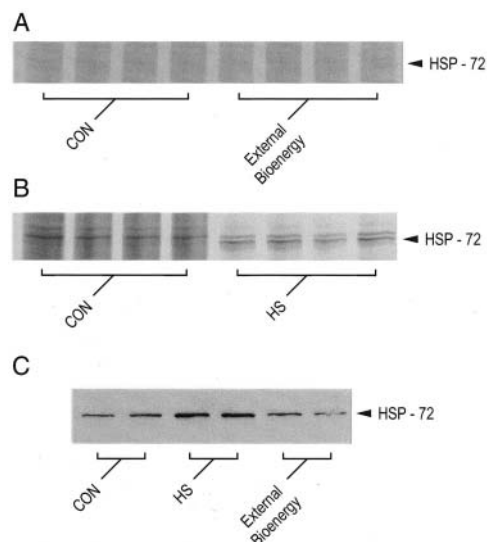
To determine whether pretreatment of cells with external bioenergy would impact the cellular response to heat





**Figure 3.** Increase in  $[Ca^{2+}]_i$  in Jurkat T cells by external bioenergy left at the site. Representative fluorometer tracings are presented. A. Cells in suspension were placed at the site where a single treatment of external bioenergy had been performed previously, for 15 minutes before  $[Ca^{2+}]_i$  measurement ( $n=10$ ). B. Cells in suspension were placed at the site where no external bioenergy had been performed ( $n=4$ ). The initial tracing is the basal level of  $[Ca^{2+}]_i$ . The arrow indicates bioenergy treatment.

stress, Jurkat T cells were exposed to  $45^\circ\text{C}$  for 10 minutes<sup>6,9</sup> and  $[Ca^{2+}]_i$  was measured. Figure 5 exhibits the different responses of cells to heat stress. In cells not exposed to external bioenergy, heat stress increased  $[Ca^{2+}]_i$  by  $305 \pm 9\%$  ( $n=6$  from one experiment, Figure 5A), whereas in cells treated with a single dose of external bioenergy 2 hours before heat stress,  $[Ca^{2+}]_i$  was increased by  $279 \pm 8\%$  ( $n=8$  from one experiment, Figure 5B), a level significantly lower than that in untreated cells ( $t=3.86$ ,  $P<0.005$ , unpaired  $t$  test). Similar results were obtained in three independent experiments. The decrease was not a result of cell death, because cell viability was not



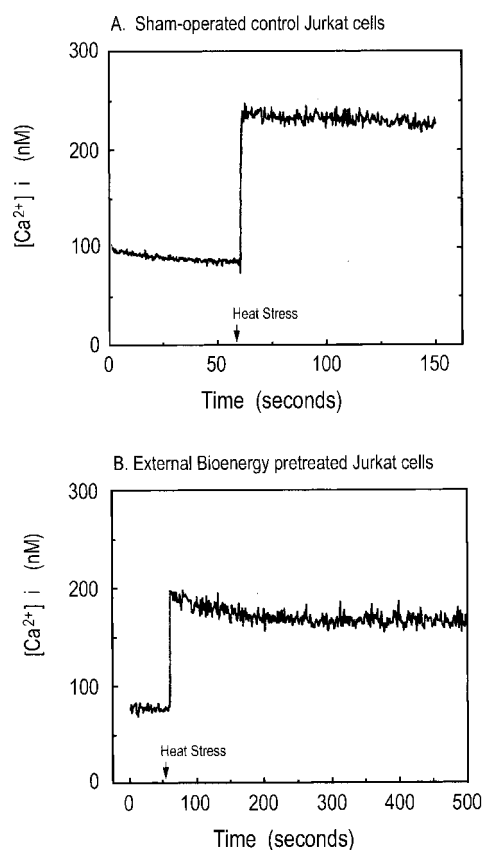
**Figure 4.** Induction of heat shock protein 72 kd by heat stress but not external bioenergy. Cells were either treated with external bioenergy for 15 minutes or exposed to  $45^\circ\text{C}$  for 10 minutes and recovered for 4 hours before cell lysate was collected for heat shock protein 72 kd (HSP-72) detection. Cells were prepulsed with  $^{35}\text{S}$ -methionine for 2 hours before cell lysate collection. Representative autograms with cells exposed to heat stress ( $n=4$ , B) or treatment with external bioenergy ( $n=4$ , A) are shown. The Western blot analysis against the HSP-72 antibody was conducted ( $n=2$ , C). A representative blot is shown. CON: control; HS: heat stress

different from those cells untreated with external bioenergy (data not shown). This reduction in the  $Ca^{2+}$  responding to heat stress was no longer present 24 hours after treatment with external bioenergy.

## DISCUSSION

In this study, the resting  $[Ca^{2+}]_i$  in suspended Jurkat T cells was  $90 \pm 3$  nM, as determined by indo-1. 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  $[Ca^{2+}]_i$ . The application of a single dose of external bioenergy significantly increased  $[Ca^{2+}]_i$  by  $22 \pm 3\%$ , which was not a result of the temperature changes in the buffer with cells or the laboratory where the experiment was conducted with the application of external bioenergy.

By denoting the chemical actions on samples in the conventional studies that correspond with “the application of external bioenergy” in this study, we recognize the complexity of experiments with distant application of energy by human emitter(s). However, we try to avoid detailed analysis of the exact impact and interaction process of all the known and unknown contributing factors, such



**Figure 5.** A reduced  $[Ca^{2+}]_i$  response to heat stress in bioenergy-treated Jurkat T cells. Cells were treated with a single dose of external bioenergy for 15 minutes. Then these were recovered for 2 hours, before a 10-minute exposure to  $45^{\circ}C$ . A. A representative fluorometer tracing of  $[Ca^{2+}]_i$  in cells only exposed to  $45^{\circ}C$  for 10 minutes ( $n=21$ , total, from three independent experiments). B. A representative fluorometer tracing of  $[Ca^{2+}]_i$  in cells treated with external bioenergy followed by exposure to  $45^{\circ}C$  for 10 minutes ( $n=21$ , total, from three independent experiments).

as the overall physical/mental state of the emitter(s) as well as that of the scientist(s) conducting the experiment. Many experiments with external bioenergy have been conducted in the past two decades, but most were not as well designed as the current one because they lacked the resources available to us now. Adding to the already complex issue of external bioenergy application, many of them also used multiple bioenergy treatments, unlike that of ours, where the effects of only one application were examined. It has been reported that repetitive treatments with external bioenergy displayed a reduced interleukin-2 level and an increased interferon activity in concanavalin A-treated spleen cells,<sup>10,11</sup> an increased phagocytotic function, activity of acid phosphatase, and amount of immunoglobulin M antibodies.<sup>12</sup> In studies with cancer

models, Cao et al<sup>13</sup> reported that when C57BL/6 mice were inoculated with B16 melanoma tumor cells via the tail veins, treatment with external bioenergy markedly reduced the number of B16 melanoma pulmonary metastases nodules in the lungs and increased survival time of rats over untreated controls. Similar results were reported with mice injected with MO4 cells<sup>14,15</sup> or U27 cancer cells.<sup>15,16</sup> Mice injected with ascitic cancer fluid followed by treatment with external bioenergy had increased hemoglobin levels and numbers of red and white blood cells and smaller tumor sizes.<sup>17</sup> Other studies reported that tumor formation was prevented in NC-Z strain mice inoculated with nasopharyngeal squamous carcinoma CNE-2 cells<sup>18</sup> or human hepatocarcinoma BEL-7420 cells<sup>19</sup> and weight reduction delayed after injection of sarcoma 180 tissue.<sup>20,21</sup> Li<sup>22</sup> reported that external bioenergy reduced the size of G422 cell neurogliomas implanted in mice. In human patients treated with external bioenergy, natural killer cell activity increased, whereas the CD4/CD8 ratio remained unchanged.<sup>23</sup>

In *in vivo* experimental models, treatment with external bioenergy has been shown to increase body weight gain, reduce blood glucose, increase insulin activity,<sup>24</sup> decrease MDA and lipid peroxides,<sup>25</sup> and promote faster recovery of bone fracture.<sup>26,27</sup> It also has been observed to increase sedative and analgesic effects,<sup>28–30</sup> elevate volume of blood flow,<sup>31</sup> and restore T-cell proliferation and activity of interleukin-2.<sup>32</sup> One should bear in mind that negative results with external bioenergy studies have been reported as well.<sup>4</sup>

The biological and therapeutic effects described above result from treatments with the application of external bioenergy. However, the underlying mechanism(s) of the effects and the underlying processes of the applications 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, inducing muscle contraction, increasing hormonal secretion, and activating metabolic systems.<sup>2</sup> Thus, the treatment of cells with external bioenergy, which increases  $[Ca^{2+}]_i$  in cultured human lymphoid T cells, likely triggers these effects. Furthermore, the association between the application of external bioenergy and the increases in  $[Ca^{2+}]_i$  may be a good index for detecting the presence of human-emitted energy.

A reduction of  $Ca^{2+}$  response to heat stress in bioenergy-treated cells was observed. A similar result was found in cells overexpressing HSP-72 induced by physical methods,<sup>9</sup> chemical methods (Kiang, unpublished data), or gene transfer<sup>33</sup> in which cells remained viable after hostile assaults.<sup>5,7,8</sup> This cytoprotection is thought to be mediated via the desensitization of  $Ca^{2+}$  machinery in the cell.<sup>2,33–35</sup> Therefore, the healing capability claimed by external

bioenergy is possibly associated with its ability to regulate  $[Ca^{2+}]_i$ .

The intended external bioenergy on  $[Ca^{2+}]_i$  in this study was applied at a distance of 3 inches between the palms of the bioenergy emitter and the cells. It should be noted that the specific scale of this distance may not be of substantial significance in general. In fact, the bioenergy practitioner Mietek Wirkus admitted that bioenergy influence over experimental subjects was also observed from more remote distances (eg, distance healing, among others) than that in our study, albeit at the price of a much higher human energy consumption required to reach the desired effectiveness.

Many experimental reports indicate that the external qi of qigong—sometimes considered a form of bioenergy but in fact a traditional Chinese practice that has earned the attention of scientific studies over the past 20 years—can act on distant samples when administered by a qigong specialist.<sup>4</sup> Yan et al<sup>36</sup> and Lu<sup>37</sup> reported that results from treatment of Fab protein crystallization at the study site are similar to those at distances from 95 to 4800 km. The question was raised about how this bioenergy or qi could be aimed at the tested subjects from such a long distance.

Dr. Yan Xin, a world renown qigong master and scientist whom J.G.K. consulted throughout the course of the experiment, stated that in spite of his previously published favorable results,<sup>4,36–38</sup> no scientific explanation is yet available for the distinct characteristic of qi except for the indication that the results obtained from the long and short distance qi emissions are in agreement. Further studies certainly are needed for better understanding the mechanisms underlying many reported effects of bioenergy and qi. This study on  $[Ca^{2+}]_i$  is just a beginning.

Notably, the variability of the lingering effect of external bioenergy was lower than its direct effect on human Jurkat T cells, suggesting that the lingering energy was probably stabilizing after some period of time. More studies are being conducted to address the possibility.

In summary, this study is the first to show that a single treatment with the application of external bioenergy significantly increased  $[Ca^{2+}]_i$  but not HSP-72 in human Jurkat T cells. Heat stress still induced an increased  $[Ca^{2+}]_i$  but at a lower level compared with that found in untreated cells. Alterations in cellular  $[Ca^{2+}]_i$  may be one mechanism by which bioenergy healing occurs. Further investigation of bioenergy and its effects are needed.

#### ACKNOWLEDGMENT

*We thank Dr. Yan Xin for his immensely valuable discussion.*

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## External Bioenergy Increases Intracellular Free Calcium Concentration and Reduces Cellular Response to Heat Stress

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*J Investig Med* 2002 50: 38-45  
doi: 10.2310/6650.2002.33516

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