Experimental

EFFECTS OF QIGONG ON CELL-FREE MYOSIN PHOSPHORYLATION: PRELIMINARY EXPERIMENTS

David J. Muehsam; M. S. Markov, Ph.D.; Patricia A. Muehsam, M.D.; Arthur A. Pilla, Ph.D.; Ronger Shen & Yi Wu

ABSTRACT

This work examines the effect of Qigong from two experienced practitioners on *in vitro* cell-free myosin phosphorylation. This system has a demonstrated sensitivity to variations in static magnetic fields above and below ambient values. The results show that both Qigong practitioners were able to consistently yield results similar to those observed for variations in applied magnetic fields near the ambient level. Qigong treatment with the myosin reaction mixture in the ambient magnetic field reduced phosphorylation in each experiment by an average of approximately 15% ($p < .05$). For trials with the myosin samples in a magnetic shield, two of four (one for each practitioner) Qi treatments yielded significant reductions in phosphorylation (average approximately 10%, $p < .05$). These effects were somewhat lower and not as consistent as those obtained under ambient field conditions. The results obtained in this study demonstrate that Qigong practice can consistently affect a biologically relevant enzyme system, requiring no physical contact between the practitioner and the sample. The mechanisms of the Qigong effects observed in this study are as yet unclear.

KEYWORDS: Qigong, myosin phosphorylation, healer, in vitro, bioelectromagnetics

INTRODUCTION

The increasing emphasis on the relationship between mind and body has led to a significant body of research and clinical applications. Evidence of mind/body effects in current medical science includes the health effects of lifestyle and stress[1,2] education[3] social interactions[4,5] work status[6] religious practice[7] the spontaneous remission of cancers,[8] and the well-documented placebo effect.[9-12] The developing field of psychoneuroimmunology reflects progress in the understanding of mind/body interactions. In addition, a growing body of evidence supports the efficacy of therapies such as Therapeutic Touch,[13-16] prayer,[17] and other forms of non-contact healing.[18-20] Therapeutic Touch has been shown to affect pulse rate, skin temperature, galvanic skin response and hemoglobin levels.[21] Experiments on the effect of human intent upon seed germination by the Sprindrift group have shown that directed attention can consistently affect biological systems.[22,23] These results indicate that, in certain situations, the possibility of significant interactions without physical contact must be considered. Non-contact interaction implies that coupling between mind and environment may play a significant role in some experimental situations.[24] Sophisticated studies on human/machine interactions performed at the Princeton Engineering Anomalies Research Laboratory provide a strong impetus for further research.[25] Nonlocal effects have been known to modern physics for some time and it has been proposed that application of quantum mechanical concepts to biological systems could help to provide a framework for understanding this relatively new perspective.[26]

BACKGROUND

QIGONG

The terminology and practice of traditional Chinese medicine is centered around the concept of Qi, or vital force. Qi is thought to be the substantive element in living systems, an essential energy permeating all of space. An essential aspect of the notion of Qi in human life involves the interaction with one's environment.[27] The body is thought to contain a supply of Qi that flows throughout the acupuncture meridia and is exchanged with the Qi in one's surroundings. From this perspective, good health involves a balance and

unobstructed flow of Qi.[28 (p. 43)] The term *Qigong* refers to approximately two hundred related disciplines associated with healing and the martial arts. According to Yan Xin, "Qigong has about a 3,000 year history, with artifacts dating back as far back as 7,000 years. It is a system of physical, mental, and philosophical training for cultivation of moral and body strength, exploring the latent ability of humans, prolonging life, and developing human potential."[29] In China, emphasis is placed on Qigong as a committed daily practice, involving the development of skills over the course of many years.[30] A distinction is drawn in the Chinese terminology between *internal Qigong* and *external Qigong*; the former referring to disciplines in which the practitioner manipulates the Qi to bring about beneficial effects, the latter referring to the practice of having effects outside the body through the manipulation of Qi. Both types of Qigong are in widespread clinical use in China and are practiced as a form of health maintenance by millions of Chinese.[28 (pp. 200-210)] The focus of this research is on external Qigong, hereafter simply referred to as "Qigong."

As evidenced by several hundreds of studies, a sustained interest in Qigong research exists in China.[31] Research to date outlines many effects and possible mechanisms for the phenomenon of Qigong. Experiments on animals and humans indicate that Qigong may accelerate bone healing in rabbits,[32] elevate T-lymphocyte counts and raise chronic low hemoglobin levels in humans,[33] decrease metastatic tumor formation in mice,[34] and reduce the size of malignant tumors in mice.[35] Qigong effects reported on *in vitro* systems include changes in Laser Raman spectra of phospholipids[36] water, saline and glucose solution,[37] changes in the phases of liquid crystals and lipids,[38] changes in the ultraviolet absorption spectra of nucleic acids,[39] decrease and increase in the activity of saccharogenic amylase,[40] increases in respiration rate and synthesis of DNA and proteins in cultured fibroblasts,[41] and increases and decreases in the growth rates of *E. coli* bacteria.[42] Studies mentioned above which report both increases and decreases state that the practitioners used different methods to emit either "lethal Qi" or "health-promoting Qi."[28 (p. 213)] There have also been attempts to evaluate the physical characteristics of Qigong. Most of these studies were aimed at the measurement of electromagnetic quantities. Researchers have reported observing low levels of photon emission,[43,44] infrared light,[45] magnetic fields,[46] alterations in the discharge behavior of a Van de Graff generator,[47] and infrasound emissions.[48] At least one group has put into clinical application an electronic device that

mimics measured electromagnetic emanations of an experienced Qigong master.[49] It should be noted, however, that many of the above studies have not yet been replicated or published in peer-reviewed journals.

The reports that Qigong may have electromagnetic components suggested that the high magnetic sensitivity of the cell-free myosin phosphorylation system developed in this laboratory might provide a means of measuring Qigong effects. The experiments described herein were conceived as preliminary trials whose purpose was to assess the potential for more comprehensive future research directed towards providing in vitro experimental evidence for the effects of Qigong treatment.

This study involved a collaboration with two Qigong masters, Ronger Shen and Yi Wu. They were amongst the first in China to learn a Qigong form called Soaring Crane Qigong and were the first to introduce it in the US. Soaring Crane Qigong was first introduced to the public in China in the 1980's and quickly became a popular form with over 20 million followers.[50] Soaring Crane Qigong, according to Ronger Shen and Yi Wu, "is a combination of physical movements and mental meditations with five routines as its basis."[50] They indicate that, for the dedicated practitioner, "it can also tap human potentials."[50]

Cell-Free Myosin Light Chain Phosphorylation

The cell-free system we have used to study Qigong effects, phosphorylation of myosin light chains, is involved in the regulation of smooth muscle contraction. Calcium plays a key regulatory role in this system. The activity of the enzyme which catalyzes this phosphorylation, myosin light chain kinase (MLCK), is calcium dependent. For activation of MLCK, calcium must bind to calmodulin, a regulatory protein. Thus, Ca^{+2}-calmodulin dependent phosphorylation of myosin occurs in the following manner: Ca^{+2} binds to calmodulin, causing a conformational change in calmodulin; the calcium-calmodulin complex then interacts with the inactive catalytic sub-unit of MLCK to form a catalytically active holoenzyme complex; the kinase then proceeds to phosphorylate myosin light chain.[51-53]

Sensitivity of Myosin Phosphorylation to Electromagnetic Fields

Weak, environmental range, electromagnetic fields (EMF) have been shown to modulate the rate of phosphorylation of myosin in a cell-free preparation.[54,55] The following is a summary of experiments reported by this laboratory demonstrating EMF sensitivity. Myosin light chains and myosin light chain kinase were isolated from turkey gizzard.[56] Calmodulin was isolated from bovine brain. The reaction mixture contained 160 nM myosin light chains, 2 nM myosin light chain kinase, and 70 nM calmodulin in 20 mM Hepes buffer, pH 7.0; 0.5 mM magnesium acetate; 1 mg/ml bovine serum albumin, 0.1% (W/v) Tween 80. This mixture was chosen to make Ca^{2+}-calmodulin interaction the rate limiting step, with Ca^{2+} maintained at 0.9 μM. Phosphorylation of myosin light chains was carried out in 1.5 ml centrifuge tubes (Eppendorf). The Eppendorf tubes were placed in a plastic water bath maintained at 37.0 ± 0.1 °C. Temperature was monitored with a thermistor system (Cole-Parmer model 8110-20) immersed in one Eppendorf tube during all experiments. The water bath was placed in the central area of the Helmholtz exposure system described below. All exposures were for 6 min. Control assays were run as sham prior to, or immediately after, magnetic field exposure, for the same time period in the same EMF setup with magnetic fields turned off. The reaction was initiated by adding 5 μl gamma^{32}P ATP to the reaction medium, and was stopped with 100 μL Laemmli stopping buffer.

Phosphorylation was determined using Cherenkov emission by counting gamma^{32}P incorporation into myosin light chains. All exposures and experiments were repeated at least five times, and the mean value at each time point is from at least 30 independent measurements. A Student's *t*-test for independent samples was performed for each time and exposure condition. Significance was accepted at $p < .05$.

The EMF exposure system consisted of two orthogonal pairs of 60 turns, 8 in. square coils by which both the vertical and horizontal static magnetic field could be adjusted. A pair of circular (8 in. diameter) coils mounted horizontally were used to apply any other signal for which the parameters vary in time. Static magnetic fields were measured to ± 0.1 μT using a Bartington MAG-01 fluxgate magnetometer.

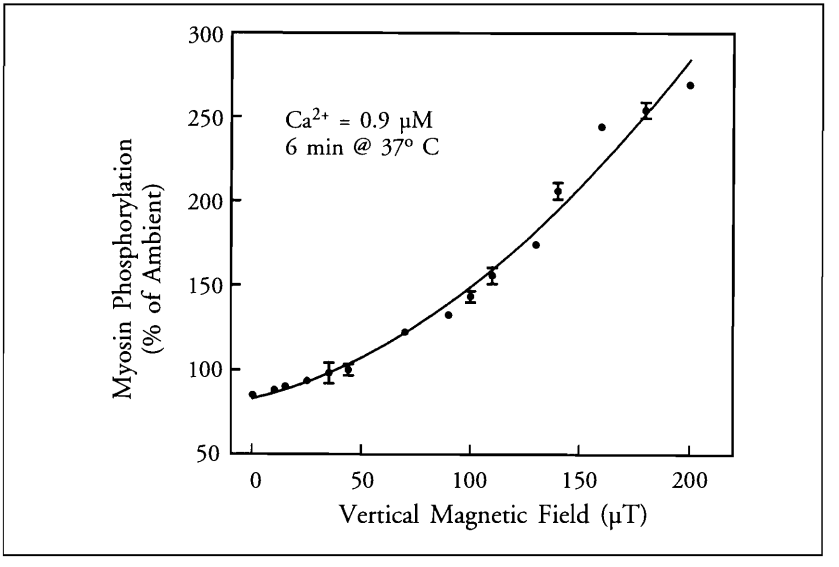

Figure 1. *Effects of vertical DC magnetic fields above and below ambient on cell-free myosin phosphorylation. The amplitude of AC or horizontal DC fields did not exceed ± 0.1 μT. It is this remarkable sensitivity to static magnetic fields that led the authors to suspect that this* in vitro *system might allow Qigong effects to be examined quantitatively.*

In order to assess the effect of ambient (geomagnetic and environmental) magnetic fields, a specially designed shielding box (mu-metal, Ammunel, Philadelphia, PA) allowed shielding of the exposure system and samples from any natural and extraneous time-varying and static magnetic fields by 50 dB (to 0.1 μT), below approximately 1 MHz. To test for static magnetic field effects, fields of magnitudes from 0.1 to 200 μT were applied inside the shield. The spatial orientation of the field, either vertical or horizontal, had no influence on the reported magnetic field effects. Ambient magnetic fields were carefully monitored in all experiments. The daily static magnetic field variations at the experimental site were as follows: 37 ± 1 μT vertical and 24 ± 1 μT horizontal, resulting in a magnitude of 44 ± 1.4 μT, 57° from horizontal. *[Note 44 μT = 0.44 Gauss]*

An example of the results obtained is shown in Figure 1. As may be seen, vertical DC magnetic fields below ambient, the 0.1-45 μT range, decreased

phosphorylation rate to about 80% of control values. In contrast, phosphorylation increased to more than 200% of the mean control value when the DC magnetic field was 200 μT. The line drawn through the points in Figure 1 indicates that a 10 μT change in ambient magnetic field produces about a 10% change in phosphorylation vs. control values. These results clearly show that very small alterations in ambient DC magnetic fields can affect myosin phosphorylation.

EXPERIMENTAL

Preliminary Experiments with Qigong Applied to Myosin Phosphorylation

Prior to Qigong treatment, all enzyme samples were prepared in exactly the same manner as described above for EMF exposure. The samples were placed in exactly the same position in the laboratory as that of the EMF exposure samples. No external fields were applied; the samples were subjected only to the ambient magnetic field at the site. No significant baseline changes in ambient field magnitudes were observed over the course of any group of Qigong treatments. Controls were run immediately before or after Qigong treatment and the Qi practitioners were not present in the laboratory during these control group experiments. For each treatment a minimum of five repeated measures in the scintillation counter were recorded, and the mean deviation from controls is reported. A Student's *t*-test for independent samples was performed for each exposure. Significance was accepted at $p < .05$.

The Qigong practitioners were requested to treat the samples as they would treat a patient. No mention was made of the type of illness to which the treatment might correspond, nor were the practitioners requested to engage in any particular Qigong form. Treatment of enzyme samples with Qigong was accomplished by allowing the practitioner to prepare for treatment and beginning the reaction at the practitioner's signal. The practitioner stood two to five feet away from the sample during treatment. Treatment of all samples with Qigong continued for six minutes, at which time the reaction was stopped as described in the previous section.

Table I
Effect of Qigong on Myosin Phosphorylation in Ambient Magnetic Field

TRIAL	PRACTITIONER	PERCENT CHANGE
1	RS	-11.1 ± 0.6
2	RS	-10.1 ± 0.5
3	RS	-14.8 ± 0.7
4	YW	-13.3 ± 0.7
5	YW	-17.6 ± 0.9
6	YW	- 8.5 ± 0.4
7	YW	-18.7 ± 0.9
8	YW	-16.9 ± 0.9
9	YW	-23.0 ± 1.2

Note: $p < .05$ for all trials.
The negative percent effect indicates a decrease vs. controls.

The results are shown in Table I, which indicates all Qigong treatments with the samples in the ambient magnetic field were found to significantly affect myosin phosphorylation. All treatments in this group resulted in reductions in myosin phosphorylation, with an average reduction of 14.9 ± 0.8% ($p < .05$). This mean reduction in phosphorylation is comparable to that observed for a 15 μT reduction in the ambient static magnetic field. Qigong practitioner Shen completed three independent treatments. Each of these treatments resulted in significant reductions in myosin phosphorylation, with an average reduction of 12.0 ± 0.6% ($p < .05$). Practitioner Wu completed six independent treatments, all of which resulted in significant reductions in phosphorylation. The mean reduction for this practitioner was 16.3 ± 0.8% ($p < .05$).

In one trial an increase in phosphorylation of 8.6 ± 0.4% ($p < .05$) was noted. Practitioner Shen indicated that she willfully caused this increase in response to the experimenter's previous observation that the practitioners had thus far always caused a reduction in phosphorylation. The trial that produced this increase was performed immediately after the experimenters had asked the Qigong practitioners if they felt that the decreases observed thus far might be intrinsic to the practice.

Table II
Effect of Qigong on Myosin Phosphorylation
in μ-metal Shielding Box

TRIAL	PRACTITIONER	PERCENT CHANGE (* $p < .05$)
11	RS	- 7.8 ± 0.4*
12	YW	-13.2 ± 0.7*
13	YW	- 7.6 ± 0.4
14	YW	- 5.8 ± 0.3

Four trials were performed for which the myosin samples were placed inside the mu-metal box used in the EMF experiments to shield from ambient fields. The results of these trials are displayed in Table II. Two trials (one from each practitioner) produced statistically significant ($p < .05$) reductions in phosphorylation, their average being 10.5 ± 0.5%. The remaining two trials performed by a single practitioner with the shielding box in place produced smaller, non-significant phosphorylation reductions.

In addition, one treatment by an untrained individual, with the sample in the ambient magnetic field produced the smallest statistically significant reduction in phosphorylation of 2.6 ± 0.1% ($p < .03$).

DISCUSSION

The above results appear to demonstrate that the Qigong practice of the two practitioners involved in this study can consistently affect *in vitro* cell-free myosin phosphorylation. For each of nine treatments with similar intent on the part of the practitioners and the samples in the ambient magnetic field, there was a significant reduction in the phosphorylation of myosin light chains, as compared to controls. For these trials, the healers were requested only to treat the samples as they would a patient, with no indication of the type of malady to be treated or particular Qigong form to be practiced. The consis-

tency of these reductions in phosphorylation suggests that Qigong practice may have well-defined effects for *in vivo* treatment conditions. One may conjecture that the effects measured in these trials portray a generic result due to a common treatment form.

As indicated by the Qigong practitioner at the time of the experiment, one atypical trial yielding an increase in phosphorylation was due to an intentional change in the treatment modality on the part of the healer. This observation raises the possibility that adept Qigong practitioners may be able to vary treatment forms at will, perhaps with profoundly different therapeutic affects. The likelihood of the existence of different types of Qi and Qigong practice, as mentioned earlier,[28 (pp. 200-210)] suggests that further research considering the healer's perceptions and the effects of varying healing intentions may yield valuable insights into the nature of Qigong therapy.

Four trials performed with the sample placed inside a mu-metal shielding box yielded two significant reductions in phosphorylation. These results are clearly ambiguous. However, the lowered Qigong effect could possibly be interpreted as providing weak support for an electromagnetic component of Qigong. It is necessary, however, to assess psychological variables such as the impact on the practitioner of placing the sample inside a large opaque container designed to block invisible energies. The mu-metal box provides shielding by 50 dB (to 0.1 μT from DC to 1 MHz). Therefore, if the practitioners can continue to affect myosin phosphorylation through the magnetic shield in future experiments, the conclusion must be that electromagnetic fields can be only one component of the Qigong practitioner's output.

The effect measured due to treatment by an untrained individual is near the level of resolution of these measurements in the myosin phosphorylation system. The 2.6% reduction in phosphorylation for this trial may not reflect an actual intentional effect upon the system. Current analyses of the myosin phosphorylation system employed at the time of these trials indicates that changes below the 5% level may not be distinguishable from the experimental errors inherent in the system itself. Therefore this particular result may have been spurious and cannot be considered meaningful until many more experiments are performed.

In light of the magnitude of the average overall reduction in phosphorylation of approximately 15% for trials in the ambient magnetic field, it is important to note that many clinically significant changes in biological parameters due to the therapeutic application of electromagnetic fields are of comparable size. For example, changes induced by EMF signals in clinical use for bone repair in calcium uptake in embryonal chick tibia *in vitro*, are of similar magnitude.[57] Thus, small changes in biological endpoints can be the measurable affects due to clinically effective EMF signals. Also, experimental results with EMF signals have indicated that bioeffective dosimetry at the tissue site is waveform dependent.[58,59] These results suggest that for EMF therapy, and perhaps also for Qigong, it may be the informational content of an interaction, rather than simply the magnitude of effects, that is decisive in the promotion of healing.

Adopting the metaphors of traditional Chinese medicine, information regarding healing and the body's state may be communicated through the acupuncture meridia. The demonstrated electromagnetic qualities[60] of the acupuncture points suggests that further study of the possible therapeutic actions of Qigong practice and acupuncture may shed light on the field of bioelectromagnetics. It may be premature to equate the notion of Qi and the concepts of Chinese medicine with electromagnetics. However, if electromagnetic components are consistently found to be associated with Qigong, further research may benefit from the hypothesis that Qigong treatment involves a form of information transfer mediated through an electromagnetic interaction.

The extreme sensitivity of the myosin phosphorylation system to weak EMF and Qigong suggests that systems of this type may be influenced by a broader range of variables than those considered traditionally. There is a growing body of data suggesting the possibility of significant variations in measurement of non-contact interactions due to environmental factors and/or experimenter interactions. Experiments evaluating human intentional effects on the measurement of radioactive decay have shown, for example, correlation with solar,[24] and geomagnetic activity.[61] Also, research on human-machine interactions[25] has routinely employed ordinary individuals with no training as healers or reporting psychic activity. If untrained individuals are found to have significant effects on biophysical systems such as the myosin phosphorylation system utilized in this study, there may be a need to account for the possibility of experimenter effects.

Conversations with the practitioners involved in this study indicated that they believe that the specific type of treatment given to a patient is dependent upon the nature of the patient's ailment. The possibility that Qigong and other healing methods have an inherently interactive nature is of consequence with regard to the methods of research directed towards these phenomena. If the Qi "emission" from a practitioner is meaningfully affected through an interaction with a patient, then the results of *in vitro* experiments and measurements of physical quantities such as electromagnetic fields near a single Qigong practitioner may not accurately reflect Qigong effects in clinical situations. Studies performed in the context of actual healing sessions may provide a clearer view of Qigong effects.

CONCLUSION

The results of these preliminary experiments demonstrate that Qigong practice can consistently affect a biologically relevant enzyme system. This interaction requires no physical contact between the practitioner and the sample. Although trials in which samples were placed inside a magnetic field shielding box produced reduced Qigong effects, hypotheses regarding mechanisms by which these effects may be understood are as yet unclear.

These results suggest strongly that more comprehensive studies will yield valuable information regarding Qigong effects. Examination, through measurement of fields and further trials utilizing shielding devices, of potential electromagnetic components involved in Qigong practice may help to clarify the results of these experiments. Further research aimed at investigating the possibility of variations due to different treatment modalities and amongst different practitioners is suggested.

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CORRESPONDENCE: D. J. Muehsam, M. S. Markov, P. A. Muehsam & A. A. Pilla • Bioelectrochemistry Laboratory • Department of Orthopaedics • Mount Sinai School of Medicine • New York, NY 10029 • Ronger Shen, Yi Wu • Qigong Center • Flushing, NY 11354

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