Electric stimulation of protein and DNA synthesis in human fibroblasts

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ABSTRACT

Human fibroblast cell cultures were employed as a model system to rapidly examine several potentially important variables involved in the use of high-voltage, pulsed galvanic stimulation (HVPGS) to increase the healing rate of soft tissue injuries. Fibroblasts were grown on Millipore filters and exposed to HVPGS of various voltages and pulse rates for 20 min in a rectangular, plastic chamber filled with growth medium. Filters with attached cells were placed either in the center of the chamber or close to the positive or negative electrode. Protein synthesis and DNA synthesis were monitored after stimulation using the radioactively labeled precursors, [^{3}H]proline and [^{3}H]thymidine, respectively. The major results obtained in this study are as follows: *1*) the rates of both protein and DNA synthesis can be significantly increased by specific combinations of HVPGS voltage and pulse rate; *2*) maximum stimulation of protein and DNA synthesis was obtained at 50 and 75 V, respectively, with a pulse rate of 100 pulses/s and the cells located near the negative electrode; and *3*) exposure to HVPGS intensities greater than 250 V (at all pulse rates and locations within the chamber) is inhibitory for both protein and DNA synthesis. In view of the results obtained in preliminary clinical studies on the use of HVPGS for the treatment of dermal ulcers, it appears that similar voltages, pulse rates, and relative electrode location may be required for maximum acceleration of human skin wound healing. — BOURGUIGNON, G. J.; BOURGUIGNON, L. Y. W. Electric stimulation of protein and DNA synthesis in human fibroblasts. *FASEB J.* 1: 398-402; 1987.

Key Words: electric stimulation • fibroblasts • protein synthesis • DNA synthesis • wound healing

ELECTRIC STIMULATION OF VARIOUS TYPES has been found to be an effective treatment technique for accelerating the healing rate of both hard and soft tissue injuries (1-4). In particular, there have been several recent reports that high-voltage, pulsed galvanic stimulation (HVPGS—a frequently utilized physical therapy modality) can significantly increase the healing rate of skin wounds (5-9). However, no systematic, controlled studies have been carried out to determine the specific conditions required for maximum effectiveness of HVPGS in the treatment of soft tissue injuries.

As a first step toward defining the optimal HVPGS treatment conditions for different types of soft tissue injuries, we have examined the effect of *1*) voltage, *2*) pulse rate, and *3*) location of the cells relative to the electrodes on protein and DNA synthesis in diploid human fibroblasts growing in cell culture. Because fibroblasts are one of the primary cells involved in all types of soft tissue healing, we believe it is likely that some of the results obtained with this simplified model system will be relevant for the complex patient treatment situation and, thereby, provide a rational starting point for future clinical investigations.

There appears to be a recent resurgence of interest in the effects of electric currents and electromagnetic fields on biological systems (10, 11). With regard to cell cultures, Yang et al. (12) have observed cell shape changes and cytoskeleton reorganization in mouse fibroblasts exposed to a low-intensity, direct current. Similar results have been reported for *Xenopus* epithelial cells (13) and rat basophilic leukemia cells (14). In addition, there have been a number of reports on the effects of different types of electric stimulation on macromolecular synthesis by cells in culture (15-20). However, to our knowledge this is the first report concerning the effects of HVPGS on cellular biosynthesis.

MATERIALS AND METHODS

Cell cultures

All experiments were done with a normal, diploid human fibroblast cell line designated IMR-90 (21). The cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Gibco, Grand Island, NY), 2% HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] buffer (pH 7.0) plus 1% penicillin/1% streptomycin at 37°C in a 5% CO_2/95% air atmosphere.

For treatment with HVPGS, the fibroblasts were plated on Millipore filters (25 mm, GSWP 02400; Millipore Corp., Bedford, MA) at a density of 5×10^3 cells/filter and incubated in DMEM plus 10% fetal calf

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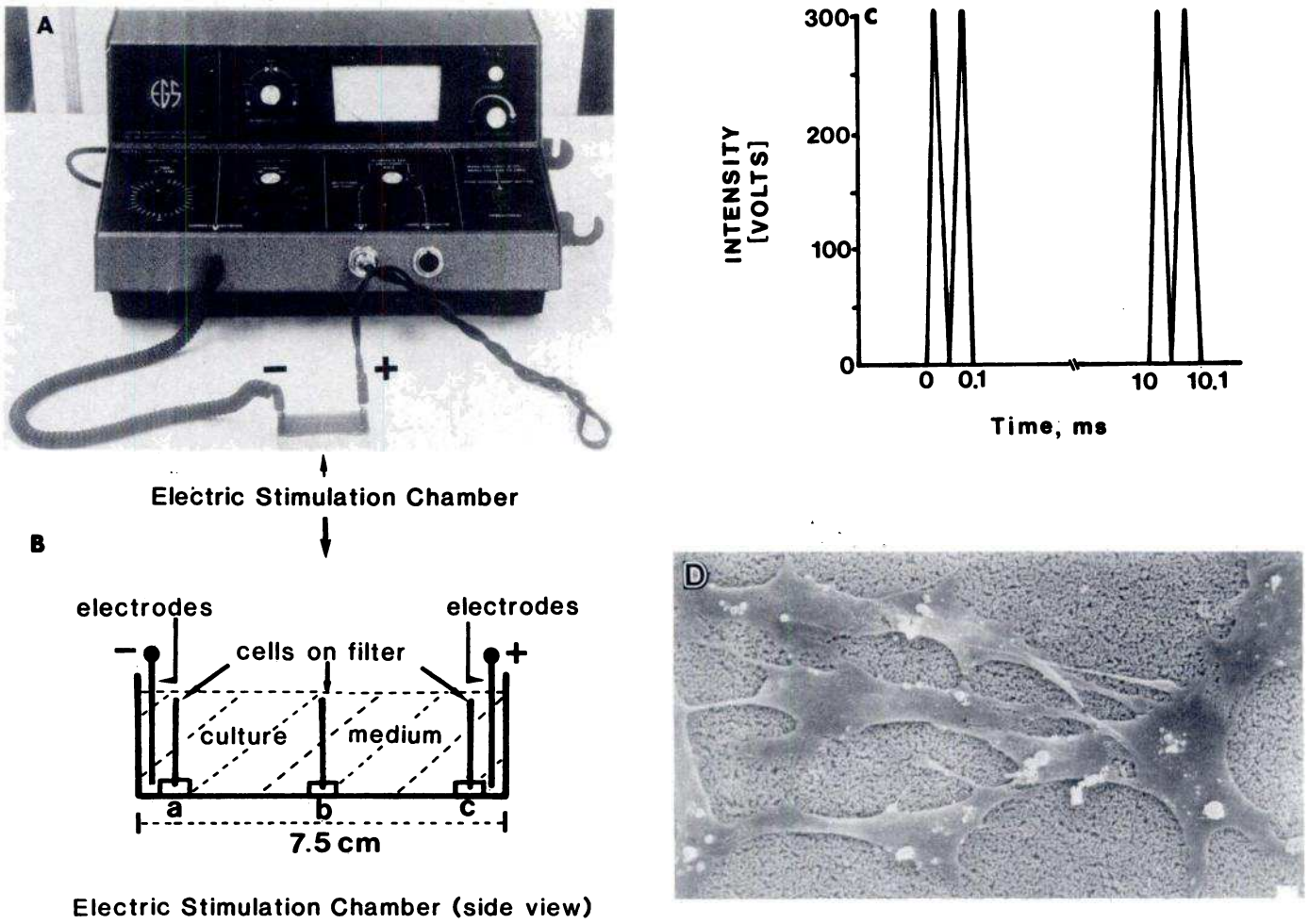

Figure 1. *A*) EGS 100-2 high-voltage galvanic stimulator connected to the cell chamber. *B*) Diagram of cell chamber. a, b, and c denote the positions at which filters were located in different experiments. *C*) HVPGS waveform—for 300 V and 100 pulses/s. *D*) Scanning electron micrograph of the IMR-90 human fibroblasts attached to a Millipore filter. × 4000.

serum for 48 h at 37°C. The cells rapidly attached to the filters and grew with the normal 24-h generation time. All cultures were in mid-log growth phase at about 50% confluency when exposed to HVPGS (see Fig. 1*D*).

The HVPGS

Electric stimulation was carried out in a plastic, rectangular chamber (7.5 × 2.5 × 1.5 cm) filled with 25 ml of culture medium. Flat, rectangular electrodes (2.2 × 1.5 cm) made of stainless steel were fixed at each end of the chamber. The filters with attached cells were placed vertically, either in the center of the chamber or approximately 5 mm from the positive or negative electrode (see Fig. 1*B*).

The HVPG stimulator used in this study was the EGS Model 100-2 (Electro-Med Health Industries, Miami, FL) (Fig. 1*A*). The HVPGS waveform consists of monophasic, twin-spike pulses that have a fixed pulse duration of 100 μs (see Fig. 1*C*). In this study voltages ranging from 0 to 300 V, with pulse rates of 60–120 pulses/s (pps), were applied across the chamber for 20 min at room temperature. Because the rectangular electrodes cover the entire area at each end of the chamber, the electric field and current are essentially homogeneous throughout the chamber. The time-averaged current that flows through the filled chamber at the maximum settings of 300 V and 120 pps was measured with a volt/ohm meter (Radio Shack, Tandy Co., Fort Worth, TX) and found to be approximately 50 μA.

Measurement of protein and DNA synthesis

After HVPGS treatment, the cells were incubated in complete growth medium at 37°C for 2 h before protein and DNA synthesis measurements were made.

The rate of protein synthesis was determined by measuring the amount of incorporation of the radioactively labeled amino acid [^{3}H]proline (1 C/ml) into protein that occurs in 20 min at 37°C. Radioactive labeling was terminated by placing the filters in ice-cold, 5% trichloroacetic acid (TCA). Filters were then washed three times with 10 ml of cold 5% TCA, dried, and counted by standard liquid scintillation counting procedures.

DNA synthesis was determined by incubating the cells in complete growth medium containing the radioactive nucleoside [^{3}H]thymidine (0.1 Ci/ml), at 37°C from 2 to 24 h poststimulation. Incorporation of nucleoside into DNA was terminated by placing the filters in ice-cold 5% TCA. Filters were then washed, dried, and counted in the same way as in the protein synthesis determination.

In all experiments, the amount of radioactivity incorporated in the HVPGS-treated samples was compared with that incorporated in identical, untreated control samples that were incubated in the filled chamber for 20 min without HVPGS. All results are expressed as a percentage of control, defined as (counts per minute in treated sample/counts per minute in identical control sample) × 100%, and are the average of three or four repetitions of each experiment. The relative SD for all the individual data points ranged from 1 to 8%.

Scanning electron microscopy

The fibroblasts attached to a Millipore filter were fixed in 2% glutaraldehyde for 1 h at room temperature, dehydrated through a graded ethanol series, critical-point dried, gold-coated, and examined by use of a JEOL-135 scanning electron microscope (JEOL, Tokyo, Japan) as described previously (22).

RESULTS

Effect of HVPGS on human fibroblast protein and DNA synthesis

Voltage and pulse rate

The rate of protein synthesis was determined at 2 h poststimulation whereas the accumulation of newly synthesized DNA was measured from 2 to 24 h poststimulation. Initially, the amount of synthesis (expressed as a percentage of that occurring in identical, untreated control samples) was determined as a function only of the applied voltage. The pulse rate was set at 100 pps and the cells were located in the center of the chamber. Under these conditions, both protein synthesis and DNA synthesis are increased at voltages 100 and 200 V with maxima of 125 and 150 V, respectively (Fig. 2). However, both types of synthesis are inhibited at 300 V. Preliminary experiments indicate that intensities greater than 300 V are increasingly inhibitory.

Subsequently, the pulse rate was also varied and the voltage maximum for protein synthesis was found to shift from about 125 V at 120 pps to 175 V at 60 pps (Fig. 3). A similar pattern was observed with DNA synthesis except that the voltage maxima were all about 25 V greater at each pulse rate tested (data not shown).

Location of cells relative to the electrodes

To determine whether the observed stimulation of protein and DNA synthesis may also depend on the location

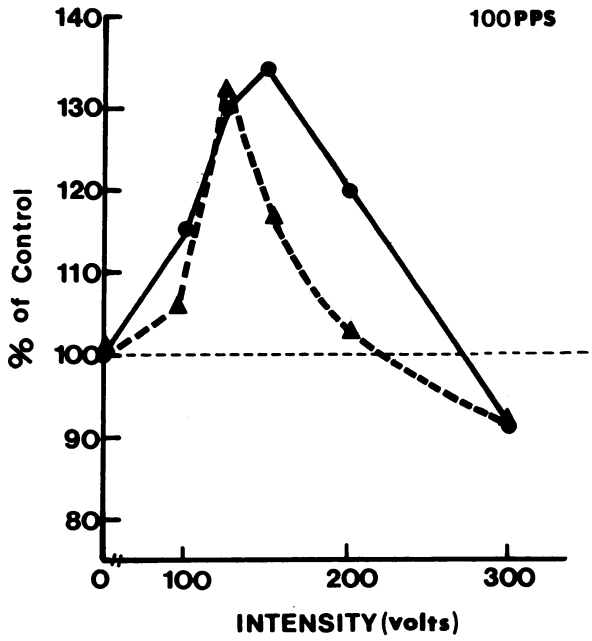

Figure 2. Effect of HVPGS voltage on fibroblast protein and DNA synthesis. Stimulation was performed at 100 pps with the cells positioned in the center of the chamber. (▲---▲) [^{3}H]Proline incorporation into proteins. (●—●) [^{3}H]Thymidine incorporation into DNA.

of the cells relative to the two electrodes, filters with attached cells were placed 0.5 cm from either the anode or cathode during HVPGS treatment (see Fig. 1*B*). As shown in Fig. 4, maximum protein synthesis (160% of the control) occurred with the cells close to the negative electrode and at an intensity of 50 V instead of 125 V. On the other hand, when the cells were located near the positive electrode, the maximum rate of protein synthesis was only 120% of untreated control samples and required an intensity of 150 V. Again, the pattern observed with DNA synthesis is similar to that with protein synthesis, except that the maxima are all increased about 25 V.

It is important to note that all of these results are independent of the filter orientation within the chamber. In other words, the same results are obtained whether the filters are placed with the cells facing toward or away from either the positive or negative electrode.

DISCUSSION

In this study we have begun to examine the effects of HVPGS on normal, diploid human fibroblasts in culture (Fig. 1*D*) as a first step in identifying the potentially important variables involved in HVPGS-mediated soft tissue healing. Our results indicate that HVPGS can induce fibroblasts to: *1*) significantly increase their rate of protein synthesis at 2 h poststimulation with a maximum increase occurring at 50 V, 100 pps, and the cells close to the negative electrode; *2*) significantly increase the amount of DNA synthesized between 2 and 24 h poststimulation with a maximum increase occurring at 75 V, 100 pps, and proximity to the negative electrode; and *3*) decrease both protein and DNA

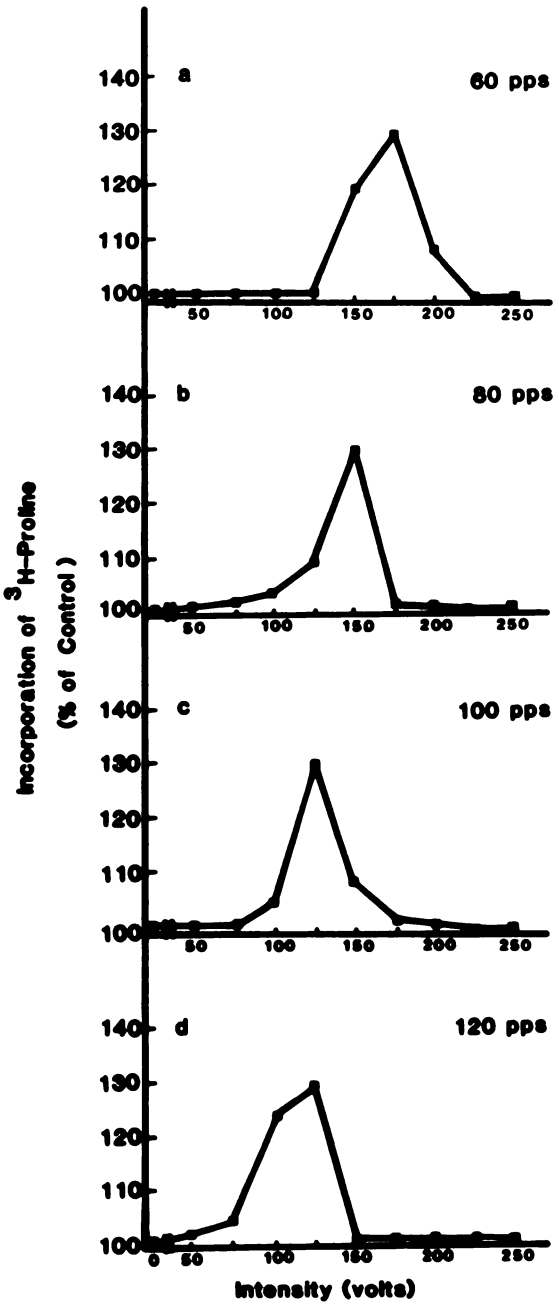

Figure 3. Effect of HVPGS voltage and pulse rate on fibroblast protein synthesis. Cells were positioned in the center of the chamber.

synthesis at intensities of 300 V and above. Results of preliminary assays for collagen production indicate that the fibroblasts exposed to HVPGS synthesize more collagen than untreated control cells. In addition, the increased amount of DNA synthesized in response to HVPGS suggests that the fibroblasts are also stimulated to proliferate. Clearly, both of these factors—increased collagen synthesis and increased cell proliferation—could explain why HVPGS is able to accelerate the rate of soft tissue healing.

The mechanism by which HVPGS (or any other type of electric stimulation) induces cellular activation is unknown at the present time. One obvious possibility is that an electric current could increase the temperature of the cells and, thereby, stimulate cellular metabolism. In this study we did not detect any change in the temperature of the medium ($\geq$ 0.1°C) during any HVPGS treatment. This is not surprising considering that, at most, there was only 50 μA of current flowing through the chamber. A second possibility is that the pH of the medium could be changed by the electric current. Again, in this study we did not detect a shift in the pH ($\geq$ 0.1 pH unit) of the medium during any HVPGS treatment.

A third possibility is that the HVPGS current could be releasing metal ions from the stainless steel electrodes that are affecting cellular biosynthesis. To test this possibility, complete growth medium alone (i.e., no cells) was first exposed to maximal HVPGS (125 V, 100 pps, for 20 min), and then the cells (attached to a filter) were placed in the conditioned medium for 20 min with no HVPGS. These cells were found to synthesize both protein and DNA at exactly the same rate as untreated, control cells. This result indicates that the observed increases in protein and DNA synthesis are probably not due to the release of a substance or substances from the electrodes, unless these substances are unstable and disappear rapidly after being generated.

Because HVPGS involves low amounts of current (i.e., in the microampere range), it has been suggested that HVPGS may trigger an electrophysiological effect in cells rather than cause an electrochemical reaction (23). Recent experiments in our laboratory support this idea. Using a fluorescent potentiometric dye, we have determined that HVPGS can also induce an immediate depolarization (approximately 20 mV) of the cell membrane. In this regard, one can make a simple calculation based on electrostatic theory concerning the effect of an external electrical field on the cell membrane potential. As outlined by Poo (24), this calculation indi-

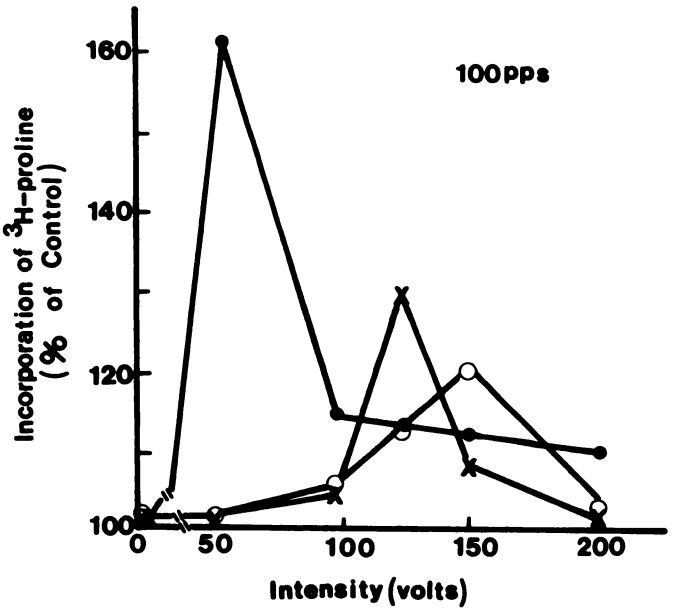

Figure 4. Effect of cell location in the chamber on fibroblast protein synthesis. (●—●) Cells positioned near the negative electrode. (×—×) Cells positioned in the center of the chamber. (○—○) Cells positioned near the positive electrode.

cates that the plasma membrane on the side of the cell facing the negative electrode will be depolarized whereas the membrane on the opposite side of the cell will be hyperpolarized by the same amount. For a 10-μm-diameter cell, the membrane potential will be decreased (on the cathode-facing side of the cell) and increased (on the anode-facing side of the cell) about 15 mV by a 10 V/cm external electric field—approximately the field strength applied to the chamber by the HVPG stimulator at 75 V. It is certainly possible that such a depolarization and/or hyperpolarization could trigger one or more important membrane-related events, e.g., gating of ion channels or activating membrane-bound enzymes. In fact, Tsong and Astumian (25) have recently reported that an oscillating electric field can stimulate membrane-bound ATPases with voltage and frequency dependencies similar to those reported in this study.

At the present time, we do not have a satisfactory explanation for the different voltage maxima and amount of stimulation that occurs when the cells are located near the negative vs. the positive electrode. It is noteworthy that bone healing has been found to be accelerated specifically by the negative electrode. We believe that further studies of the effects of electric stimulation not only will help to identify the important treatment parameters for optimal utilization clinically, but will also provide important new information regarding the mechanism or mechanisms of cell activation by external stimuli. FJ

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