Activation of signal-transduction mechanisms may underlie the therapeutic effects of an applied electric field

J. C. Seegers,[1] C. A. Engelbrecht,[2] D. H. van Papendorp[1]

[1]Departments of Physiology and [2]Physics, University of Pretoria, Pretoria, South Africa

Summary   Successful treatment of various medical complaints with an applied electric field has been reported over the years. The identities of the cellular mechanisms that are influenced by this type of treatment and facilitate the positive effects, remain elusive. A study of many in vitro and in vivo reports revealed that the beneficial effects can be attributed to the activation of membrane proteins, and specifically proteins involved in signal-transduction mechanisms. Not only may the proteins be affected but it is now well established that enhanced Ca^{2+} influx, observed to follow electric stimulation of cells, also contributes to many calcium-dependent cellular processes which can be linked to the therapeutic effects discussed in this paper. An hypothesis of the physical changes caused by an applied, relatively small (10^3 to 10^4 V m^{-1} range), electric field with low to moderate frequency (below 150 Hz), is postulated. © 2001 Harcourt Publishers Ltd

INTRODUCTION

There are numerous reports of positive clinical effects after exposure to applied electric fields of relatively small strength (in the 10^3 to 10^4 V m^{-1} range) which are variable with low to medium frequency (below 150 Hz). Effects reported include the alleviation of pain, decreased inflammation, wound and bone fracture healing, enhanced blood circulation as well as various other conditions (1–4). Most functions in the human body are tightly controlled, and activation of the processes underlying these functions must take place at the cellular level. The two most important communication systems in the body, which affect cellular functions, are the nervous system and the hormonal system. A crucial point in the discussion undertaken in this paper is that these two systems act mainly on the *membranes* of cells. Since so many macroscopic functions of the body have been reported to be positively affected by an applied electric field, we hypothesize that a remarkably wide range of processes involved in cellular membrane signal transduction mechanisms are decisively affected by an applied electric field of specific strength and form. Activation of these mechanisms may then cause the observed beneficial effects.

The effects of an applied electric field on cellular controlling mechanisms located in the cell membrane, reported in the literature, were investigated. Physical models to explain the efficacy of applied electric fields were also considered. Because it is difficult to study cellular control in the intact body, many experiments determining the effects of an applied electric field are performed in vitro. The extent to which the results obtained in vitro may be extrapolated to the human body in vivo is not yet totally clear; the physical arguments to explain the effects observed in vitro are equally compelling to the situation in vivo.

PART 1: EFFECTS OF ELECTRICAL STIMULATION ON PROTEIN FUNCTION IN THE CELL MEMBRANE

The cell membrane with its phospholipid backbone is normally impermeable to all compounds carrying a charge. Ions can move through their specific protein-membrane channels only when these channels are opened by an appropriate first messenger. Ion channels are voltage- or ligand-dependent. Furthermore, the conformation and function of membrane proteins can be affected by an applied electric field (5–7). Specifically, it has been shown that the charge distributions on macromolecules are affected when the electric environment is changed. As a result, most biological molecules have different accessible conformational states with different electric properties. It follows that an applied electric field may affect membrane proteins with various functions, including enzyme activity (Na^+/K^+ATPase and Ca^{2+}ATPases), ion channels, transport systems and receptors, by affecting the charge distribution (i.e. the conformation) on these molecules.

SPECIFIC MEMBRANE PROTEIN-LINKED FUNCTIONS AFFECTED BY AN ELECTRIC FIELD

The effects on specific proteins and the cellular functions connected with these proteins will be discussed.

Ion-channels

There is ample evidence that an applied electric field of appropriate strength opens Na^+-, Na^+/K^+ and Ca^{2+}-channels (10–13). After opening of the Na^+-channels, enhanced Na^+ inflow causes an action potential leading to depolarization of the cell. Opening of the Ca^{2+} channels causes an increase of Ca^{2+} influx which affects numerous cellular activities at the basis of the optimal functioning of the body, including cell shape changes, signal transduction mediation, muscle contraction, cytoskeletal reorganization (14–19), cell orientation and migration (14), immune-cell functions (18), cell proliferation (20,21), and metabolic processes (22).

Proteins involved in the activation of other membrane signalling mechanisms

Noradrenaline release

Application of an external electric field causes the release of the neurotransmitter noradrenaline (NA) (23,24). The released NA will further activate many other cellular processes through the activation of the membrane signal-transduction pathway leading to Ca^{2+} inflow and the formation of second messengers including cAMP (25).

Numerous metabolic processes are subsequently activated by cAMP-dependent protein kinases through the phosphorylation of specific proteins by using ATP (25). Inflow of Ca^{2+} into the cells, apart from the effects listed above, will also activate many cellular processes through the binding to calmodulin, which will then activate calmodulin-dependent kinases. Increased cellular Ca^{2+} in conjunction with diacylglycerate will furthermore activate protein kinase C. The function of all these kinases is to phosphorylate proteins using ATP. The phosphorylation of intracellular proteins causes conformation changes. The conformation changes cause the proteins to perform specific cellular functions in target cells. Therefore, an exogenous electric field, through its enhancement of Ca^{2+} influx, C-AMP production and NA release respectively, should have a far-reaching effect on the functions of cells and consequently on the optimal functioning of the body (13–25). Since no serious side effects have been reported after electrical treatment using fields in the range 10^3 to 10^4 V m^{-1} and of low to moderate frequency, it can be assumed that cellular functions are positively affected.

Growth hormone/factors production and cell growth

For cells to start multiplying as part of wound healing and tissue regeneration, the appropriate cells must first be activated by a specific growth factor/hormone, which acts on receptors on the cell membrane. This activation also leads to protein phosphorylation using ATP. The phospho-proteins activate the tyrosine-kinases linked to the control processes involved in cell cycle control and therefore in cell growth. Recently, it was shown that an applied electric field increases the production and activation of several growth factors (26–28). The positive effects of electric therapy on wound healing can in part be explained by these effects (see Part 2 of this paper).

The following growth factors are affected by an applied electric field:

Vascular endothelial growth factor (VEGF). Increased VEGF-mRNA production, translation and secretion after exposure to an electric field was recently reported by Kanno et al. (1999) (26). VEGF is necessary for angiogenesis and the authors suggested that electrical treatment may be beneficial for patients with serious ischemic diseases. They further proposed that this type of treatment, which is simple as well as practical and is without side effects, may prove to be of great therapeutic value (26).

IGF-II implicated in bone fracture healing. There are many reports described in which bone cells exposed to low frequency pulsed electric fields showed enhanced proliferation (13). In one report (27), enhanced bone cell growth was associated with increased IGF-11 (insulin growth factor-11) mRNA synthesis and subsequently increased secretion of this growth factor.

Prostaglandin E2 (PGE2) implicated in bone cell healing. Prostaglandin E2 (PGE2) is another growth factor, apart from Ca^{2+}, VEGF and IGF-II, which is implicated in electrically stimulated bone cell growth (4). Lorich et al. 1998 (4), found that electric stimulation increased Ca^{2+}-dependent phospholipase-A_2 activity, which caused an increase in PGE2 which then acted as mitogen in bone cell proliferation (4).

EGF receptor-accumulation at the cathode-facing pole after exposure to an electric field. The redistribution of the receptor of epithelial growth factor (EGF) has been reported by Guigni et al (1987) (28). This redistribution enhanced the growth effects of EGF.

It is well established that an applied electric field causes increases in cell growth, although the controlling mechanisms were not always identified. Apart from the effect on the growth factors shown above, which indicate how the control of cell growth is affected, increased DNA synthesis, mRNA synthesis, optimal cell orientation, and cell differentiation processes associated with cell growth have been reported after exposure to an applied electric field. Many observations of increases in protein and DNA synthesis (20,21,29,30) confirmed the positive effects of electrical therapy on cell proliferation.

Role of ATP in applied electrical stimulation

All the above listed signal-transduction mechanisms, which activate various cellular processes, do so through phosphorylation of target proteins. ATP is necessary for this activation process. Furthermore, all the ion pumps use ATP to remove the cations from the cells after influx through the electrically opened channels. None of the reportedly stimulated mechanisms could function without attendant stimulation of ATP-production. Indeed, several reports on the effects of a small applied electric field on ATP production were found (5,31–33).

The necessity of cytoplasmically generated ATP for cytosolic Ca^{2+} homeostasis after exposure to an applied electric field was also shown (33).

After Ca^{2+} ions move into the cell the calcium must be removed fairly quickly from the cytoplasm into the smooth endoplasmic reticulum, mitochondria, or pumped across the cell membrane into the interstitial fluid, in order to retain the calcium balance in the cells. In all three cases mentioned above, Ca^{2+} is removed from the cytoplasm by $Ca^{2+}ATPases$. These pumps utilize ATP for the removal of the calcium. Numerous reports of increases in ATP levels after exposure to an applied electric field have been published (31–33). As discussed above, ATP is utilized for maintaining Ca^{2+} levels and for the phosphorylation of proteins responsible for the final activation of cellular functions.

The metabolic processes responsible for increased ATP production during electrical stimulation remain unclear. The enhanced inflow of Ca^{2+} may be involved. It is known that an increased influx of Ca^{2+} activates the Crabtree (anaerobic ATP production) effect (34), which enhances glycolysis in the cytoplasm but inhibits ATP-synthase in the mitochondria. Therefore, the ATP produced in the cell under these circumstances would most likely be anaerobic. The decrease in ATP production in the mitochondria is a result of the increased mitochondrial Ca^{2+} (35). Since an applied electric field enhances ATP production, the observed increases in ATP production due to an applied electric field may be explained as a result of the electrically stimulated Ca^{2+} influx activating glycolysis and subsequently anaerobic ATP production. Wojtczak et al. (1999) (35) showed in detail that Ca^{2+} influx will inhibit the mitochondrial F(1)F(0) ATP synthase, and favour the Crabtree effect in the cytoplasm.

There is also evidence for enhanced glucose uptake after electric stimulation that may ensure an adequate glucose supply for the anaerobic production of ATP (36,37). Electrical stimulation enhanced the levels of Glut-4 (glucose transporters) in the membranes of muscle cells and subsequently increased the glucose uptake in these cells. The increased glycolysis that followed resulted in increased ATP production. The Glut-4 glucose transporters were more affected than Glut-1 in skeletal muscle exposed to an electric field (36,37).

ATP-release in cells exposed to an electric field

Electric field exposure not only stimulates cytoplasmic ATP production, it also stimulates the *release* of ATP from the stimulated cell. Extra-cellular ATP can function as an autocrine as well as a paracrine signal which may influence many cellular functions through the activation of purinergic receptors (38–40).

The release of ATP after exposure to an electric field indicates a further possible mechanism of enhanced signal transduction. Both ATP and NA were released after the tissue was electrically stimulated (41).

Conclusion of Part I

From the above data obtained in the literature it is clear that a relatively small applied electric field affects many membrane-linked controlling mechanisms, which may explain the observed therapeutic effects reported as a result of electric therapy. It furthermore also increases ATP levels which provide the necessary energy for the activated cell to perform the necessary cellular processes underlying optimal body functioning. The next part discusses the therapeutic effects linked to the protein functions discussed in Part 1.

PART 2: THERAPEUTIC EFFECTS OF A SMALL APPLIED ELECTRIC FIELD THAT CAN BE LINKED TO ENHANCED SIGNAL TRANSDUCTION

A short description of the following healing processes after treatment with a small applied electric field will be given.

Wound healing and tissue regeneration

Healing in an experimental wound model was ascribed to increased cell proliferation (29). Wound healing, however, entails many processes, including: tissue regeneration, new capillary formation, enhanced local blood flow, and inhibition of microbial growth. As described in Part 1, an applied small electric field enhances cell proliferation (27–30) and therefore tissue regeneration. Kanno and co-workers (1999) also showed that capillary formation may be initiated by an applied electric field (26). There is also evidence that electric treatment of this nature may enhance local micro-circulation by increasing the levels of the vasodilater nitrogen oxide (NO) (42–45). These reports show that an applied electric field enhances NO-synthase activity and by so doing enhances NO production which has a vasodilatory effect. This effect of NO may be implicated in improved micro-circulation. This latter effect may also contribute to the decrease in local edema seen after electric treatment. The activation of NO synthase is in part regulated by Ca^{2+} influx (42).

An important factor in activating all of the above, is amplitude and frequency modulation of cellular metabolic oscillations which contribute to intracellular mitotic signalling synergy and NO production (46). This issue is expanded in section 3. The positive effects of electrical stimulation were discussed above (26–29,42–44).

It has been suggested that the presence of an electric field enhances wound healing because of its bactericidal effect. An applied low strength electric field enhanced the killing action against *Pseudomonas aeruginosa* (47). There is more evidence of antimicrobial effects of a small applied electric field (48,49).

Antibacterial activity was also enhanced by H_2O_2 production at the anodal side in the presence of a low amperage (10–100 µA) direct electric current (DC). Bacteria killed were *Staphylococcus epidermis* and *Staphylococcus aureus* (48,49).

Further evidence of wound healing using electrical stimulation is the orientation of newly synthesized collagen (50). This process will take place even in the absence of neural influences (50).

Bone-fracture healing

Worldwide more than a quarter of a million patients with non-union fractures have benefited from the non-invasive effects of electric treatment (13).

Although the mechanisms, by which this therapeutic effect on bone-fracture healing is manifested, have not yet been fully elucidated, many studies on the effects of an electric field on basic bone cell processes are increasing our understanding of the healing effects. The inflow of Ca^{2+} into cells after exposure to a small electric field activates many processes specifically involved in the healing process of bone cells specifically (see Part 1).

Evidence of specific effects which may help to clarify the bone-fracture healing process is the calcium-related production and release of growth factors in bone cells; including, PGE_2, IGF-II TGFβ-1, discussed previously (4,17,21,27). Another important paper on bone fracture healing described that osteochondral repair was stimulated in a fresh fracture healing experiment (51). Enhanced blood flow was also seen in this experiment (51).

Pain alleviation

Pain has been treated successfully with applied electric fields for many years. However, the cellular mechanisms responsible for the decrease in pain perception remain, in part, unresolved. Enhanced release of dynorphins and enkephalins (especially increases of β-endorphins) in experimental animals and in humans, exposed to low (2 Hz) and moderate (100 Hz) frequencies has been implicated in raising of the pain threshold (1). It was shown by Ulett et al. (1998) that electrical stimulation alone was as effective in pain relief as electroacupuncture. Although the role of substance P in the alleviation of pain after electric treatment remains unclear, recent research indicated that the internalization of substance P receptors may also contribute to the hyperalgesia experienced after electric treatment (52).

Odendaal and Joubert showed that patients with chronic back ache, due to osteoporosis, were successfully treated with electric therapy (53). The effects of low frequency electric stimulation on pain and mobility in osteoarthritis patients were also studied (54). In this study, patients responded positively to the treatment. DH Van Papendorp et al. (2000) (55) showed a fourfold increase in β-endorphin levels in patients with chronic pain compared to healthy volunteers after electric therapy.

Anti-inflammatory effects

Electrical therapy is used across the world for the relief of pain and inflammation. Although widely used, the physiological action mechanism explaining the analgesic effects is not perfectly clear. The mechanism of mid-range frequency (100 Hz) treatment is usually explained by the

gate control theory of pain, while low frequency (4 Hz) treatment is usually explained as due to the release of endorphins. Recently Sluka et al. (1999)(1), showed that the mid-range frequency (100 Hz) and low frequency (4 Hz) application produced the analgesic effects through different opioid receptors in arthritic rats. Both types of electric treatment were 100% effective.

Enhanced local circulation

Apart from the possible contributing effects of electrical therapy on blood flow specifically in wound healing, the effect on local circulation in general has also been reported. Again enhanced NO production was implicated (42,56).

Scott and McCormack (1999) (57) also showed that electrical stimulated vasodilation in guinea-pig pulmonary arteries was mediated by NO. Enhanced peripheral micro-circulation reported by many (42,56–58) after electrical stimulation may also contribute to improvement in inflammatory conditions in the treated area. From the short discussion above it is clear that an applied small electric field reduces pain and inflammation. Electric therapy may also reduce local swelling because of enhanced local circulation. All these factors may contribute to promote pain relief and enhance joint mobility.

Electric stimulation reduces pain in arthritic patients

In a multicenter, double-blind, randomized, placebo-controlled study, it was found that pulsed electrical stimulation was an effective treatment of osteoarthritis of the knee. The device used was a portable, battery operated device that delivers a mid-range frequency (100 Hz), low amplitude, voltage sourced, monophasic, spiked signal to the knee via skin electrodes (3).

A PHYSICAL MODEL FOR UNDERSTANDING THE VARIETY OF HEALING EFFECTS ASCRIBED TO THE APPLICATION OF AN EXTERNAL APPLIED ELECTRIC FIELD

The most attractive proposal to explain the effects discussed in the prior parts of this paper is due to Tsong and co-workers (5,31). This model suggests resonant coupling between an oscillating electric field and changes in the conformational state of membrane proteins involved in signal transduction processes. In physical terms, each type of membrane protein is an oscillating system that may be driven by an applied electric field of the appropriate strength and frequency. A crucial element of this model is the dramatic amplification of the applied electric field in the cell membrane of an intact cell. This effect allows the application of a benign electric field on the macroscopic level, avoiding the dielectric breakdown of

H_2O and chemical burning from taking place. Another important aspect of this model is that a continuously oscillating electric pulse is needed to effectively drive the described signals to the cell. Furthermore, the complexity of the chemical environment in the cell prescribes that the most efficient driving frequency will show some variation along the dynamic changes occurring in the cellular environment around the cell membrane.

Tsong (5) indicates experimentally how different transmembrane processes respond most effectively to oscillating electric fields of particular frequencies specific to each particular cellular process.

Both a pulsed DC field and a sinusoidal AC field have the required oscillatory character. However, an argument may be made that the energy contained in a pulsed DC field with short pulse width is more effectively concentrated than the energy distribution in an AC field. Direct comparison of the two types of field is suggested to establish whether this is indeed the case.

In conclusion: the judicious application of the driven oscillator model to the wealth of cellular transmembrane effects discussed in this paper promises a rich harvest of benefits to human health.

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