Cellular mechanisms of direct-current electric field effects: galvanotaxis and metastatic disease

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Summary

Endogenous direct-current electric fields (dcEFs) occur in vivo in the form of epithelial transcellular potentials or neuronal field potentials, and a variety of cells respond to dcEFs in vitro by directional movement. This is termed galvanotaxis. The passive influx of Ca^{2+} on the anodal side should increase the local intracellular Ca^{2+} concentration, whereas passive efflux and/or intracellular redistribution decrease the local intracellular Ca^{2+} concentration on the cathodal side. These changes could give rise to 'push-pull' effects, causing net movement of cells towards the cathode. However, such effects would be complicated in cells that possess voltage-gated Ca^{2+} channels and/or intracellular Ca^{2+} stores. Moreover, voltage-gated Na^{+} channels, protein kinases, growth factors, surface charge and electrophoresis of proteins have been found to be involved in galvanotaxis. Galvanotactic mechanisms might operate in both the short term (seconds to minutes) and the long term (minutes to hours), and recent work has shown that they might be involved in metastatic disease. The galvanotactic responses of strongly metastatic prostate and breast cancer cells are much more prominent, and the cells move in the opposite direction compared with corresponding weakly metastatic cells. This could have important implications for the metastatic process and has clinical implications. Galvanotaxis could thus play a significant role in both cellular physiology and pathophysiology.

Key words: Galvanotaxis, Ca^{2+}, Direct-current electric fields, Metastatic disease, Na^{+} channel

Introduction

The effects of exogenous and endogenous electric fields (EFs) on physiology and their possible relatioship to disease states have interested researchers for years. Most organs (especially glands) and embryos surrounded by a layer of epithelial cells produce potential differences or transepithelial potentials (TEPs) of a few millivolts to tens of millivolts. These correspond to transcellular direct-current EFs (dcEFs) of 50-500 mV/mm, as measured in vivo or in vitro in small airways of sheep lungs (Al-Bazzaz and Gailey, 2001), guinea pig trachea (Dortch-Carnes et al., 1999), mouse rectum (Wang, Q. et al., 2000) and rat prostate (Szatkowski et al., 2000). Endogenous EFs might also exist in the central nervous system owing to the presence of extracellular field potentials across the blood-brain barrier (Sorensen et al., 1978; Nicholson, 1980), including specific transendothelial potentials (Revest et al., 1993; Revest et al., 1994).

Endogeneous dcEFs play a significant role in major biological processes such as embryogenesis, wound healing and tissue regeneration (reviewed by Nuccitelli, 1988). TEP values vary depending on the physiological condition or the pathophysiological state of the tissue. For example, in cystic fibrosis, which is associated primarily with impaired Cl^{-} transport across epithelial membranes, the TEP of the nasal airway epithelium is hyperpolarized (–51 mV in cystic fibrosis patients, compared with –15 mV in normal nasal airway epithelium) (Hofmann et al., 1997).

A major cellular effect of dcEFs is galvanotaxis, which is directional movement towards the cathode or the anode. Application of in vitro dcEF strengths comparable with those detected in vivo produces galvanotaxis in a variety of cultured cells. In most cases, cells move towards the cathode; for example, bovine corneal epithelial cells (Zhao et al., 1996), bovine aortic vascular endothelial cells (Li and Kolega, 2002), human retinal pigment epithelial cells (Sulik et al., 1992), human keratinocytes (Sheridan et al., 1996), amphibian neural crest cells (Cooper and Keller, 1984), C3H/10T1/2 mouse embryo fibroblasts (Onuma and Hui, 1985), fish epidermal cells (Cooper and Shliwa, 1986) and metastatic rat prostate cancer cells (Djamgoz et al., 2001). However, some cell types move towards the anode; for example, human granulocytes (Rapp et al., 1988), rabbit corneal endothelial cells (Chang et al., 1996), human vascular endothelial cells (HUVECs) (Zhao et al., 2004) and metastatic human breast cancer cells (Fraser et al., 2002). Although human dermal melanocytes were recently reported to be insensitive to an external dcEF of 100 mV/mm (Grahn et al., 2003), this might have been due to these cells having a higher threshold (Onuma and Hui, 1988). We should also note that species and/or cell subtype differences might affect galvanotaxis. For example, HUVECs move towards the anode (Zhao et al., 2004), whereas bovine aortic vascular endothelial cells show a cathodal response (Li and Kolega, 2002).

In this Commentary, we discuss the subcellular mechanisms by which dcEFs could change the intracellular milieu, in particular the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, and thus induce galvanotaxis. We then examine the possible role of galvanotaxis in cancer metastasis.

Cellular mechanisms of galvanotaxis

There are five main steps in cell migration (reviewed by Friedl and Wolf, 2003): (1) elongation of cortical actin filaments that connect to adaptor proteins and 'push' the membrane in the direction of movement; (2) formation of adhesive contacts through integrins, which interact with several signalling proteins and structural components of adhesions; (3) 'focalized proteolysis', involving cleavage of extracellular matrix (ECM) components by proteases; (4) contraction driven by binding of myosin II to actin filaments; and (5) disassembly of focal contacts and detachment of the rear margin of the cell. Galvanotaxis should involve the same cytoskeletal rearrangements, although the mechanisms controlling the different aspects of cell movement (e.g. speed versus directionality) might vary (Zhao et al., 2002a; Trollinger et al., 2002). One link between the galvanotactic stimulus (the dcEF) and these fundamental mechanisms of cell migration is probably Ca^{2+}.

The importance of Ca^{2+}

In most cells studied, galvanotaxis is thought to depend on changes in $[Ca^{2+}]_i$. This is consistent with the initial response to dcEFs being fast (see below) and hence is likely to involve a 'small' molecule. Measurements of $[Ca^{2+}]_i$ in mouse embryo fibroblasts, using the Ca^{2+}-sensitive photoprotein aequorin, show that application of a dcEF produces a significant overall $[Ca^{2+}]_i$ increase (up to mM), which is maintained throughout exposure to the field (Onuma and Hui, 1988). Ca^{2+} channel blockers, such as Co^{2+} or D600, or removal of extracellular Ca^{2+}, can block the dcEF-induced rise in $[Ca^{2+}]_i$ (Onuma and Hui, 1988) and, in most cases, this inhibits galvanotaxis without influencing the basic ability of the cell to move (Nuccitelli et al., 1993). dcEF stimulation also more than doubles $[Ca^{2+}]_i$ in a rat osteoblast-like cell line (Wang et al., 1998). Moreover, note that Ca^{2+} influx induced by various factors, including membrane depolarization, has a role in cellular contraction (see below) in hepatic stellate cells (Bataller et al., 2001), pulmonary artery smooth muscle (Zhang et al., 1997) and skeletal muscle cells (Mickleson and Louis, 1996). The commonly seen cathodal galvanotaxis of cells can be explained if one assumes that, as a consequence of the dcEF, a rise in $[Ca^{2+}]_i$ in a given part of the cell causes contraction of that side whereas the opposite occurs at the other side. This should bring about a 'push-pull' movement (Cooper and Keller, 1984). Such Ca^{2+}-dependent cellular contraction/protrusion could involve at least two different mechanisms (Horwitz and Parsons, 1999) (Table 1), the most obvious being actin polymerization/depolymerization and actomyosin contractility.

Note that in some cell types, such as mouse NIH 3T3 fibroblasts (Brown and Loew, 1994) and certain frog spinal neurites, the response to dcEFs is not always sensitive to extracellular Ca^{2+} (Palmer et al., 2000). Interestingly, however, the galvanotactic response of another mouse fibroblast cell line, C3H/10T1/2, does require extracellular Ca^{2+} (Onuma and Hui, 1988). The reason(s) for this difference is not clear at present but probably involves differences in the experimental conditions. In particular, the different concentrations of fetal calf serum used might have affected the expression of ion channels (Ding and Djamgoz, 2004) and/or the different surfaces could have altered the electrotactic response (Sheridan et al., 1996). Nevertheless, Ca^{2+} sensitivity does suggest a potential galvanotactic mechanism in the majority of cells where it is evident.

Table 1. Effects of dcEFs on intracellular Ca^{2+} levels and cytoskeletal components on the cathodal and anodal sides of a typical motile cell

	Cathodal side (leading edge)	Anodal side (rear margin)
$[Ca^{2+}]_i$	Decreased	Increased
Actin	Depolymerization/ polymerization (increased)	Polymerization/ depolymerization (increased)
Actomyosin	Relaxation	Contraction
Adhesion	Increased	Decreased

Actin polymerization/depolymerization

Elongation of actin filaments, usually at the leading edge of the cell (Chan et al., 1998), is the main driving force for cell movement. The mechanism involves generation of free barbed ends of cortical actin filaments by gelsolin- and/or cofilin-induced severing. A reduction in $[Ca^{2+}]_i$ releases gelsolin from these barbed ends (Condeelis, 2001), which could thus promote polymerization, thereby causing protrusion of that part of the cell (Onuma and Hui, 1988). Although the mechanisms responsible for actin dynamics at the rear of migrating cells are still not well understood, $[Ca^{2+}]_i$ might be increased, resulting in depolymerization of actin (Wehrle-Haller and Imhof, 2003; Small et al., 1998). Externally applied dcEFs transiently increase the total amount of filamentous actin in the cell. The lamellipodia projecting towards the cathode become selectively enriched in filamentous actin in these cells (Li and Kolega, 2002; Zhao et al., 2002b), which indicates that actin polymerization is indeed an important aspect of galvanotaxis.

Actomyosin contractility

Myosin II is the most common molecular motor of muscle and non-muscle cells and is regulated by $[Ca^{2+}]_i$ (Somlyo and Somlyo, 2000). Myosin light chain kinase (MLCK), found in differentiated smooth muscle and non-muscle cells, is Ca^{2+}/calmodulin-dependent and phosphorylates the regulatory light chain of myosin II. This phosphorylation stimulates the actin-activated myosin ATPase and is thought to play a major role in cell contraction (Stull et al., 1998; Goeckeler and Wysolmerski, 1995). Myosin disassembly follows a transient increase in $[Ca^{2+}]_i$ (Rees et al., 1989), and Ca^{2+} triggers contraction of non-muscle bile canaliculi in freshly isolated monolayer cultures of rat hepatocytes (Watanabe and Phillips, 1984). Although it is not clear whether actin polymerization/depolymerization and actomyosin-based mechanisms have the same $[Ca^{2+}]_i$ requirement (i.e. respond to the same quantitative changes in $[Ca^{2+}]_i$), both should displace the cell in the same direction (towards the cathode) in a dcEF, along the axis of a high to low $[Ca^{2+}]_i$ gradient. In such a model, there could be some redistribution of intracellular Ca^{2+} as 'Ca^{2+} waves' (Perret et al., 1999) (Fig. 1B).

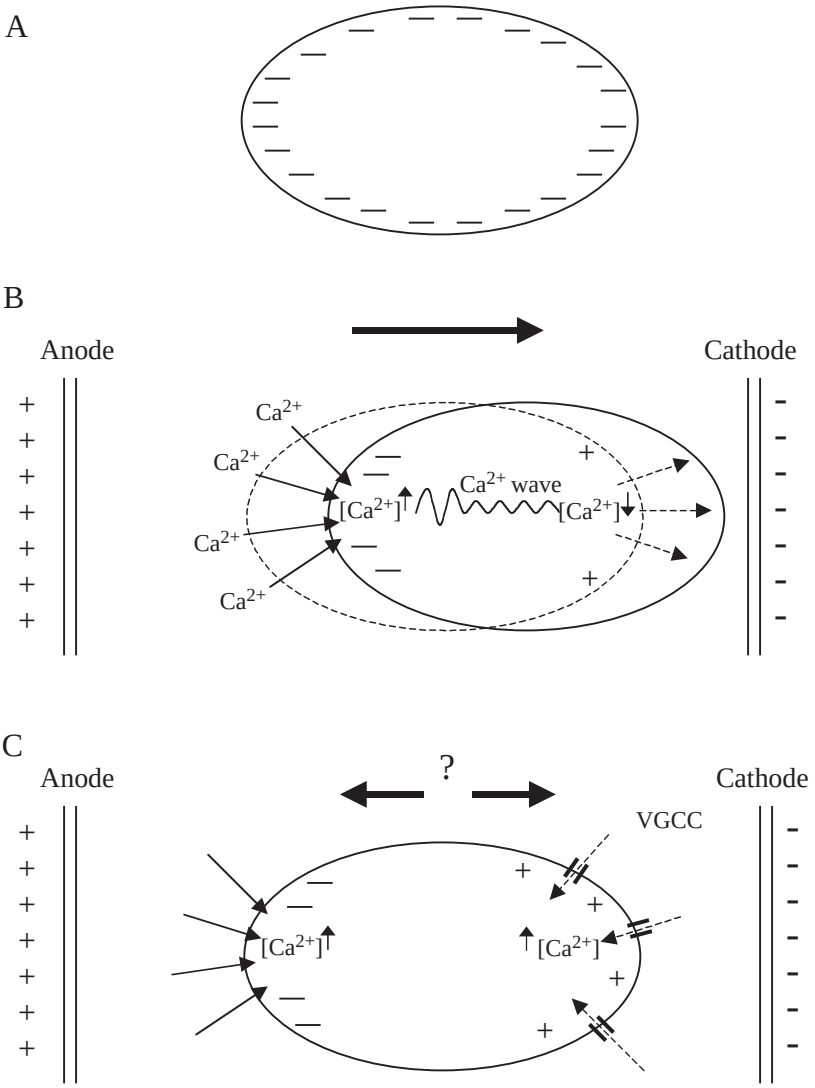

Fig. 1. Responses of cells with different membrane properties to dcEFs. (A) A simple cell in the resting state has a negative membrane potential. (B) A cell with negligible voltage-gated conductance exposed to a dcEF. The membrane towards the anode is hyperpolarized and attracts Ca^{2+} by passive electrochemical diffusion. Consequently, this side of the cell contracts, thereby propelling the cell towards the cathode. (C) A cell with voltage-gated Ca^{2+} channels (VGCCs). Channels near the cathode (depolarized) side open, thereby allowing Ca^{2+} influx. In such a cell, intracellular Ca^{2+} levels will rise both on the anodal side (as in B) and on the cathodal side, owing to depolarization-induced activation of the VGCCs. The direction of cell movement, if any, then depends on the balance between the opposing contractile forces.

Other effects

The change in $[Ca^{2+}]_i$ should also affect cell adhesion (Table 1). To move, the cell must attach where it is protruding and detach where it retracts (Libotte et al., 2001; Gerisch et al., 1999). Changes in $[Ca^{2+}]_i$ could cause these effects (Hendey and Maxfield, 1993). Indeed, an increase in $[Ca^{2+}]_i$ is responsible for rear-margin detachment during the movement of keratocytes (Lee et al., 1999). There should thus be a tendency for the cell to detach and attach where $[Ca^{2+}]_i$ rises and falls, respectively. Such changes would be consistent with the net direction of movement. Ca^{2+} influx could also have several secondary effects, such as activation of Ca^{2+}-dependent K^+ channels (Schwindt and Crill, 1995) and Ca^{2+}/calmodulin-dependent kinases (CaMKs), which could also affect migration (Kobayashi et al., 1999).

dcEF-mediated direct regulation of $[Ca^{2+}]_i$

Passive influx

It has been estimated that for dcEFs of 10-100 mV/mm applied to a cell 10 μm across, the cathodal side depolarizes by ~5 mV whereas the membrane facing the anode hyperpolarizes by the same amount (Patel and Poo, 1982; Poo, 1981). This hyperpolarization could increase the electromotive force driving cations passively into the cell (Cooper and Keller, 1984). Since the transmembrane electrochemical gradient for Ca^{2+} is ~10,000:1, there could be a significant passive influx of Ca^{2+}. In cells with few or no voltage-gated ion channels, application of a dcEF would cause an influx of Ca^{2+} into the anode-facing part of the cell and consequent movement towards the cathode through the mechanism described above (Fig. 1A,B). The effects of intracellular Ca^{2+} have been studied in human prostate cancer LNCaP cells, which do not possess voltage-gated Ca^{2+} channels (VGCCs) (Skryma et al., 1997; Laniado et al., 2001). Measurement of $[Ca^{2+}]_i$ by high-speed confocal imaging of fluo-3 has shown that an externally applied dcEF hyperpolarizes the anode-facing membrane, depolarizes the membrane facing the cathode and induces an intracellular Ca^{2+} wave that starts from the hyperpolarized end of the cell (Perret et al., 1999). Similarly, in fish keratocytes, Ca^{2+} entry starts at the hyperpolarized side of the cells (Brust-Mascher and Webb, 1998). In both cases, the Ca^{2+} waves measured are ‘fast’ (22.5 μm/s and 50 μm/s respectively).

Influx through voltage-gated Ca^{2+} channels

If VGCCs are present, membrane depolarization (on the cathodal side) should open them and allow Ca^{2+} influx, which would then tend to cause the cell to move towards the anode – in the opposite direction to the effect of passive Ca^{2+} influx alone (Fig. 1C). The net movement, if any, would then depend upon the balance of the two opposing forces. Application of ‘large’ voltage pulses across keratocytes causes Ca^{2+} influx through VGCCs (Brust-Mascher and Webb, 1998). Several other excitable and non-excitable cell types respond similarly, including myeloma cells, osteoclasts, astrocytes and fibroblasts (Rink and Merritt, 1990; Cho et al., 1999). In several cases, Ca^{2+} channel blockers reduce galvanotaxis (e.g. Brust-Mascher and Webb, 1998; Trollinger et al., 2002). Experiments on frog myoblasts confirmed that the orientation of these cells in

response to dcEFs depends on the presence of Ca^{2+} in the extracellular medium and that application of the general VGCC blocker Co^{2+} suppresses the responses completely (McCaig and Dover, 1989). In human keratinocytes, galvanotaxis can be blocked by Ni^{2+} and Sr^{2+}, which also inhibit VGCCs (Trollinger et al., 2002). The effect of another Ca^{2+} channel blocker, verapamil, is not consistent (Brust-Mascher and Webb, 1998; Trollinger et al., 2002); however, this might be owing to its complex action involving other ion channels (Fraser et al., 2000). Interestingly, Sr^{2+} specifically blocks the directed migration of the cells yet the speed of motility remains the same. This suggests that these parameters are controlled by separate mechanisms (Trollinger et al., 2002).

Internal Ca^{2+} stores

dcEFs might stimulate the release of Ca^{2+} from intracellular stores. As already noted, they can induce propagated intracellular Ca^{2+} waves (Perret et al., 1999), which normally require interplay between Ca^{2+} influx and Ca^{2+} released from internal stores (Bootman et al., 2001; Himpens et al., 1999). Indeed, in LNCaP cells and fish keratocytes, thapsigargin, which depletes internal Ca^{2+} stores, blocks the response to dcEFs (Perrett et al., 1999; Brust-Mascher and Webb, 1998). Inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] can also release Ca^{2+} from intracellular stores and produce oscillatory changes in $[Ca^{2+}]_i$, including waves (Berridge, 1993; Dawson, 1997). Neomycin, an antagonist of phosphoinositide signalling, inhibits the response of chondrocytes to dcEFs (Chao et al., 2000). Similar experiments on embryonic muscle cells showed that galvanotaxis is not restored even in the presence of a 16-fold increase in the level of extracellular Ca^{2+} (McCaig and Dover, 1991).

Mechanosensitive channels

Additional secondary rises in $[Ca^{2+}]_i$ could occur by mechanosensitive (e.g. stretch-activated) cation channels (Lee et al., 1999). Indeed, Gd^{3+}, which is a general blocker of mechanosensitive channels, suppressed galvanotaxis of human keratinocytes (Trollinger et al., 2002).

The role of voltage-gated Na^+ channels

The dcEF-induced change in membrane potential might also affect other voltage-gated conductances, which in turn could influence the cytoskeleton with or without the further involvement of intracellular Ca^{2+}. In response to an externally applied dcEF (≤300 mV/mm), rat prostate cancer Mat-LyLu cells show a strong cathodal galvanotactic response (Djamgoz et al., 2001). The highly specific voltage-gated Na^+ channel (VGSC) blocker tetrodotoxin (TTX) significantly reduces this response, whereas veratridine, which prolongs VGSC opening, has the opposite effect.

The highly metastatic human breast cancer MDA-MB-231 cell line is also galvanotactic, but the cells migrate anodally (Fraser et al., 2002). Application of TTX greatly suppresses the directionality of this response of the MDA-MB-231, which is consistent with the involvement of VGSCs (Fraser et al., 2002).

How VGSCs control galvanotaxis is not well understood, but several possibilities exist (Fig. 2). First, Na^+ influx through VGSCs could increase $[Ca^{2+}]_i$ locally by inhibiting Ca^{2+} exchange across the plasmalemma (Blaustein and Lederer, 1999) and/or alter the release and uptake of Ca^{2+} from intracellular stores through disruption of normal pH-regulating mechanisms (Ishibashi et al., 1999). Second, the Na^+ influx could activate protein kinase A (PKA) to phosphorylate cytoskeletal components (Liu et al., 2001; Senter et al., 1995). Third, VGSCs could interact directly with the cytoskeleton (Komada and Soriano, 2002) or calmodulin (Herzog et al., 2003). In particular, the β subunit is necessary for cytoskeletal linkage and could also function as a cell adhesion molecule mediating interaction with the ECM, cell migration and aggregation (Malhotra et al., 2000; Isom, 2002).

Thus, a variety of mechanisms might link VGSCs and/or intracellular Na^+ with galvanotaxis (Fig. 2). Interestingly, directional migration and patterned growth of neurons in vivo is controlled by VGSC activity (Dubin et al., 1986; Catalano and Shatz, 1998; Meyer, 1982; Penn et al., 1998; Shatz, 1990). However, it is not known whether these phenomena involve endogenous dcEFs.

The influence of surface charge

Many cells possess a surface charge owing to the presence of charged residues in intrinsic plasma membrane proteins and/or ‘free’ ions. This charge ‘cloud’ could shift in response to an exogenous dcEF, generating a spatial variation in membrane potential (Heberle et al., 1994; Scherrer, 1995). In turn, this could affect VGCCs, VGSCs or other voltage-dependent ionic activies. Experiments on embryonic muscle cells showed that concanavalin A receptors, which normally accumulate on the cathodal side of cells under the influence of an externally applied dcEF, can be induced to migrate to the opposite side if they are preincubated with neuraminidase (an enzyme that digests sialic acid moieties, which are the most abundant charged molecules on mammalian cell surfaces) (McLaughlin and Poo, 1981). Interestingly, surface charge can change with the pathophysiological state of the cell, and this could affect galvanotaxis. Indeed, increased negative surface charge is known to be associated with malignant cancer cells (Abercrombie and Ambrose, 1962; Carter and Coffey, 1988; Carter et al., 1989; Price et al., 1987), and a surface charge effect could underlie the fast, cathodal galvanotaxis of rat prostate cancer cells (Djamgoz et al., 2001).

Other mechanisms: growth factors and protein kinases

Several other mechanisms have also been implicated in the control of galvanotaxis. These include those involving protein kinases such as protein kinase C (Nuccitelli et al., 1993), cGMP-dependent protein kinase and PKA, MLCK (Pullar et al., 2001), CaMKs (Zhuang et al., 1997) and mitogen-activated protein kinase (MAPK) (Zhao et al., 2002a; Zhao et al., 2002b; McBain et al., 2003). Growth factors and their receptors are also implicated, especially epidermal growth factor (EGF), fibroblast growth factor, transforming growth factor (TGF)-β1/α (Zhao et al., 1996; Zhao et al., 2002a), hepatocyte growth factor (McBain et al., 2003) and vascular endothelial growth factor (VEGF) (Zhao et al., 2004). The limited work on growth factor involvement in galvanotaxis suggests that speed and

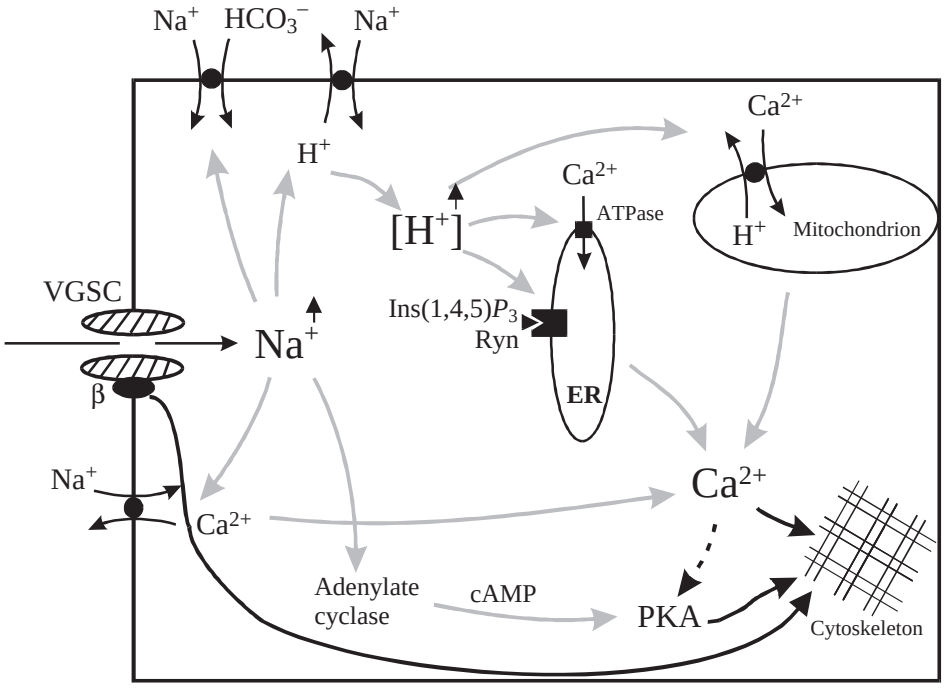

Fig. 2. Various mechanisms that might underlie involvement of VGSCs in galvanotaxis. Contraction could be driven by Ca^{2+} originating from a variety of sources secondary to Na^+ influx through VGSCs. Alternatively, it could be stimulated by protein kinase A (PKA) activated by cAMP, synthesized by adenylate cyclase, stimulated by Na^+. Direct interaction between the β subunits of VGSCs and the cytoskeleton is also possible.

directionality are controlled by different mechanisms and also potentially by different MAPK subtypes (Fang et al., 1999; Zhao et al., 2002a; Wang et al., 2003; Tani et al., 2000). Moreover, the effects are cell-type specific, and growth factors appear to have differential effects on motility characteristics (e.g. directionality versus speed) within a given cell (Fang et al., 1999; Wang et al., 2003). However, much work remains to be done on the exact modes of action of growth factors before these tentative conclusions can be generalized.

Short-term and long-term responses to dcEFs

The initial reactions of cells to externally applied dcEFs can be very fast. *Amoeba proteus*, for example, responds to reversal of a dcEF of 600 mV/mm within ~1 second by contracting the anode-facing part of the cell; the cathode-facing part continues to move in the original direction for tens of seconds (Korohoda et al., 2000). The strongly metastatic rat prostate cancer Mat-LyLu cells respond within ~30 seconds of reversing a dcEF of 300 mV/mm (Djamgoz et al., 2001). The reaction time of amphibian neural crest cells to a dcEF (500 mV/mm), resulting in a withdrawal of all protrusions, is also seconds (Cooper and Keller, 1984). Thus, these initial responses can be very fast and are consistent with the involvement of passive Ca^{2+} fluxes, voltage-gated ion channels and/or surface charge effects. However, long-term responses are evident in some cells.

Rat prostate cancer cell lines (Djamgoz et al., 2001), human keratinocytes (Pullar et al., 2001), amphibian neural crest cells (Cooper and Keller, 1984) and human corneal epithelial cells (Zhao et al., 1997) show no sign of habituation. However, in the case of *A. proteus*, cells subjected to a dcEF for 5-10 minutes react to field reversal 10 times more quickly than they did initially, which can be viewed as sensitization (Korohoda et al., 2000). Analysis of the responses of Mat-LyLu cells indicate that, although an initial galvanotactic reaction to an applied dcEF occurs within 30 seconds, steady state is not achieved for 30 minutes (Siwy et al., 2003). Such long-term responses could involve translocation by electrophoresis of proteins involved in galvanotaxis (Jaffe, 1977; Brown and Loew, 1994), or changes in enzyme activity and gene expression (Fang et al., 1999). Interestingly, fast and slow components of the responses to dcEFs might interact in the same cell. For example, the dcEF-induced asymmetrical distribution of epidermal growth factor receptors (EGFRs; or other growth factor receptors) in the cell membrane, and associated EGF signalling, could further affect galvanotactic reactions by modulating VGSC expression and/or activity (e.g. Toledo-Aral et al., 1995) (Y. Ding, Cellular studies of ionic activity in prostate cancer metastasis and pain signalling, PhD Thesis, University of London, 2002).

Metastatic disease

Metastasis involves tissue invasion by cancer cells translocating across extracellular barriers both at primary and secondary sites (Woodhouse et al., 1997). The mechanisms of cancer cell motility are probably similar to those in normal cells, but the regulation of motility might be very different (Levine et al., 1995). Several studies have shown that cell transformation also modifies responses to dcEFs. For example, under identical experimental conditions, primary human lens epithelial cells migrate towards the cathode, whereas their transformed counterparts migrate towards the anode (Wang, E. et al., 2000). Similarly, the highly metastatic human breast cancer cell line MDA-MB-231 shows an anodal galvanotactic response, whereas weakly (or non-) metastatic MCF-7 cells move cathodally (Fraser et al., 2002). Human epidermal keratinocytes continue to migrate towards the cathode when they become undifferentiated but their migration becomes faster (Obedencio et al., 1999).

We compared the effects of external dcEFs on two prostate cancer cell lines of markedly different metastatic potential: Mat-LyLu (strongly metastatic) and AT-2 (weakly metastatic) cells (Isaacs et al., 1986). Mat-LyLu cells move towards the cathode, as noted earlier, whereas AT-2 cells migrate in the opposite direction (Djamgoz et al., 2001). Galvanotaxis of the Mat-LyLu cells depends on the activity of VGSCs, which are expressed specifically in highly metastatic cells (Grimes et al., 1995). By contrast, VGSC activity plays no role in the

galvanotactic reaction of AT-2 cells, which probably involves voltage-gated K^+ channel (VGPC) activity since it is suppressed by verapamil (Djamgoz et al., 2001).

Cancer cell galvanotaxis could be important in vivo. The rat prostate has a TEP of ~10 mV, which corresponds to a gradient of ~500 mV/mm (Szatkowski et al., 2000). One can therefore imagine the following scenario. Early in metastasis, when the epithelial ducts are intact, epithelial cells expressing functional VGSCs (i.e. with the potential to metastize) tend to migrate into the lumen and be detectable in semen or urine (Couture et al., 1980; Barren et al., 1998; Bockmann et al., 2001). However, as metastasis progressed, the ducts would deform, the TEP would disappear and galvanotaxis would slow down and might even reverse, which would encourage invasion of the surrounding tissue (Djamgoz et al., 2001). Furthermore, circulating metastatic cells would be subject to the endothelial potential (Revest et al., 1993) and this could similarly influence intra/extravasation, a potentially crucial step (Wyckoff et al., 2000). Note that TEP changes could be further influenced by alterations in the tight junctional coupling of epithelial cells (Tobioka et al., 2002; Kominsky et al., 2003) and, therefore, might be more complex.

Other cells might also undergo galvanotaxis in this context. Endothelial cells themselves might be galvanotactic since they possess functional ion channels, including VGSCs (Chang et al., 1996), and are associated with endogenous dcEFs in the form of transendothelial potentials and/or tissue field potentials (Revest et al., 1994). Indeed, two studies have shown that bovine and human endothelial cells are galvanotactic (Li and Kolega, 2002; Zhao et al., 2004). An intriguing question is whether endothelial galvanotaxis is involved in angiogenesis, a process of fundamental physiological and pathophysiological importance (Papetti and Herman, 2002). Interestingly, application of dcEF to HUVECs stimulates VEGF production (Zhao et al., 2004), which is known to induce angiogenesis in vivo (Kanno et al., 1999).

Another important cell type in cancer is tumour-infiltrating lymphocytes, which can be a significant prognostic determinant (Nzula et al., 2003; Marincola et al., 2003). Galvanotaxis might be involved in intra/extravasation of lymphocytes since these possess a variety of ion channels, including VGSCs (Cahalan et al., 2001), and there is an endothelial potential difference in areas that infiltrate (e.g. ~25 mV in bullfrog cornea) (Graves et al., 1975).

Possible clinical applications

The galvanotactic and electro(patho)physiological characteristics could be useful for diagnostics. The electrical characteristics of tissues probably change in malignancy (Jossinet, 1998; Toso et al., 2000). As described above, as metastasis progresses and the cells start to migrate out of the epithelium, TEPs probably gradually decay and eventually disappear completely. Electrodiagnosis could form the basis of early detection because epithelial transformation would occur before the metastatic cells entered circulation. Davies et al. have used impedance and TEP analyses to examine differences in the electrical properties of the distal colon in cancer-susceptible (CF1) and cancer-resistant (DBA) mouse strains. After application of the carcinogen dimethylhydrazine (DMH), there was a significant (≈35%) increase in the electrical conductance of the surface colonic epithelium in CF1 mice compared with DBA mice, which showed about a fivefold decrease (Davies et al., 1989a). The value of TEP changed significantly in animals treated with DMH: –2.3 mV in the control group and –1.5 mV after DHM treatment (Davies et al., 1989b).

There are also therapeutic implications. Electrotherapy for adenocarcinoma could work in two ways. First, since malignant cells are strongly galvanotactic, one could ‘draw them out’ of the diseased gland by applying small dc voltages across the epithelium. In the case of the prostate gland, this would be possible by insertion of an ‘active’ electrode into the urethra, which would be similar to the surgical procedure used in trans-urethral resection of the prostate (TURP), and a reference electrode nearby. Electrotherapy might also be applicable to breast cancer, exploiting the methodology already developed for breast lavaging (e.g. Gray et al., 2000). Second, assuming that endogeneous TEPs facilitate galvanotaxis in vivo, it might be possible to suppress cell migration by reducing TEPs by using inhibitors of the ion pumps and exchangers that generate them.

Conclusions and future perspectives

Many cells in the body can potentially respond to an exogeneous dcEF, at least by means of passive Ca^{2+} influx. Moreover, endogenous dcEFs are present, at least in the form of TEPs, and are likely to be involved in physiological and pathophysiological processes. However, in most cases, the precise mechanisms by which cells detect and respond to endogenous or exogenous dcEFs are still not known. The way a cell responds to an externally applied dcEF probably reflects the equilibrium between several processes operating in the short- and long-term. It is also possible that different cells use different ionic mechanisms to influence the cytoskeleton and move within dcEFs. Further work is required to elucidate the subcellular and molecular bases of the responses to dcEFs in different cells, as well as to relate the observed effects of exogenous dcEFs and in vitro studies to situations that exist in vivo.

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