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Ion Gradients in Tissue and Organ Biology

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Abstract

This Review article focuses on a general property of cells: the membrane potential and the ion gradients that arise from the segregation of charges by molecular machines like pumps, transporters and ion channels mostly situated in the plasma membrane of the cell. Such patterns are able to form tissues (e.g. in the early embryo and organs, e.g. the neural tube and the lens). Furthermore, these patterns arise in pathophysiological states like wound healing (forming e.g. a transepithelial potential) and regeneration of amputated limbs. Furthermore, not only small ions like sodium or potassium can be involved in this field patterning but also larger biomolecules like tissue factors, growth hormones, transmitters and signaling molecules like serotonin and others are driven by these gradients. By this these gradients of charged molecules coupled into classical signaling pathways hitherto described in cell biology. An upcoming field regarding these endogenous ionic gradients and resulting electric fields is also influence on stem cells and tumour formation.

Keywords: Cell organelles; Electromagnetic fields; Plasma membrane

Introduction

The subject of this review is the intrinsic property of all cells and tissues to generate electric potentials of low magnitude and ion gradients, which are not spikes of electric activities but smooth membrane potentials that can change over a longer period and even form ultra low frequency electromagnetic fields (UL-EMF) below 1 Hz, or of some few Hz. It should be clearly noted: we are not talking about typical action potentials of nerve or muscle cells but of small and steady gradients which are often at the beginning of a cascade of events which then is linked to the classical signaling pathways known in cell and molecular biology.

In the last decade many elaborated studies could show that these phenomena are intrinsic to biological systems and the electric field gradients are not only created by small ions but are also affected and can be driven by larger biomolecules and pathways such as tissue factors, growth hormones, transmitters, and signaling. In former times this kind of "bioelectricity" - a term used from the times of Galvani on - was disregarded in history not only because of many curious medical devices and mis- and over interpretations of measured fields but also because of the lack of appropriate methods to observe these phenomena and to link these to modern cell and molecular biology [1-3].

However, since about ten years new methods like membrane potential– and ion– sensitive *in vivo* dyes and other constructs for imaging and molecular tracing are available, which allow a direct observation of the mentioned processes in living organism and even in cells and in cell organelles [4]. Thus, this review should not also compile recent publications in this field but also give some ideas and hints for further studies.

General Overview

Membrane potentials and electrical – and ion gradients arise from the segregation of charges by molecular machines like pumps, transporters and ion channels that are mostly situated in the plasma membrane [5]. Such ion gradients generate electric fields and direct currents as well as ultra low electromagnetic fields [1], which are able to form patterns within cell membranes e.g. in the early embryo [6], within cell arrays e.g. in the developing lens [7] and within tissues e.g. during neural tube formation [3]. Furthermore, not only small ions like protons, sodium or potassium can be involved in this field patterning but also larger biomolecules (nearly all possess electrical charges) like tissue factors, growth hormones, transmitters and signaling molecules like serotonin and others [6]. Also, cell processes and extensions (from microvilli to tunneling nanotubes) [8] should be involved in the spreading of these gradients also gap junctions [4] as well as extracellular material like collagen fibers or the extracellular material of tissue [9,10].

What has not been studied until recently [1,11] is: embryonic development [12], tissue and organ formation [13], regeneration and wound healing [3]; as well as influence on cell migration [14-16], cell differentiation and proliferation [6].

In the last years scientists also become aware of these endogenous ionic gradients and the resulting electric fields especially for tumor formation [17]. Another emerging field are the "channelopathies", e.g. by mutations in genes encoding ion channel proteins [5] and the influence of the mentioned gradients and fields on stem cells [4]. The "electric" interior of the cell is also the border of our present knowledge because only first attempts were made to analyze intracellular fields of organelles like mitochondria using "nano – pepple" sensors [18,19]. The influences of such fields and gradients on biomolecules, their charge characteristics in the electric field and in the intracellular surrounding (including nuclear envelope and the influence on epigenetic changes like histone methylation or acetylation) are just being discovered.

Early Embryonic Development

During early development of amphibian and chicken embryos, endogenous ionic currents can be measured. The currents and related fields are actively generated by passive Na+ uptake from the environment that leads to a transepithelial potential difference (TEP). Differences in TEP between various regions form intraembryonic voltage gradients. The magnitude of the arising endogenous static electric fields (EF) is

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in the order of 1-5 V/cm and therefore well above the minimum level needed to affect morphology and migration of embryonic cells in vitro [2,20,21]. It has also been stressed by McCaig et al. (2005) [3] that "it is important to put the dimensions of the electric fields into the right context": a depolarization of a neuron by surface electrodes requires a field of 10-20 V/cm. Nerve cell action potentials are confined to cell membranes and are propagated along the cell membrane. In contrast to short-lived action potentials, small endogenous EF last very long and build up gradients that persist from UL-EMF to minutes and weeks.

Transepithelial potential differences are also the source of current loops detected an axolotl embryo (using non- invasive vibrating electrodes). Outward currents are found at the lateral edges of the neural ridges and at the blastopore, whereas inward currents are found at the center of the neural groove and at the lateral skin [13]. A strong electrical gradient has also been detected across the wall of the neural tube [3]. Additionally, voltage drop across the neural tube wall, division and differentiation of neurons in the presumptive CNS lumen, and axis of cell division can be influenced by applied and endogenous EF [22,23]. Ion flows are also involved in differentiation control. Recent findings have implicated the calcineurin pathway, which upregulates myogenin and MEF2 activity, as linking K+ channel (Kir2.1)-mediated hyperpolarization with differentiation in human myoblasts [24]. Therefore, vertebrate embryos possess steady voltage gradients particularly in areas where major developmental events related to cell movement and cell division occur. Disrupting these electrical fields disrupts normal development [14].

Patterning

From the fertilized egg and very early developmental stages on cell membrane potentials and ion gradients like pH (proton) gradients build a kind of pre-formative pattern, which later on is imprinted into genes via signaling pathways and then is morphologically seen in distinctive folding, proliferation and migration patterns of different cell groups. Disrupting this cascade of information (in –formation also in a literally sense) by blocking of e.g. cell membrane bound proton transporters will ultimately lead to malformations, dislocation of organs and other severe defects. In this sense one of the major patterning events is left – right patterning because it directs the position of organs like heart, liver and further organs of the digestive tract asymmetrically to the left – right axis. Also other body axes (anterior – posterior and ventral – dorsal) have to be pre formed in a similar cascade of events. On the other hand, the regulation of body axis specification is explained in classical textbooks e.g. by a Cartesian coordinate system of signaling proteins like Wnt and BMP [25].

With respect to left – right patterning different models exist till now [6]: unidirectional motion of cilia is suggested to set up an asymmetric fluid flow. This triggers a cascade of asymmetric gene expression by the movement of morphogens or an activation of Ca++ signaling in sensory cilia. Ultimately this should lead to asymmetric gene expression and biased organ placement [26].

Some authors set the starting point of left right patterning in the polarization of a cytoskeletal-organizing element like the centriole and then an executive action by cytoskeletal elements [27-29]. However, the centriole must get its position information from the periphery of the cell and/or its neighborhood. Furthermore, numerous species have left - right asymmetry without cilia or a node as well as zebrafish, pig or chick. Also mouse mutants without cilia exhibit left right patterning [30]. These authors could show that chirality in tubulin associated proteins affects very early steps of left–right patterning in nematode and frog embryos as well as in human cells in culture.

Another model begins at earlier stages with a patterning of cell membrane components, possibly by more subtle fields arising e.g. also in the fertilized egg ending in an asymmetric distribution of ion channels and pumps. This can result in asymmetric ion gradients [31,32], which subsequently drive the establishment of physiological gradients of molecules (e.g. serotonin) [33,34]. After the first embryonic cell divisions the cells on the right side are more negative due to the polarized distribution of ion gradients. A network of open gap junctions can then distribute left - right signaling molecules to the right- and ventral-most blastomere [11].

Carneiro et al. [35] found that these signaling molecules ultimately control the expression of asymmetric genes by a histone deacetylase (HDAC)-dependent intracellular receptor. Thus, HDAC activity is a new LR determinant controlling the epigenetic state of defined genes in early developmental stages. The HDAC binding partner Mad3 may then be the new serotonin-dependent regulator of asymmetry linking early physiological asymmetries to stable changes in gene expression during organogenesis [35].

Electro-physiological parameters have also been found for craniofacial patterning in Xenopus embryos [36] found a complex pattern of voltage gradients, driven by the regionally different activities of the V – ATPase proton pump at the primitive oral opening and the neural tube. Interestingly, a perturbation of the voltage domains and pH gradients results in expression changes of genes driving this orofacial patterning and ultimately in malformations.

The activities of EMF observed in embryos may also apply to patterning and differentiation of single organs. For example in the vertebrate lens, basolateral membranes of anterior epithelial cells produce a DC EF by Na+/K+ pumps [7]. Using published values for equatorial and polar lens resistivity (0.5 and 500 k Ohm/cm) [3] has calculated that lens currents give rise to steady DC EF of between 0.02 and 6 V/cm, a normal physiological range. Ion current flow draws associated water through the avascular lens, and this may flush out metabolites [37]. The main current efflux is concentrated at the lens equator where important aspects of lens physiology, such as growth of new cells, take place. During adult life, lens epithelial cells move towards the equator, probably by active migration, proliferate and transdifferentiate into lens fiber cells [38].

The studies reported above shed light on how gradient formation in single cells can lead to large-scale morphogenetic gradients. In this sense, Adams and Levin (2012) [11] mentioned that (after a classic paper on Alan Turing, 1952) [39] a spatially periodic, temporally stable pattern, which arises from the instability of a homogenous steady state, represents a mechanism for embryonic development. This means also that this information must be transduced to the genetic machinery by a kind of "bootstrapping" process where the subtle fields are linked to transmitter molecules like serotonin, which are involved in molecular gradients and later to direct switches of common cellular signaling pathways. Until now it was not shown consistently how such cues are transferred into the genome but the examples above show how this can happen.

Migration

Since the beginning of electrophysiological experiments in cells, the phenomenon of cell migration was in focus of the studies. Migration of cells is constitutive for tissue and organ formation and also important

for regeneration and wound healing. Thus, in many experimental approaches an electrical field has been applied externally to observe the cell behavior and the cell migration.

It has been reported that in vitro many cell types often migrate to the cathode at externally applied field strengths of 0.1-10 V/cm like neural crest cells, fibroblasts, keratinocytes, chondrocytes, rat prostate cancer cells and many epithelial cell types [40-44]. In contrast, fewer cell types move to the anode like corneal endothelial cells, bovine lens epithelium, human granulocytes and human vascular endothelial cells. Both speed and movement direction in this case is voltage dependent. As described in a previous review [45], current data suggest that species and cell subtype differences affect electrotaxis. For example, human vascular endothelial cells migrate towards the anode, whereas bovine aortic endothelial cells move towards the cathode. In SAOS – an osteosarcoma cell line, rat calvaria osteoblasts and fibroblasts we found that during movement, ruffled membranes, lamellipodia and filopodia are formed preferentially in the direction of the anticipated electrotaxis migration and the cells oriented and elongated perpendicular to the electric field lines [46,47]. Several cell types were even reported to change their initial movement direction when current polarity was reversed [48-51].

Interestingly, Sun et al. (2004) [52] noticed directed fibroblast migration at field strengths as low as 0.1 V/cm in three-dimensional collagen gels, but not in conventional two-dimensional cultures. Thus, three-dimensional conditions have the potential to reflect in vivo situations in which DC EF of 0.1-0.2 V/cm are known to be present during many events, including embryonic development. Also genes are activated in electrotaxis phenomena [14]: during wound healing (see below) also phosphatase and tensin homolog (PTEN) enzymes are directly involved. This again shows that there must be a kind of bootstrapping mechanism together with signaling pathways.

Regarding sensing of such fields, our group [16] found using electrically polarized cell chambers for migration (electrotaxis) assays that the function of Na, K-ATPase and a Na+/H+ exchanger isoform (NHE3) can act also as directional sensors. The information is transferred via a mechanism that involves PIP2 as a potential mediator and the cell membrane potential may act as a regulatory cue. This maintains the persistent direction in electrotaxis. In similar experimental set ups, we could now get first hints that there must be a feedback loop from sensing in the periphery (cell membrane) to a polarization of the centriole via still unknown signaling pathways. NH3 and proton (pH) gradients are involved in this sensing and subsequently distinctive signaling pathways are triggered (manuscript submitted).

Regarding internally arising gradients and cell migration one example will be given: in the axolotl embryo neural crest cells of the trunk migrate out laterally as single cells between somites and epidermis, and medially between somites and the neural tube. The lateral neural crest cells give rise to pigment cells and the medial ones form neurons and glia of the peripheral nervous system [53]. It is very possible that these migrating cells are also guided by ion gradients creating relative far-reaching electric fields. Until recently most studies of migrating neural crest cells have considered only the intrinsic properties of the extracellular matrix and the guidance by signaling molecules [53].

Wound Healing

Cell membrane surface charges can also change with pathophysiological states. Electrotaxis is also found in the process of wound healing: here, enhanced DC-EF are present. In epithelial layers a TEP is always generated and immediately upon wounding this is enhanced with the cathode at the wound center. It is possible that this DC EF is the earliest signal that an epithelial cell receives to initiate directional migration into the dermal wound bed [54,55]. Even transient breaches in an epithelium, also during natural turnover, induce short-lived, local electrical signals that influence cell regulation [3]. During disruption of an epithelium, the potential difference becomes short-circuited, either across the whole epithelial sheet, or across a single cell membrane [3]. A wound-induced electrical signal comes very early and lasts for many hours [3] and regulates different cell behaviors within 500 μm to 1 mm from the wound edge. After complete covering by the epithelium the signal fades.

Interestingly in corneal epithelial wound healing, the electric field lines control even the orientation of the mitotic spindle in the proliferating epithelial cells as well as the orientation of the re-growing nerve sprouts [23,56]. Also in cultured hippocampal neural and glial cells the cleavage planes were oriented perpendicular to the DC-EF field lines [57]. Regarding the time course of corneal epithelial wound healing in vivo, Kucerova et al. [58] could show that the electric fields after wounding trigger only the initial signals like planar polarization of the cells and later on other factors (like growth factors etc.) may take over.

In cultured bovine corneal endothelial monolayers linear narrow wounds heal by actin cable formation at the wound border. Chifflet et al. [59] found here that membrane depolarization, not the increase in intracellular Na+ concentration, is responsible for the formation of the actin cable whereby a depolarization of the plasma membrane potential of the cells occurs determined by a rise in the epithelial Na channel activity. This may constitute an additional factor in the intermediate cellular processes leading to wound healing in some epithelia.

Simple reasoning about physiological phenomena leads to the conclusion that an electrical short-circuiting must be the fasted signal together with the signal of autonomic sensory nerves and diffusion of signaling molecules and hormonal actions normally are much slower. These processes represent a very interesting topic for future studies.

All these examples together show that the mentioned small DC electric fields are ideally suited to bridge the information gap for short time periods and for the spatial dimensions between the short-range action of molecules e.g. by local hormones, growth factors etc. and the far reaching influences from the organism e.g. hormones distributed via blood stream, nerves. On the other hand, the cell membrane is a Faraday cage for the cell interior because of the relative high membrane potential - the voltage across the membrane is 0.05 V-0.1 V; with membrane thickness of only 10 nm a potential of 10^7 V/m arises. This prevents the interior of the cell from being triggered directly and shelters the cell interior from irrelevant or stochastic EMF- also from environmental "noise" [1].

Regeneration

Regarding regeneration, the present models (e.g. limb regeneration in salamanders and newts [60-62] encompass so many facets that it is at the moment very difficult to find out all molecular aspects of ion transporter location. It is known from a paper by Adams et al. (2007) [63] that H+ pump (V-ATPases)-dependent changes in membrane voltage are an early mechanism, which is necessary and sufficient, to induce tail (spinal cord, muscle and vasculature) regeneration in Xenopus. After amputation, the normal regeneration bud depolarizes, but after 24 h it repolarizes due to V-ATPase activity. The mentioned paper [63] reveals active upregulation of a pump mechanism

specifically during regeneration, in contrast to passive injury currents that result from breaks in ubiquitously polarized epithelia during limb regeneration [64]. Here, the cell-surface V-ATPase is upregulated at the mRNA and protein levels within 6 h of amputation and V-ATPase-dependent expression of downstream gene expression indicates that this is an extremely early step in the regeneration process. More recently, Ozkucur et al. (2010) [65] could show that ion contents in the axolotl tail blastema change dynamically during regeneration and, in most cases, are still fluctuating at 48 h *post amputationem*. After 6 h the membrane potential was depolarized by five-fold in the bud region blastema compared with other regions and to the uncut tail. Especially, the epidermal as well as mesenchymal cells were involved in early events (6 h) both having elevated membrane potential compared with others such as melanophores.

Thus, several ion channels and pumps have roles as information cues for different cell biological processes unrelated to their ion transport [2]. Tseng et al. (2010) [66] could tackle these regeneration events more precise in the amputated Xenopus tail model: 6 h after amputation a V-ATPase dependent proton extrusion occurs, followed by an increased Na+ flux in the regeneration bud and after 24 hours activation of downstream pathways (BMP, Notch, Msx, Wnt and Fgfs). After 7 days the regeneration is completed. Artificial modulation of wound physiology by addressing the right ion transporters may therefore be a promising direction for augmenting and inducing regeneration in otherwise non-regenerating tissues.

Stem Cells

Stem cells and ion gradients and electric fields were mentioned in the chapters above and indeed, this field is rapidly emerging [67] showed that an artificial membrane hyperpolarization induces differentiation of mesenchymal stem cells. Also in embryonic stem the cell membrane potential can trigger differentiation. Human embryonic stem cells and human induced pluripotent stem cells showed a completely different electrotaxis in DC-EF: the embryonic stem cells migrated to the cathode whereas the latter migrated to the anode [68]. Regarding electrotaxis, Zhao et al. (1999) [22] could demonstrate that EF is a potent guidance cue in bone marrow mesenchymal stem cells and that the migration is reduced with higher passage numbers in these cells. The physiological importance of the stem cell migration behavior is largely unknown, however it is presumably involved in functionally useful system like during skin wound healing where it has been shown that the stem cells migrate to the site of lesion.

In neural tissues Arias-Carrion could show that transcranial magnetic field stimulation enhanced neurogenesis by the subventricular zone cells in nigrostriatal lesions. More recently, UL–EMF of low intensity could promote neurogenesis in the adult hippocampus in mice – possibly by triggering stem cells [69]. In rats, Sherafat et al. [70] were able to show increased proliferation and migration of neural stem cells and enhanced repair of myelin repair after demyelination. A DC electric field could elicit a clear cathodal migration in neural stem cells and Babona-Pilipos demonstrated that adult subependymal neural precursors but not differentiated cells undergo a rapid cathodal migration in a externally applied DC electric field. Moreover, electric fields could elicit enhanced differentiation and perpendicular neural process growth in hippocampal neural progenitor cells [71,72].

Tumorigenesis and Cancer

Our group could observe an opposite migration behavior e.g. in SAOS tumor osteoblast cells compared to primary cultures of osteoblasts. This migration behavior is caused by a different localization of NH_3 transporters and proton gradients on opposite sides of the cells – a phenomenon possibly being important for metastasis of tumors (manuscript submitted). Also e.g. a misexpression of a potassium channel with its regulatory accessory subunit (KCNQ1/KCNE1) can switch an embryonic cell type to a highly invasive cell type – a fact that was demonstrated in embryonic stem cells of the neural crest pigment cell lineage of the Xenopus embryo by Morokuma et al. [73].

This links the influence of ion gradients and electric fields to tumorigenesis and cancer in general. Interestingly, Tseng and Levin [17] could bridge the gap between ionic field gradients and epigenetics in Xenopus tadpole regeneration suggesting a novel role of sodium butyrate transporters: they may link influx of small molecules with modification of the chromatin state. Furthermore genome-wide methylation changes precede tumor development at very early stages. In this regard hypomethylation can result in expression of otherwise repressed tumor suppressor genes and hypermethylation acts vice versa [74,75].

Coming from the side of the ionic and electric fields, the Levin group demonstrated in the Xenopus embryo that exposure to a carcinogen can induce a change in the transmembrane potential of the whole body, a hyperpigmentation and an induction of localized tumors Lobikin et al. (2012) Furthermore, tumors induced by oncogenes show very high sodium content, a fact that can be used for diagnostics [76].

Regarding electrotaxis, Djamgoz et al. [77] found that the highly metastatic MAT-LyLu cells (prostate cancer cells) responded to DC-EF by migrating towards the cathode whereas the weakly metastatic AT cells did not respond to the field – interestingly, the electrotaxis phenomenon could be also controlled by Na+ channels.

Outlook

Many aspects in the context of ionic gradients and endogenous and exogenous bioelectric fields have only been scratched in this review. The studies cited have demonstrated that this topic is very attractive because it is intrinsic to nearly all known biological processes. Thus, this emerging scientific field is also widely open for many more investigations especially those, which link the mentioned phenomena to modern molecular biology. Here, many processes should be studied: e.g. in embryology the migration, in regeneration and tumorigenesis it is the linking to epigenetic and genetic phenomena. Another topic is the study of physical and molecular mechanisms that couple ion gradients and electric fields to specific molecules (e.g. charged receptors) and then link to classical intracellular signaling pathways. Finally also the methodological strategies should be described how to tackle these phenomena with advanced techniques of molecular and cell biology [11,4].

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