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invited review

Cellular target of weak magnetic fields: ionic conduction along actin filaments of microvilli

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Gartzke, Joachim, and Klaus Lange. Cellular target of weak magnetic fields: ionic conduction along actin filaments of microvilli. *Am J Physiol Cell Physiol* 283: C1333–C1346, 2002; 10.1152/ajpcell.00167.2002.—The interaction of weak electromagnetic fields (EMF) with living cells is a most important but still unresolved biophysical problem. For this interaction, thermal and other types of noise appear to cause severe restrictions in the action of weak signals on relevant components of the cell. A recently presented general concept of regulation of ion and substrate pathways through microvilli provides a possible theoretical basis for the comprehension of physiological effects of even extremely low magnetic fields. The actin-based core of microfilaments in microvilli is proposed to represent a cellular interaction site for magnetic fields. Both the central role of F-actin in Ca^{2+} signaling and its polyelectrolyte nature eliciting specific ion conduction properties render the microvillar actin filament bundle an ideal interaction site for magnetic and electric fields. Ion channels at the tip of microvilli are connected with the cytoplasm by a bundle of microfilaments forming a diffusion barrier system. Because of its polyelectrolyte nature, the microfilament core of microvilli allows Ca^{2+} entry into the cytoplasm via nonlinear cable-like cation conduction through arrays of condensed ion clouds. The interaction of ion clouds with periodically applied EMFs and field-induced cation pumping through the cascade of potential barriers on the F-actin polyelectrolyte follows well-known physical principles of ion-magnetic field (MF) interaction and signal discrimination as described by the stochastic resonance and Brownian motor hypotheses. The proposed interaction mechanism is in accord with our present knowledge about Ca^{2+} signaling as the biological main target of MFs and the postulated extreme sensitivity for coherent excitation by very low field energies within specific amplitude and frequency windows. Microvillar F-actin bundles shielded by a lipid membrane appear to function like electronic integration devices for signal-to-noise enhancement; the influence of coherent signals on cation transduction is amplified, whereas that of random noise is reduced.

calcium signaling; cell differentiation; Brownian motor hypothesis; cyclotron resonance; hair cell; mechanoelectrical coupling; physiological effects

MORE THAN A DECADE OF RESEARCH on field effects in biological systems has yielded rather compelling data for the involvement of the Ca^{2+} signaling pathway as a primary and main target of magnetic fields (MFs). As first demonstrated by Liburdy and colleagues (52, 53) and later on by a number of other authors, the Ca^{2+} influx pathways of isolated or cultured cells are severely affected by rather low MF energies.

The physiological relevance of this finding is high, because Ca^{2+} represents the most important intracellular signal governing almost all physiological functions in differentiated cells. Most importantly, cytoplasmic Ca^{2+} ultimately determines the rate of cell proliferation, which is an essential factor in the promo-

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tion of tumor growth. Any increase in the rate of cell growth above the normal (evolutionally adapted) level favors the escape of rapidly growing mutants from the natural defense systems, DNA repair and immune response.

Further studies revealed some surprising features of MF action on biological systems, complicating the interpretation of the underlying mechanisms. First, distinct effects were observed at relatively low MF energies. Second, the simultaneous presence of static and alternating MFs turned out to be optimal for MF interaction with biological systems. Third, biological responses could be detected only within certain "windows" of MF parameters at unexpectedly low amplitudes ($\leq$1 Gauss) and frequencies (8–60 Hz).

According to a recent analytic survey of theoretical studies (8), the most important unsolved problems associated with the action of weak MFs on biological systems are centered on the influence of thermal energy (the "kT problem"). First, what interaction mechanism is able to convert the very low quantum energy of the applied MFs to the biochemical response? This problem appears to be rather sophisticated because the energy scale of biochemical processes (on the kT level) is orders of magnitude greater than the energy quantum of the extremely low frequency (ELF) MFs found to be effective. Second, why does thermal fluctuation not interfere with the conversion of the so much smaller ELF MF energy and its transduction to the biological target(s)?

Until now, a simple explanation for the action of weak MFs (at or slightly above the geomagnetic level) on biological processes is lacking. Nevertheless, as assessed by numerous experiments on isolated cell systems, the Ca^{2+} signal pathway appears to be a very likely target of MF action. On physical arguments, the observed frequency dependency of biological responses points to direct transfer of field energy to calcium ions. Therefore, any theoretical discussion starts with the problem of how calcium ions can take up energy from MFs. Several physical models for the energy coupling between Ca^{2+} and MF directly are discussed. Some years ago, Lednev (ion parametric resonance model; Ref. 50) and, later on, Binhi (ion interference model; Ref. 7) published hypotheses for resolving this energy transfer problem using different quantum theoretical approaches. In both cases, the frequency of optimal MF interaction with a biological system, which is identical to the so-called cyclotron frequency for Ca^{2+}, is an essential component of the physical models. The cyclotron frequency represents a MF frequency that is able to accelerate a Ca^{2+} ion moving on a curved pathway. The cyclotron frequency appears to be the only physical parameter established by experiments that clearly points to an essential role of Ca^{2+} in magnetobiology. However, direct energy transfer via cyclotron resonance (35) is considered unlikely because an appropriate biological target system is lacking. In principle, charged clusters or vortices formed by ion clouds with an angular momentum of their mass centers are postulated as interaction sites. However, until now, a specific biological target system with these properties has been unknown.

The interaction mechanism postulated by Binhi (7) is based on the interference of quantum states of Ca^{2+} and other cations bound to biomolecules. The predicted frequency dependence of this interaction is compatible with experimental data. The Binhi theory provides a mechanism by which MFs with very low field strength can interact with bound Ca^{2+} by changing the spatial pattern of probability densities of the bound ion(s) in a manner that may result in altered interaction properties of these cations to biomolecules. The theory also predicts the existence of windows for optimal field parameters as established by experiments.

The other notion, called "ion parametric resonance theory," as discussed by Lednev (50), starts from classic Zeeman splitting of excited states of bound ions by a static MF (e.g., geomagnetism). Modulation of these states by ELF MFs is proposed to serve as a mechanism for the interaction of MFs with Ca^{2+} or other cations in biological systems. This hypothesis also reflects the observed optimal frequency and amplitude windows of MF action as well.

However, transduction of field energy to a bound cation is only the first step on the way to biological effects. The second step concerns the functional response of the biomolecule to the altered energy state of the bound cation. The preferred assumption is dissociation of the cation from the biomolecule, e.g., calmodulin, which may cause a change in its biological activity. As it appears, this problem is the more complicated part. Up to now, it is highly mysterious how the proposed changes in the energy state of Ca^{2+} can induce the observed biological effects, because the dissociation energy of bound Ca^{2+} in organic molecules exceeds by orders of magnitude the energy level of ELF MFs. The discussion about the transfer mechanism is further complicated by the problem that thermal fluctuations are also several orders of magnitude higher than the energy quantum of the effective ELF MFs. Thus the question arises as to how biological systems can discriminate transferred MF energy from the much higher thermal energy supply.

In conclusion, although the primary step of MF interaction, the transfer of MF energy to Ca^{2+}, can be explained on the physical level by different interaction models, further research is severely hindered by the lack of an idea about the biochemical interaction site for MFs. Because essential conditions and limitations of the used experimental approaches depend on the specific properties of this interaction system, all hitherto acquired experimental results have varied in an unpredictable manner.

Proceeding from presently known relevant experimental and theoretical data, three essential properties of a qualified biochemical MF interaction system can be defined: *1*) the interaction system must be a Ca^{2+}-containing molecule or structure that is critically involved in Ca^{2+} signaling; *2*) the interaction site should exhibit rather weak Ca^{2+} binding, although the overall biological effect of Ca^{2+} dislocation must be of high biological rele-

vance; and *3*) the interaction mechanism should display molecular properties, allowing efficient discrimination of thermal energy in favor of MF energy.

The recently outlined concept of microvillar regulation of ion and substrate fluxes (44, 45) offers a potential biophysical mechanism for interaction of low-energy MFs, which may have some or even all of the above mentioned characteristics.

The present study is aimed at introducing the notion of microvillar ion transduction into the discussion of MF actions and to survey the relevant properties of this model on the basis of known experimental data and theoretical studies. The actin cytoskeleton, which has been supposed to be involved in ion channel regulation via microvillar pathways, represents an ion conducting system with molecular and electrical properties ideally matching the postulates for interaction of MF with cells (45). Moreover, microvillar ion conduction is intimately involved in Ca^{2+} signaling of differentiated (or resting) cells (44).

The development of microvilli on the cell surface accompanies cell differentiation or cell cycle arrest at the predifferentiation point G_0/G_1 (1, 2, 6, 9, 31, 71, 83). Most of the characteristic properties of differentiated tissue cells are due to the expression of microvilli and the differentiation-specific localization of integral proteins within the membrane domain of the microvilli tips (42, 44, 45). The novel view of the functional relevance of microvilli contradicts the current opinion that microvilli expression only serves to increase uptake of metabolic substrates by cell surface enlargement. In fact, the formation of microvilli cuts short the rapid unregulated uptake of substrates via transporters, ion channels, and other functional proteins because the microfilament bundle of the shaft region represents a diffusion barrier, which effectively hinders diffusion of ions and transported substrates from the tip compartment into the cytoplasm (42). The diffusion barrier is subject to a variety of regulatory mechanisms conducted not only by the known receptor-mediated signaling pathways but also by different chemical, osmotic, thermal, and physical effects.

The preferred or exclusive localization of various types of ion channels on microvilli including nonselective cation channels, Na^+, K^+, and anion channels is documented for a multitude of different cell types. Most likely, stable segregation of membrane proteins into microvilli affords direct or indirect binding of their cytoplasmic domains to microvillar actin filaments. The central role of specific linker proteins between membrane proteins and actin filaments, such as the ezrin/radixin/moetin (ERM) protein family, in the formation and maintenance of the microvillar surface organization has recently been demonstrated by Yonemura and Tsukita (94), as well as Oshiro et al. (67). Various receptor-regulated effector systems, known to be localized to microvilli including K^+ channels (33), epithelial Na^+ channels (79, 97), erythrocyte anion channels (77), CFTR anion channels (78), insulin-sensitive glucose transporters, GluT4 (34), the Na^+/K^+-ATPase (15), and Na^+/H^+ exchangers (16), are able to interact with the actin cytoskeleton via specific linker proteins of the ERM family. As it appears, a specific group of functional proteins is selected to reside exclusively within microvillar membranes because of their stable binding to cortical actin filaments, whereas other proteins lacking binding sequences for linker proteins may have limited residence times within microvilli.

The localization of functionally important integral membrane proteins on microvilli tips is a new, hitherto unknown aspect of cellular differentiation (42). This type of surface organization implies a number of intriguing consequences that essentially result from sealing the cell surface against the unrestricted influx of metabolic substrates and ions. Even more relevant is the new property of differentiated cells that various cell functions become subject to regulation by external signals via receptor-mediated pathways.

Another important consequence of the microvillar surface organization in differentiated cells is the effective influx restriction for divalent cations and the establishment of a high-affinity storage system for cytoplasmic free Ca^{2+} (41). As recently proposed (reviewed in Ref. 44), both F-actin-based functions are essential components of the Ca^{2+} signaling pathway, the most important signal system of differentiated cells.

The concept of actin-based Ca^{2+} signaling (44) essentially rests on the dual function of the microvillar F-actin system acting both as diffusion barrier and high-affinity Ca^{2+} store (39–41, 43, 48). Receptor-mediated F-actin dissociation/reorganization of the microvillar actin filaments at the same time releases Ca^{2+} from the F-actin store and opens the influx pathway for external Ca^{2+} into the cell. This double function of microvillar F-actin effectively couples Ca^{2+} release to the influx pathway for external Ca^{2+} (store-dependent Ca^{2+} influx).

Thus essential cellular functions such as contraction, secretion, modulation of membrane potential, and cell growth are stimulated via the phospholipase C-coupled Ca^{2+} signal pathway by depolymerization and reorganization of actin filaments (27, 28, 49). In addition to receptor-mediated signaling, the effectiveness of the cytoskeletal diffusion barrier can be modulated by several other mechanisms:

1) Membrane stress generated during hyposmotic cell swelling shortens microvilli, thereby activating regulatory K^+ and Cl^- efflux (46).

2) Exposure to lipophilic compounds alters physical properties of the plasma membrane (17, 18), giving rise to dissociation of the bindings between membrane and cytoskeleton in microvilli. This type of interaction of xenobiotics with the cell surface is characterized by typical shape changes of microvilli indicative for an activated Ca^{2+} influx pathway by impairment of the cytoskeleton-membrane interaction (25, 26).

3) Subtle mechanical load such as small bending of microvilli on vascular endothelial cells induced by streaming blood activates the microvillar ion conduction pathway.

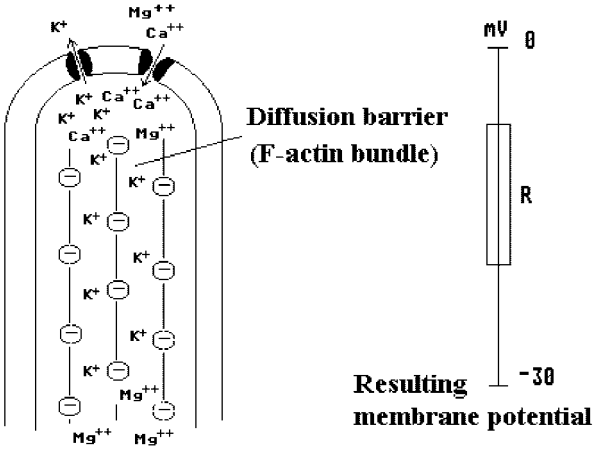

Fig. 1. Polyelectrolyte nature of the microvillar diffusion barrier.

4) Even physical forces such as light and sound can interact with microvilli to generate electrical responses in photoreceptor cells of the retina and sound receptor cells (hair cells) of the inner ear.

Moreover, the interaction with electromagnetic fields (EMF) may represent a further mechanism for modulating the microvillar diffusion barrier and stimulating influx of external Ca^{2+} into the cytoplasm.

IONIC CONDUCTION ALONG ACTIN FILAMENTS

The observed structural organization of ion channels within microvilli strongly suggests that the ion conduction properties of F-actin may govern at least some aspects of overall ion channel behavior (reviewed in Ref. 45). Microfilaments that are enwrapped in a cable-like manner with an isolating lipid membrane are cell organelles with unique electrical properties.

THE F-ACTIN DIFFUSION BARRIER IS A CATION EXCHANGER

As pointed out above, the formation of microvilli on the surface of differentiated cells may be considered as a physiological arrangement for sealing the surface of differentiated cells against Ca^{2+} influx via membrane channels and to establish the intracellular ionic conditions necessary for Ca^{2+} signaling. The barrier function of the microvillar actin filament bundle rests on its structure of a dense polyelectrolyte matrix closely resembling a cation exchanger (Fig. 1). The high number of fixed positive charges within F-actin bundles (5–6 binding sites for Ca^{2+} or Mg^{2+} per subunit) imparts to this structure considerable resistance to transduction of associated counter ions. Whereas free anions are largely excluded from the negative matrix of actin filament bundles because the counter ion charges are highly compensated by the anionic polyelectrolyte, cation conductivity depends on charge number and supplied external energy of either electrochemical or thermal origin. Because of tighter binding of divalent cations to fixed charge centers, divalent cation conduction is more restricted.

The resulting high influx resistance for Ca^{2+} (and Mg^{2+}) enables differentiated cells to maintain the extremely steep Ca^{2+} gradient of 1:10^4 between the intra- and extracellular space and to generate the rapid Ca^{2+} increases following receptor-mediated disorganization of the microvillar bundle structure (40, 43, 44).

In contrast to divalent cations, the monovalent cations Na^+ and K^+ can pass microfilament bundles with lower resistance (54). As shown in Fig. 1, low membrane potential is an important consequence of the resistance of microvillar ion pathways for K^+ conduction (29). Hepatocytes, for instance, display membrane potentials of only −30 mV, which can be significantly enhanced by shortening of microvilli by hypotonic cell swelling or receptor-mediated activation (89, 90).

MECHANISM OF ION CONDUCTION ALONG ACTIN FILAMENTS

Fixed anionic charges within the matrix of F-actin bundles are neutralized by cationic counter ions in the immediate vicinity of the charge centers. The spatial coordination of counter ions to these centers corresponds to a moderate binding of these cations to the sites of fixed charges. Consequently, free ionic movement along an electrical field is restricted. The transport of cations along a linear matrix of fixed charges requires the simultaneous movement of counter ions from one charge center to the next along the whole length of the conducting path (Fig. 2). Simultaneous jumping events, however, afford that all moving ions along the whole conduction path must be provided with the necessary activation energy at the same time. According to the Boltzmann equation of statistical mechanics, the probability, that an ion acquires the extra energy (E_a) needed for translation to the next cationic binding site, is proportional to exp ($-E_a/kT$). Simultaneous activation of a number (n) of such events affords very much higher total activation energy. The probability to reach this state is proportional to $[\exp(-E_a/kT)]^n$, i.e., exponentially decreasing with the length of the conducting pathway.

However, as discussed in the following section, the picture of single counter ions is a simplification that

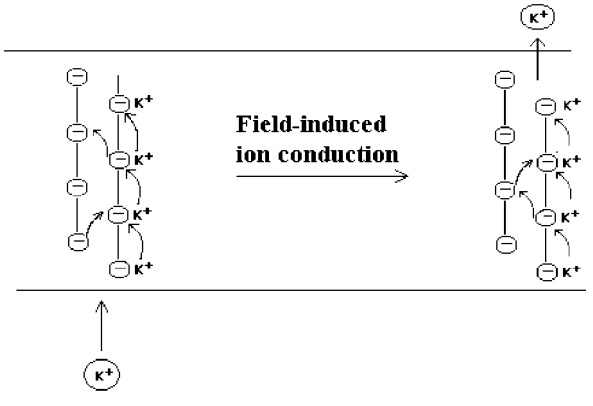

Fig. 2. The "hopping model" of electrodiffusion. Ion transfer by the simultaneous one-step mechanism.

does not account for all conductance properties of microfilament bundles, because fixed charge centers on the polymer are opposed by counter ion clouds. Thus single ions may change from one charge center to the next, giving rise to locally restricted excess of partial charges along the conducting pathway. In a recent study, Lin and Cantiello (54) impressively proved the unique electrical features of this type of ion conduction for a monovalent cation.

CABLE-LIKE CONDUCTANCE IN F-ACTIN BUNDLES

Lin and Cantiello (54) demonstrated cable-like conductance along F-actin filaments in an aqueous environment. Cable-like conductance is defined as the property of extended polymers to conduct ionic currents better along the filament axis than through the surrounding salt solution. The theory of this phenomenon is based on the hypothesis of "condensed counter ions" as proposed by Manning (62) and confirmed for F-actin by Tang and Janmey (82) and Xian et al. (91).

In linear polymers with high charge density, cable-like conductance occurs when the thermal energy of the counter ions is identical to or larger than the energy necessary for its transfer to the next charge center, but lower than the dissociation energy into the free solution (Fig. 3; $E_1 > kT > E_2$). Under these conditions, ions can move along the polymer filament but are hindered from leaving the zone of condensed counter ions (Fig. 3).

As shown by Lin and Cantiello (54) (Fig. 4), cable-like conductance occurs in F-actin bundles. Specifically, K^+ conduction along microfilaments is characterized by the decomposition of an electrical input pulse into discrete delayed charge portions (solitons). Delayed nonlinear discharge patterns clearly indicate the existence of charge centers with corresponding counter ion clouds along the polymer axis.

Formation of series of delayed discharge events can be explained by the capacitor-like action of the counter ion clouds. At high input energy, more cations can accumulate within the clouds than necessary for neutralization of the corresponding charge center (Fig. 5). Consequently, ionic conduction systems of this type are equivalent to electron-conducting systems of coupled oscillators, each consisting of a resistance (intercloud potential barrier) and a capacitor (condensed ion clouds). Both systems, the electron- and the ion-conducting, exhibit nonlinear transmission properties characterized by delayed oscillating output signals.

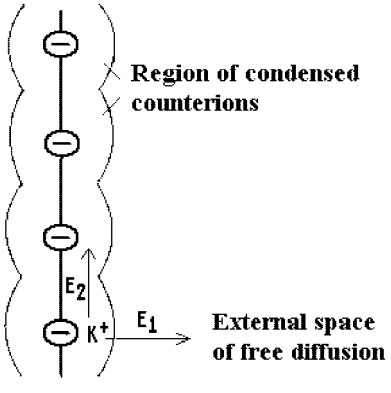

Fig. 3. Schematic presentation of Manning's theory of condensed counter ions in linear polyelectrolytes.

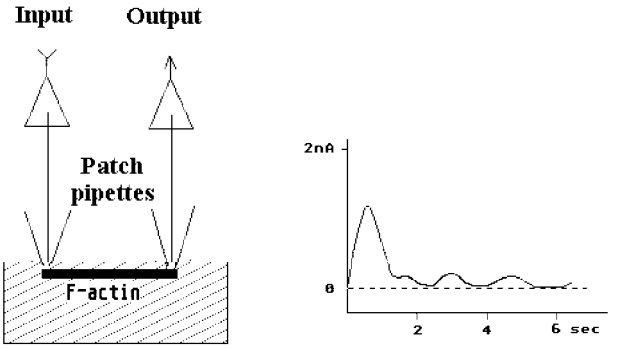

Fig. 4. Experiment of Lin and Cantiello demonstrating cable-like conduction along actin filaments of 50 μm length in aqueous 100 mM KCl. The diagram illustrates the output signal resulting from a 500-μs lasting square input pulse with an amplitude of ~2 nA.

One of the general characteristics of ionic conduction is the large time constants of the output response, which results from the slower migration velocity of ions in aqueous solutions compared with that of electrons in metallic conductors. Consequently, the later part of the output response in an F-actin bundle of 50 μm length is delayed by up to several seconds (Fig. 4).

Evolution of living organisms has brought about a variety of specific sensor cells designed for detection of different physical phenomena such as light, sound, chemical compounds (taste and odor), osmotic force, and mechanical deformations. All these receptor cells appear to use the specific ion transduction properties of microvillar F-actin bundles for potential generation. The audio receptor (hair) cell of the inner ear is the most extensively studied sensory cell type. Hair cells represent an excellent example for electric field interaction with biological systems.

MECHANOELECTRICAL COUPLING IN AUDIO RECEPTOR CELLS

The audioreceptor cell is an extensively used experimental model for studying mechanoelectrical coupling (3, 32, 59, 75). This highly specialized cell type is

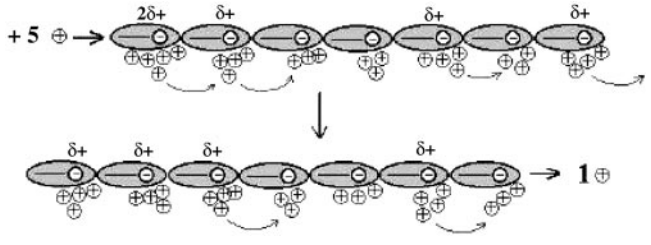

Fig. 5. Generation of delayed discharge patterns of ion currents along F-actin bundles. Discharge of overloaded counter ion clouds only occurs in direction of the next cloud with less positive charge. *Top*: initial state after cation injection. *Bottom*: state after the first step of charge transfer.

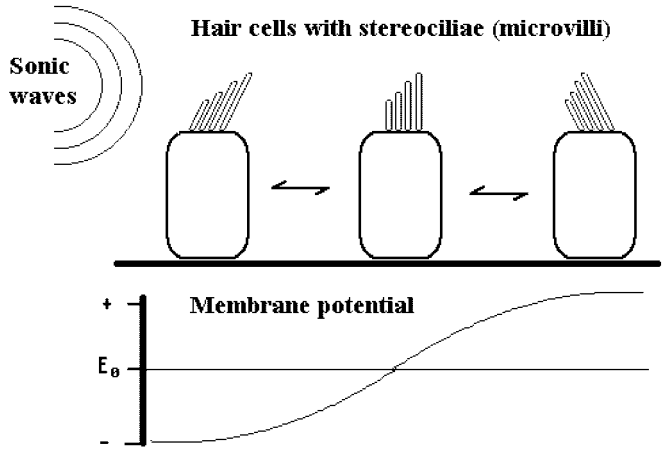

Fig. 6. Acoustic stimulation of hair cell microvilli resulting in oscillating membrane potentials synchronized with sound frequency.

equipped with a characteristic arrangement of large (apical) microvilli that can be laterally moved by applied acoustic fields (Fig. 6). The swinging movement of their microvilli bundle generates a synchronously oscillating membrane potential changing with an amplitude of about 10 mV around its normal value (E_0). Dislocation of the microvilli tips by only 10–50 nm significantly increases the cation permeability of the hair cell microvilli.

In addition to mechanoelectrical transduction, vertebrate hair cells display high sensitivity and frequency selectivity by adding self-generated mechanical energy to low-level signals. This allows detection of signals that are much smaller than thermal molecular motion. Two different sources of mechanical energy fed into sound detection have been identified. One source, occurring in all vertebrate hair cells, is active and spontaneous microvilli movement. Another source of mechanical movement found in mammalian hair cells is membrane potential-driven shortening and elongation of the whole cell body.

The mechanism by which deflections of hair cell microvilli produce changes in membrane potential and the mechanism of the reverse process are still a matter of debate. The most cited hypothesis for mechanotransduction assumes the opening of cation channels at the tips of microvilli due to membrane strain generated via the microvillar tip links. This notion further postulates a direct connection of the tip links with myosin molecules within microvilli, believed to impart to the system an adaptive motile response, as well as the ability for active sound amplification (reviewed in Ref. 32). However, recent findings not only appear to disprove the involvement of the tip link in mechanotransduction (65) but even the hitherto widely accepted notion that regulation of the transduction current occurs at the level of the channel protein (59).

Using the Ca^{2+} fluorescence technique, Lumpkin and Hudspeth (59) were the first to demonstrate directly that Ca^{2+}-permeable channels are located at the tip of hair cell microvilli. These channels were shown to be open in the resting state. Accordingly, high Ca^{2+} concentrations were present in the tip compartments of hair cell microvilli. After sound-induced mechanical stimulation, a flow of Ca^{2+} from the tip compartment toward the microvillus base was observed. Lumpkin and Hudspeth are not alone in stating that the ionic composition of the microvillar tip compartment rather resembles that of the extracellular medium than that of the cytoplasm (see Refs. 45 and 46). These findings strongly support the diffusion barrier concept delineated in the introductory section. Rapid equilibration of the ionic conditions between the internal tip compartment of microvilli and the external medium is one of the consequences following from the presence of cation and anion channels in the microvillar tip membrane and the existence of a cytoskeletal diffusion barrier between tip compartment and cytoplasm (as discussed in Ref. 46). Thus the main conclusion from the findings of Lumpkin and Hudspeth (59) should be that regulation of ion fluxes does not occur at the channel protein itself. Instead, the regulatory mechanism must be located within the microvillar shaft region, which is tightly filled with bundled actin filaments.

Further data supporting this idea come from Meyer et al. (65), who showed that removal of the tip link does not close the transduction channel; instead, the cells display a maintained inward current of similar magnitude to that of the receptor current before treatment. The authors stated that this result is not in accordance with the tip link model of mechanotransduction.

Nevertheless, recent work of Martin and Hudspeth (63), as well as that of Manley et al. (64), offers compelling evidence that mechanotransduction, as well as the intimately associated amplifying mechanism for sound detection, is connected to oscillations of the stereociliar bundle. The underlying bioelectrical processes, however, are unknown (reviewed in Ref. 21).

A possible mechanism of mechanotransduction in microvillar F-actin bundles is proposed in Fig. 7. This mechanism rests solely on theoretical considerations following from the diffusion barrier concept and the electrochemical properties of actin filament bundles as depicted above. The sound-induced bending of microvilli causes mutual dislocation of charge centers by

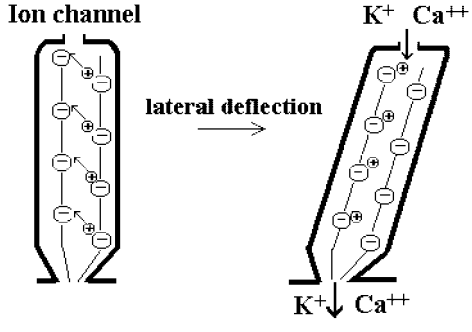

Fig. 7. Hypothetical mechanical induction of ion currents in F-actin bundles by filament gliding.

the sliding of filaments along the bundle axis (24). Filament gliding approximates the charge centers of neighboring filaments and facilitates the passage of a limited amount of counter ions through the bundle. The general design of this mechanism is that of an ion switch operated by mechanical energy, i.e., a cellular device for coupling mechanical movement with the membrane potential.

Until now, no experimental work has been done to detect ion currents induced by filament gliding in isolated microfilament bundles. However, several studies on the polyelectrolyte properties of F-actin have clearly shown that specific molecular interaction forces exist between aligned actin filaments (82). Specifically, in the presence of only monovalent cations, F-actin exhibits sufficient unshielded negative surface charges to inhibit bundle formation. In the presence of polyvalent cations, however, increased counter ion condensation by the shielding of fixed negative charges results in filament alignment and bundle formation. Under these conditions, attracting forces between the filaments become predominating. The observation of specific spatially ordered arrangements of the filaments as 2- and 3-dimensional paracrystalline arrays clearly points to the existence of energetically preferred mutual alignment positions with minimal internal energy (81). The unique arrangement of F-actin bundles in combination with ion channels within the microvillar structure opens a multitude of possible effects mediated by field application. As recently discussed (45), the conduction properties of the microvillar pathway can be modulated under the influence of an electrical field. By driving divalent cations into or out of the core bundle, the electrical conductance via monovalent cations (and mechanical properties) through the microvillar filament bundle can be widely modulated.

The reversible gliding-filament mechanism would provide an explanation for both mechanotransduction and reverse transduction (including signal amplification) based on only one common electrochemical process. Because transduction of cations through the microvillar filament bundle only affords a simultaneous one-step transfer of ions between charge centers along the polyelectrolyte, potential generation via this mechanism may be fast enough to meet the frequency requirements of sound perception.

Moreover, the filament gliding hypothesis may be helpful in explaining transducer adaptation. Calcium ions permeating into the peripheral region of the microfilament bundles act like blockers of monovalent cation transduction. Because of the much higher binding affinity of divalent cations to the fixed charge centers of the filaments, the transduction of monovalent cation is severely inhibited. Simultaneously, the mechanical properties of the filament bundle are altered because incorporation of divalent cations into the condensed counter ion cloud considerably increases shielding of fixed charges on the filaments (82). The immediate consequence of higher mutual filament attraction (bundle density) is enhanced mechanical stiffness. Thus the experimental finding that the degree of transducer channel adaptation is proportional to the amount of entered Ca^{2+} is in accord with this idea.

MICROVILLI AS CELLULAR INTERACTION SITES FOR ELECTRIC FIELDS: REVERSE TRANSDUCTION IN HAIR CELLS

Reverse transduction has been demonstrated by using two different experimental approaches. Hair cells from different locations on the auditory organ display a marked resonance of the membrane potential generated in response to a specific sound frequency (potential resonance; see Refs. 3 and 32). Similarly, nonphysiological mechanical stimulation of hair cell microvilli with this selective frequency results in maximal amplitudes of the oscillating membrane potential. The same frequency of potential resonance is also generated by injection of a current pulse into the hair cell ("induced ringing") (Fig. 8). The frequency of this oscillation is the same as that of sound to which the cell is most sensitive (13). The length of hair cell microvilli inversely correlates with this frequency.

Because hair cells also respond to application of an electrical potential difference with lateral deflections of microvilli in the nanometer scale (12, 73), the finding of induced ringing strongly suggests that such movements are potential driven.

These experiments show that microvilli of hair cells represent cellular interaction sites for external electric fields at which field energy can be transformed into mechanical and electrical responses. Most importantly, the reverse transduction experiments clearly point to the microvillar F-actin bundles as the primary interaction site for both the mechanical and the field-induced responses. Because the mechanical properties of the microvilli are exclusively determined by the cytoskeletal core, potential-induced ringing of microvilli must be due to the electromechanical properties of microfilaments. This notion contrasts with the common view that mechanoelectric coupling occurs by direct action of mechanical forces on the ion channel protein in the tip membrane of microvilli. Although the latter conception could explain mechanoelectrical transduction, it is unable to account for the field-induced swinging movement of hair cell microvilli at their resonance frequency.

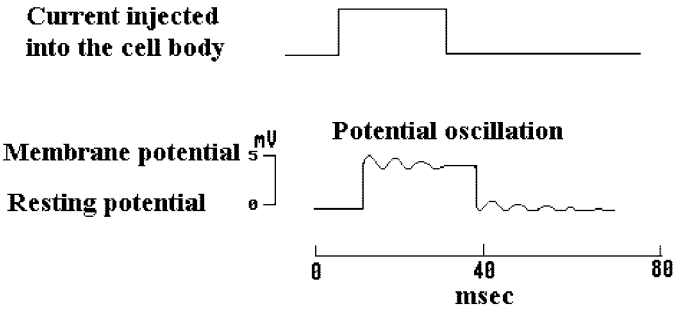

Fig. 8. Tuned electrical resonance in hair cells ("induced ringing").

Summing up, the electromechanical properties of hair cells point to a possible natural mechanism for perception of electric fields. Many arguments support the assumption that the microvillar actin filament bundle is intimately involved in this mechanism. The hair cell bundle system comprises unique electrical and mechanical properties that enable cells not only to detect environmental mechanical and electrical signals but even to discriminate such signals from thermal energy.

A HYPOTHETIC MECHANISM FOR MF-INDUCED ACTIVATION OF MICROVILLAR CATION PATHWAYS

Experimental data from Torbet and Dickens (84) suggest that MFs are able to induce a movement of condensed counter ions along the filament axis. These authors demonstrated the alignment of actin filaments in a strong MF, indicating that actin filaments are able to convert MF energy into mechanical movement. Because MFs can only interact with molecules exhibiting magnetic dipole moments, this finding clearly demonstrates that MFs can induce molecular magnetic moments. However, an interaction of ELF MFs with biologically relevant polyelectrolytes has not been described as yet.

The unusual electrical properties of F-actin and the specific structural combination of microfilaments with ion channels within microvilli as proposed by Lange in Ref. 45 reveal a novel aspect for MF interaction with cellular signaling. One of the most important properties of the microvillar ion pathways follows from its discontinuous conduction mechanism. Partitioning of the whole conduction pathway into many different, distinct capacitive steps qualifies this structure for discrimination of thermal energy against other nonstochastic forms of energy supply. Specific molecular and electric properties of F-actin bundles potentially render microvillar structures responsive to EMFs of low energy.

Condensed ions on F-actin behave as nearly free cations. The activation energy for an ion transfer between charge centers is lower than or equal to the thermal energy level (precondition for cable-like conduction). Therefore, MF energy at or below the thermal energy level is able to move ions between the charge centers.

Even the small binding forces of ions to the fixed charge centers of the polymer prevent thermal (stochastic) induction of ion transfers through the whole array of fixed charge centers, because the transfer probability exponentially increases with the number of charge centers (Fig. 9).

In contrast to stochastic activation of ion transfer by thermic effects, energy transfer from the applied EMF may results in time-, space-, and vector-coherent excitation of ions within the whole conducting path, i.e., a simultaneous jump of ions between all centers at the same time and in the same direction.

Thus an array of coupled low-potential barriers between the charge centers efficiently discriminates thermal activation of ion conduction along the polyelectrolyte. The discriminating effect exponentially increases with the number of fixed charge centers along the conduction pathway (Fig. 9).

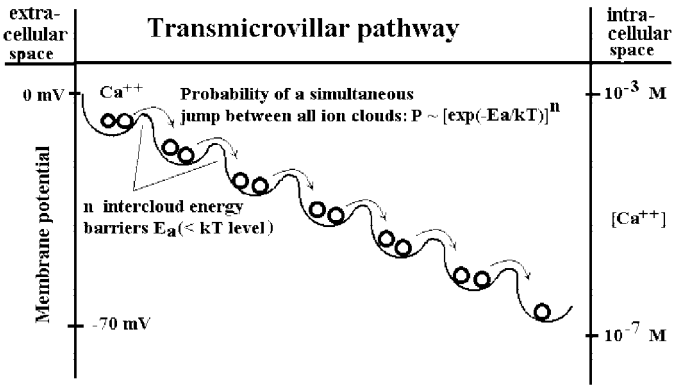

Fig. 9. Cascade model of coupled low-energy barriers along the microvillar actin filament bundle. Curved arrows indicate the simultaneous transfer of one cation through the whole cascade.

Assuming, for example, a diffusion barrier length of only 0.1 μm (microvilli are between 0.5 and several micrometers long), corresponding to ~37 actin subunits (= number of fixed ionic charge centers), the number n of the array of potential wells is 37. According to the above mentioned Boltzmann equation, the probability P for the transfer of an ion from one potential well (ion cloud) to the next is $P_1 \sim \exp(-E_a/kT)$, and the simultaneous passage of one ion through the whole array is $P_n \sim [\exp(-E_a/kT)]^{37}$. In the instance that an applied time- and vector-coherent field induces a twofold increase of the ion transfer between charge centers probability for all 37 charge centers simultaneously, the open probability for the complete pathway would be enhanced by 11 orders of magnitude (2^{37}). In other words, a transfer probability of $P = 10^{-11}$ for the whole pathway in the ground state is increased to $P = 1$ by a simultaneous twofold increase of the transfer probability. Although a doubling of P by MFs may be unrealistic, the example illustrates the enormous discrimination power of the system for thermal energy. A potential barrier array like this also illustrates the relevance of a low binding strength of the involved cations. Otherwise, the very low MF energy would not change the one-step transfer probability by a significant factor.

Cation transduction through a cascade of multiple low-energy barriers represents an ideal biophysical device for discriminating thermal or other forms of undirected energy supply in favor of time- and space-coherent excitation. Coherent stimulation of ion transduction through microvillar pathways is further potentiated by the uniform arrangement of microvilli on the cell surface, by which all ionic pathways have an identical orientation to the field vector. Thus all, or at least a large portion of, ionic pathways on the surface of an individual cell are simultaneously activated.

As discussed in the introduction section, one of the most relevant objections against ELF MF action on ion

fluxes is that the high thermal noise energy would disturb field-mediated particle motion. The above-delineated discriminating action of coherent stimulation gives a reasonable answer. However, the specific impact of stochastic thermal movement on field-induced ion transduction along the polyelectrolyte matrix remains unconsidered. Several theoretical approaches have been used to treat this problem from different points of view and with different tools, e.g., stochastic resonance and the theory of the "thermal ratchet engine." As it appears, the general results of these approaches are identical.

Because the kinetic approach is always more intelligible and most qualified for the nonmathematical treatment, the thermal ratchet model is used to demonstrate the specific function of thermal noise in the process of ionic transduction through potential barrier arrays. Most intriguingly, this model clearly demonstrates that directed movement of ions does not occur in spite of, but rather because of, thermal energy.

THE THERMAL RATCHET MODEL: HOW THERMAL MOTION OVERCOMES POTENTIAL BARRIERS

The theoretic model of the thermal ratchet engine, as recently reviewed by Astumian (4), illustrates the general conditions under which molecular motors can bias directed motion from random thermal noise, although no overall driving net force is applied.

As postulated by the second law of thermodynamics, the effect of thermal noise at equilibrium is symmetrical, and no structural feature alone is able to extract energy from Brownian motion. However, the introduction of a specific device, called ratchet, into the motor construction changes the situation. One of the most intelligible models of Brownian motors is the "ratchet in a thermal bath."

As shown in Fig. 10, a sawtooth rack is perpendicularly connected to a piece of wall. Gas molecules hit both sides of the wall. When the tooth rack is not arrested (lever up), Brownian movements push the rack forward and backward by statistically identical distances. Because of the asymmetric tooth arrangement, repeated arrests of the sawtooth rack (lever down), followed by readjustment of the rack by sliding to the next minimum lever position, result in a systematically right-directed motion of the rack.

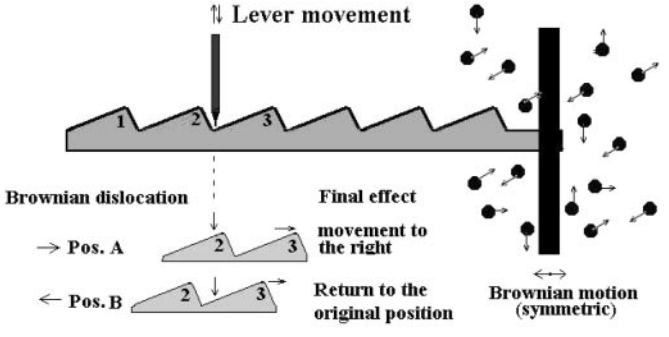

Fig. 10. Scheme of a Brownian motor periodic lever movement resulting in systematic rightward movement of the sawtooth rack. *Pos. A* and *B* indicate the rack positions after a right and a left rack movement, respectively, with identical amplitude.

In principle, the shift of the rack to the next ratchet position requires energy input applied by the lever. In their original paper, Feynman and Sands (22) discussed a motor that exclusively used thermal energy even for lever operation. They showed that when all components of such a device are in thermal equilibrium, net movement is not achieved. However, a thermal gradient between the lever and the rack can cause directed motion and may be used to do work. Because thermal gradients are very unlikely in biological systems of microscopic dimensions, other energy sources such as light and electrical field energy have been used to drive the lever function.

In principle, the Brownian motor only yields work when the lever action feeds energy into the system. Part of this energy is again added to the system's thermal energy, while another part is used for directed movement of the ratchet. Under ideal conditions, in the absence of any frictional loss within the motor, this latter part of the energy input can be infinitely low. On the other hand, when the thermal energy of the environment approximates zero, motor activity is also abolished. With increasing thermal energy, the motor velocity is enhanced.

From this rather simple theoretical model of a Brownian motor, the three main components of this device become visible: *1*) stochastic thermal energy to cause Brownian motion of the ratchet, *2*) anisotropy of the motor structure (sawtooth rack), and *3*) external variation of a constraint (pawl motion), which provides for the sole energy input into the system.

The most convincing experimental verification for this type of molecular motor has been presented recently by Faucheux et al. (19) by using a single colloidal particle in a circular optical trap that was intensity modulated to generate a sawtooth potential. According to the anisotropy of the optical potential, directed circulation of the particle occurs. A further very exciting application of the Brownian motor concept on the biological ion pump, Na^+/K^+-ATPase, has been described recently (58, 91, 93). These experiments show that energy from external periodic field exposure can substitute for energy from the hydrolysis of ATP to power the uphill transport of cations.

F-ACTIN—AN ARRAY OF THERMAL RATCHETS

A potential Brownian ratchet may consist of F-actin with fixed negative charge centers on the polymer subunits (Fig. 11). The cations within each potential well undergo small movements around the potential minimum due to thermal excitation. However, thermal energy is too low to push a relevant number of cations simultaneously over the potential barriers. Sudden modulation of the potential ratchet by externally applied alternating fields changes the positions of the potential wells and allows some of the cations to pass through the barrier and slide down to the next potential minimum. Anisotropy is generated by the direction

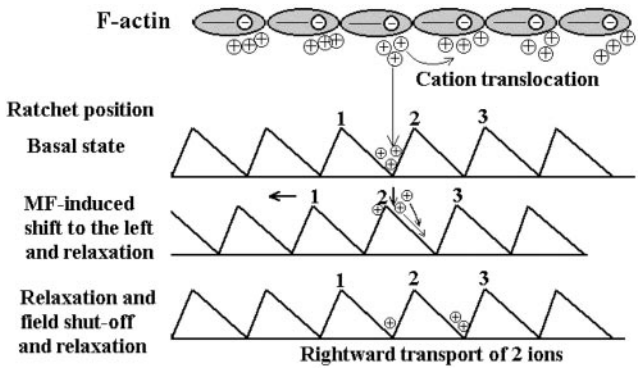

Fig. 11. Model of a Brownian cation pump consisting of actin filaments exposed to a fluctuating potential. MF, magnetic field.

of the applied field and/or the specific arrangement of the fixed charge centers on the polymer.

This arrangement is analogous to the thermomechanical ratchet. The mechanism of ion transfer through this type of polyelectrolyte-based ion ratchet also resembles that of the thermal ratchet. The ion pump function of the polyelectrolyte depends on the biasing of stochastic thermal movement by a fluctuating potential or "flashing ratchet," as recently described by Astumian (4). The specific arrangement shown in Fig. 11 does not need anisotropy within the original potential pattern, because this property is introduced by the periodically applied field or by a static field. This biological device would represent a Brownian ion pump, a molecular motor specifically designed to transform weak field energy into directed motion of particles. Combination of thermal noise with a periodically applied field-induced modulation of the potential ratchet results in unidirectional cation transport along F-actin bundles.

A very convincing feature of the kinetic interpretation of the Brownian motor mechanism is its ability to account even for one of the most puzzling properties of MF-induced Ca^{2+} signaling, the existence of frequency windows. As depicted in Fig. 11, the shifted, as well as the following basal, state of the potential ratchet has to be maintained for a specific time period sufficient for the involved cations to slide down to their minimum potential position within the potential well (relaxation). Again, because relaxation depends on thermal motion, the applied oscillating potential must have specific frequency requirements. Because the experimentally determined optimal ion-specific frequencies for transduction closely obey the predictions of the cyclotron resonance (35), a direct energy transfer from applied MF to cations via the cyclotron mechanism appears most likely.

In contrast to the original thermal ratchet motor, the F-actin model is composed of a large number of coupled particle systems instead of only one. The ion cloud of each F-actin subunit represents a separate molecular ratchet motor containing several particles that are subject to thermal motion. The linear arrangement of these ionic centers generates specific coupling phenomena. As soon as one or more ions have changed the fixed charge centers, the arising localized electric fields amplify the ion transfer induced by the following MF periods. Thus an array of potential barriers, as verified in F-actin bundles, is able to store MF energy through several cycles of the alternating field, each potentiating the overall transfer probability in the following cycles. This assumption is supported by the recent work of Derenyi and Viscek (14), who demonstrate that coupling many particles in a modulated potential ratchet gives rise to high thermodynamic efficiencies up to 50%.

An unresolved problem is the possible mode of interaction of MFs with the counter ion clouds of F-actin. Apart from the different physical hypotheses of MF/ion interaction mentioned in the introduction section, the structural arrangement of the fixed charge centers on F-actin bundles and the specific frequency and amplitude windows render a cation/field interaction via cyclotron resonance very likely.

CYCLOTRON RESONANCE

There are two possible modes of MF interaction with ion clouds of F-actin via cyclotron resonance effects, one on the level of the microfilament structure and another on the ion cloud structure.

Taking into account the molecular arrangement of F-actin filaments as a double helix, the requirements of the ion cyclotron resonance theory are met. As shown in Fig. 12, condensed ion clouds are spirally arranged along the filaments. Thus the double helix of an actin filament can be characterized as a cylindrical molecule, on the surface of which the cation clouds are arranged as two spirally conduction pathways confined to two rows of fixed negative charge centers on the two polymer chains. Charges moving along the condensed cation pathways of the polymer exhibit an angular mass momentum, as postulated by the cyclotron resonance theory. Because, under physiological conditions, K^+ is the main component of the ion cloud, a permanent streaming of the charged particles conducts a small K^+ current that determines the membrane potential. Alternating fields with the specific cyclotron frequency can accelerate K^+ and other streaming cations on their curved pathways.

A further possible site of MF interaction may be the ion cloud itself. Externally applied MFs may accelerate the weakly bound counter ions to form vortices of cations near the fixed charge centers. Such ion vortices

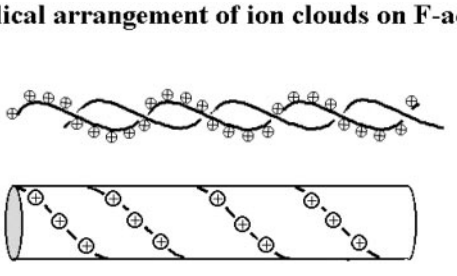

Fig. 12. Helical arrangement of the cation cloud (+) along the F-actin double helix (clouds of only one strand are drawn).

are able to take up further energy from alternating MFs in a time-dependent manner until the energy barriers are surmounted. It remains a matter of physics to evaluate the true relevance of the F-actin-bound cation system for cyclotron resonance interactions.

MF INTERACTION WITH ACTIVATED MICROVILLAR IONIC CURRENTS

In some studies, MFs strongly amplify the Ca^{2+} influx into cells with prestimulated Ca^{2+} signal pathways (52, 53, 86). Under these conditions, Ca^{2+} currents flowing through activated microvillar pathways may give rise to magnetic microfields around the ionic pathways of microvilli, which can interact with external MFs.

On the other side, even under resting conditions, the slow potential-generating leak current of K^+ through the diffusion barrier and the microvillar K^+ channels may cause an induced magnetic microfield around the diffusion barrier. External MFs would be able to interact with the microvillar MFs modulating the currents through this pathway.

PHYSIOLOGICAL EFFECTS OF MF EXPOSURE

Most epidemiological studies of field effects have concentrated on MF exposure because, unlike electric fields, MFs deeply penetrate the body tissue. Electrical fields are much more effectively shielded by the dielectric properties of water-rich tissues than MFs. An analysis of five recent studies has demonstrated a modest increase of risk for leukemia and brain cancer in electric utility workers from France, Canada, and the United States (36, 70, 85). Furthermore, a list of suspected field-induced cancer types including male breast cancer and malignant melanoma has been put forward (66).

Because Ca^{2+} signaling accelerates cell cycle progression, field-induced physiological effects are mainly centered around the general process of tumor promotion. The main cellular mechanisms of defense against life-threatening genetic mutations are DNA repair and immune response. The activity of both mechanisms has been adapted by natural selection to the demands of environmental hazards. However, further increases of the mitotic rate by additional Ca^{2+} signaling agents (tumor promoters) additionally increases the mitotic advantage of cell mutants to override the repair and immune mechanisms.

Experiments with different cell types consistently point to the Ca^{2+} signaling system as the primary cellular target of MF interactions (5, 10, 23, 52, 53, 60, 69, 86, 87). In many cases, alternating MFs alone or in combination with static fields increase intracellular Ca^{2+}, as, for instance, in HL60 cells (10), T-lymphocytes and leukemia cells (60), the human T cell line (Jurkat) (55, 56), bone cells (23), pituitary cells (5), and human astrocytoma cells (69).

Other studies showed an increase of Ca^{2+} influx in prestimulated rat thymocytes (86) and lymphocytes (52, 53).

A third group of reports describes an inhibition of Ca^{2+} signaling by strong static MFs in GH3 cells (72) and retardation of metabolic activity in HL60 cells (74), as well as a block of action potentials by static MFs in cultured neurons (11). Similarly, stimulation of cells with MF frequencies apart from the active frequency windows has been shown to act as an inhibitor to prestimulated Ca^{2+} signaling (87, 95). A variety of other MF-induced physiological effects indirectly suggests the involvement of the Ca^{2+} signal pathway (20, 37, 55, 68, 72, 76, 80, 96, 98).

Moreover, a recent study (96) has demonstrated that EMFs severely affect the structural organization of microvilli. Pulsed EMFs at frequencies between 50–70 Hz and 0.6 V/cm caused loss of microvilli and collapse of apical parts of endoderm cells in embryonic yolk sack. These ultrastructural alterations were accompanied by severe embryo toxicity. Similar observations were reported by Lisi et al. (57) and Santoro et al. (76), who applied ELF MFs (50 Hz, 2 mT) to human lymphoid cells. Field exposure resulted in a reorganization of the cortical actin cytoskeleton accompanied by loss of microvilli. Similar results were recently reported by Manni et al. (61). Exposure of keratinocytes to low-energy 50-Hz MFs resulted in changes of shape and morphology of the cells, as well as differential actin distribution. In addition, cells showed increased clonal capacity and growth, as well as a modified pattern of adhesion and differentiation markers.

These findings are consistent with the proposed role of microvilli in MF interaction. Static or alternating MFs outside of the active frequency windows do not interact with resting electric charges but stabilize their spatial arrangement. Consequently, counter ion clouds along the polyelectrolyte are fixed and cellular cation uptake via the microvillar cation pathways is inhibited.

According to the mechanism of electromechanical coupling shown in Fig. 7, charge dislocation along the filament axis may generate mutually repulsive charged centers in neighboring filaments aligned in the bundle structure. As shown in the reverse transduction experiment, microvilli of hair cells respond to this induced state of intrinsic strain with an evasive deformation of the filament bundle. The energy used for this deformation process is taken from the applied EMFs or MFs. Relaxation from this state of higher energy can be achieved in two different ways.

First, the produced mechanical stress may damage the subtle structural organization of the microvillar diffusion barrier and activate the Ca^{2+} signaling pathway. Second, the intrinsic strain of the system can be relieved by ion translocation, generating a short current pulse through the filament bundle similar to that observed during electromechanical coupling. Both induced ion fluxes and mechanical damage should be considered in further studies of field-induced biological hazards.

CONCLUSIONS

The interaction of weak EMFs with living cells is a most important but still unresolved biophysical prob-

lem. Thermal and other types of noise appear to cause severe restrictions in the action of weak signals on relevant cellular systems. Up to now, experimental data indicating low-intensity field effects could not be explained on a reasonable mechanistic basis and, consequently, became questionable as a result.

As recently stated by Kruglikov and Dertinger (38) "One way out of this dead end is to search for possible general mechanisms of signal amplification in which a weak signal is amplified by system noise itself." This type of signal amplification was discovered several years ago in physics and is known as stochastic resonance. It was shown that stochastic resonance might exceed an amplification factor of 1,000, which renders existing estimations of EMF thresholds highly speculative.

Several physical explanations for the direct transmission of MF energy to bound Ca^{2+} ions have been put forward. However, a mechanism for the transmission of weak field energy on relevant biological systems, especially the Ca^{2+} signal system, is still unknown. To close this gap, a cellular target system for MFs is proposed that combines physiological relevance for Ca^{2+} signaling with unusual electrical properties capable of explaining the effects of low-energy MFs on biological systems. This target, the ion-conducting actin filament bundle within microvilli, was previously shown to exhibit nonlinear, cable-like cation conduction through arrays of condensed ion clouds. The proposed interaction of ion clouds with periodically applied EMFs resulting in cation pumping through a cascade of potential barriers within the polyelectrolyte rests on well-known physical principles of signal discrimination such as stochastic resonance or the Brownian motor hypothesis. The involved interaction mechanism is in accord with our present knowledge about Ca^{2+} signaling as the biological main target of MFs and the postulated extreme sensitivity for excitation by very low field energies within specific amplitude and frequency windows. Even the disturbing role of thermal noise is severely qualified. Instead, thermal noise becomes an essential and necessary mechanistic component.

Over all, microvillar F-actin bundles shielded by a lipid membrane appear to function like electronic integration devices for signal-to-noise enhancement. Coherent signals on cation transduction are amplified, whereas stochastic (thermal) noise is reduced. This novel modulating principle of microvillar cation transduction might have relevance also for cell-cell interaction by oscillating EMFs or auto-activation of ionic pathways by localized changes of the membrane potential on the same cell (signal propagation).

A further, more important implication of the proposed mechanism is the synergistic action of MFs on the uptake of xenobiotics into the cell. Toxic compounds can enter the cell more readily under the influence of EMFs, activating the Ca^{2+} signaling pathway. As discussed in detail in a recent survey (47), the natural barrier function of epithelial cells strongly depends on the maintenance of intact microvillar surfaces. Any disorganization of this surface morphology severely accelerates the entrance of ionic and lipophilic xenobiotics into the cytoplasm. Long-term exposure to MFs has been shown to completely abolish the microvillar morphology (57, 76) of lymphocytes and embryonic neural endoderm (98). Thus the sensitizing effect of EMFs on the action of cytotoxic compounds can be used for treatment of drug-resistant tumors (30, 51, 88). However, apart from therapeutic use, long-term exposure to EMFs must be considered as an additional risk factor potentiating the action of environmentally hazardous compounds.

Until now, the discussion of EMF effects on health has been dominated by contradicting experimental results. The obvious main dilemma is the lack of knowledge about the true nature of the cellular target for MF interaction. Although there is solid knowledge about the existence of MF perception in animals even at the geomagnetic field level, the presence of similar mechanisms in human beings has been generally doubted. Elucidation of the molecular basis of MF interaction with biological systems would help to substantiate the discussion and establish proper experimental approaches to determine the true hazardous potential of field exposures.

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