CELL MEMBRANES, ELECTROMAGNETIC FIELDS AND INTERCELLULAR COMMUNICATION

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(Adey,1983, 1986, 1987; Adey and Lawrence, 1984; Lawrence and Adey, 1982).

In the interim, there has been an increasing focus on the role of Ca^{2+} ions in excitatory processes. There is clear evidence that Ca^{2+} ions (Adey, et al., 1982; Bawin and Adey, 1976; Bawin et al., 1978a; Bawin, et al., 1978b; Blackman et al., 1979, 1985a and b; Lin-Liu and Adey, 1982) and calcium-dependent processes at cell membranes (Dixey and Rein, 1981; Kaczmarek and Adey, 1974; Lyle et al., 1983) initiate intracellular enzyme responses (Adey, 1986; Luben et al., 1982; Luben and Cain, 1984; Byus et al., 1987a and b) that are modulated by extracellular EM fields inducing transmembrane currents millions of times weaker than required for threshold excitation in accordance with a Hodgkin-Huxley model.

1.1 Pathways for Electric Current Flow in Tissue.

The crux of the problem becomes apparent from a consideration of distribution of current flowing in a tissue composed of cells with a high membrane resistance and bathed in a strongly conducting fluid (Cole, 1940). Typical cell membrane resistances are in the range 3,000-100,000 ohm.cm^2. Extracellular fluid has a specific resistance of only 50 ohm. cm^{-1}. Thus, although the extracellular space forms only about 10 percent of the conducting cross-section of typical tissue, it is clearly a preferred pathway, carrying at least 90 percent of any imposed or intrinsic induced current.

As a corollary to this scheme of divided current pathways, most current flows along membrane surfaces, following narrow intercellular gutters that separate cell membranes. As discussed below, the longitudinal current flow at cell surfaces appears of considerable importance in the action of pericellular fields on Ca^{2+} binding at cell surfaces (Bawin and Adey,1976; Bawin, Adey and Sabbot, 1978; Bawin, Kaczmarek and Adey,

between contacting epithelial cells in seconds, it became apparent that the phenomenon of ionic coupling was not confined to excitable tissues nor to ions alone (see Fletcher, Byus and Walsh, 1987 for review). This initiated the concept that intercellular communication could have broad significance in cellular regulation (Loewenstein, 1968; Bennett and Trinkhaus, 1970; Gilula et al., 1972). Added support for this concept has come from evidence that there is exchange of metabolic products between intimately contacting cells in "metabolic cooperation" (Pitts and Finbow, 1986). As discussed below, a morphologic substrate for this cell-cell communication resides in the narrow but regular intercellular space at the junctional region designated as a gap junction (Revel and Karnovsky, 1967).

1.3 Imposed EM Fields as Tools in Studies of Transmembrane Signalling and Intercellular Communication.

Long accepted functional models of the plasma membrane have emphasized the existence in most cells of a major electrical gradient (the membrane potential) across the 40 A° width of the lipid bilayer. In these models, this gradient of 10^5V/cm has been considered an effective electrical barrier against stimulation by weak EM fields in surrounding fluid. However, much recent research has shown that imposed weak EM fields in the ELF spectrum below 100 Hz at intensities in the pericellular fluid many orders of magnitude weaker than the membrane potential gradient can modulate actions of hormone, antibody, neurotransmitter and cancer promoting molecules at their cell surface receptor sites. Similar sensitivities have been observed for radiofrequency fields that are amplitude-modulated at ELF frequencies. These modulating actions of EM fields suggest highly cooperative processes in the underlying physical mechanisms. The observed sensitivities are as low as 10^{-7}V/cm in the ELF

membrane, leading to the generally accepted fluid mosaic model of the cell membrane (Singer and Nicolson, 1972). Thus, these intramembranous protein strands form signalling pathways by which external stimuli are sensed and conveyed to the cell interior.

2.1 Cell Membrane Receptor Proteins for Human Epidermal Growth Factor (EGF) and Nerve Growth Factor (NGF) as Models of Membrane Coupling Proteins.

Much attention now focuses on membrane receptor proteins for the human epidermal growth factor (EGF) and the nerve growth factor (NGF) as models of coupling proteins in studies of the nature of transmembrane signals.

The entire 1210 amino acid sequence of the EGF receptor protein has been deduced by Ullrich et al. (1985), with striking findings on the sequences that make up the extracellular, intramembranous and cytoplasmic portions of the chain. Extracellular and intracellular segments are each composed of appoximately 600 hydrophilic amino acids. The molecule appears to cross the membrane only once. The salient and surprising finding is the extremely short length of the intramembranous segment of 23 amino acids, predominantly hydrophobic, and with only a single amino acid with a side chain capable of hydrogen bonding.

Subsequent studies have shown that the NGF receptor protein also has a strikingly similar segment of 23 hydrophobic amino acids within the membrane (Radeke et al., 1987), suggesting that this configuration plays a fundamental role in processes of transmembrane signaling. This view is strengthened by studies with a chimaeric protein constructed of the extracellular portion of insulin receptor protein joined to the transmembrane and intracellular domains of the EGF receptor protein (Riedel et al., 1986). In this molecule,the EGF receptor kinase domain of the chimaeric protein is activated by insulin binding. The authors conclude that insulin receptors and EGF receptors employ closely related

cancer-promoting phorbol esters which have a specific membrane receptor (Ca^{2+}-dependent protein kinase C). EM field interactions with these cancer promoters are discussed below.

2.2 Morphogenetic Concepts Involving Cell Surface Proteins in Brain Tissue

Cell migration in the embryo leading to tissue and organ formation requires varying degrees of cell adhesion that change in the sequence of migratory events. The glycoprotein strands on the cell surface described above in the Singer-Nicolson fluid mosaic membrane model have sialic acid (amino sugar) terminals that are strongly negatively charged, producing a huge polyanionic surface. Edelman (1984) has described patterns in the mosaic of surface glycoproteins in brain tissue as a manifestation of gene expression. These are the cell adhesion molecules (N-CAMs in nervous tissue) that determine cell adhesion in embryogenesis. N-CAM levels are high at primary sites near the neural tube, are low during migration, and reappear as they reach their destination and interact with other cells. N-CAMs change form radically in a shift from embryonic (E) to adult (A) forms, with a reduction from 30 to 10 percent of the total mass of glycoproteins. This ontogenetic change fails to occur in reeler disease in mice, manifested as a gross failure in cerebellar development.

These findings are noteworthy in three respects. Until recently, it had been a matter of speculation whether cell surface proteins included enzymes that would participate directly in the initial steps of transduction of electrical and chemical stimuli. Ehrlich et al. (1986) have reported the presence on nerve cell surfaces of an ecto-protein kinase that is selectively stimulated during cell depolarization. It performs the typical functions of protein kinase enzymes in phosphorylating other protein substrates. N-CAMs are a substrate for this ecto-protein kinase system. Also, the histology of cell surface

hydrophilic channel in the center of each connexon that appears to span the entire membrane; so that when connexons of adjacent cell membranes come into register, the cytoplasms of connected cells are effectively in continuity. These connexons thus provide the physical substrate for ionic coupling, fluorescent dye transfer and metabolic cooperation discussed above (Gilula et al., 1972).

Although gap junctions in excitable tissues clearly function as electrical synapses and may mediate ionic and metabolic coupling, much less is known about their role in non-excitable tissues where these junctions are most numerous and diverse. They appear to play a critical role in embryogenesis, where monoclonal antibodies to gap junction proteins injected at an early stage leads to arrest of embryogenesis (Warner et al., 1984). In adult tissues where cells are united by gap junctions, there have been implications of involvement in growth control, including tissue repair and neoplastic transformation (Loewenstein, 1966, 1979, 1981). As discussed below, initial studies by Loewenstein (1968) indicated a correlation between controlled cell growth and the presence of gap junctions, and uncontrolled growth in their absence, although later studies have shown that this correlation is only partial. Nevertheless, our studies indicate a synergic action of chemical cancer promoters and EM fields at cell membranes in modification of gap junction functions (Fletcher et al., 1986).

3. MODULATION OF CELL MEMBRANE TRANSDUCTIVE COUPLING BY PERICELLULAR ELECTROMAGNETIC FIELDS.

Based on the Singer-Nicolson fluid mosaic model discussed above, there is a minimum sequence of three steps in transductive coupling (Adey, 1984), and each is calcium-dependent: (a) cell surface glycoproteins that are stranded protrusions from intramembranous helical proteins (IMPs)

fields and static magnetic fields. Effects are maximal around 16 Hz and less at higher and lower frequencies. Neither size nor geometry are determinants of these interactions, which occur over an enormous range of physical dimensions, from intact cerebral cortex to cultured neurons and finally in cerebral synaptosomes with mean diameters around 0.7um. The maximum tissue electric gradients induced by these imposed fields are at the levels of the EEG in fluid around brain cells (0.1 V/cm), seen with exposures to radio-frequency fields in the studies of Bawin et al., Blackman et al., and Dutta et al. cited above. At these levels, they are at least 6 orders of magnitude less than the electric barrier of the membrane potential. However, with low-frequency fields (in the spectrum below 1.0 KHz), coupling to tissues and cell cultures is far weaker, inducing electric gradients typically in the range 10^{-7}-10^{-3}V/cm. These fields also modify tissue Ca^{2+} binding (Bawin and Adey,1976) and modulate calcium-dependent cell mechanisms, including neurotransmitter release at gradients of 10^{-4}V/cm (Dixey and Rein, 1981) and bone matrix formation at 10^{-7}V/cm (Fitzsimmons et al., 1986). These interactions emphasize the importance of amplification in their ultimate effects on intracellular mechanisms discussed below.

3.2 Effects of Combined Static Magnetic and Oscillating ELF Electromagnetic Fields.

About 20 percent of pineal cells in pigeons, guinea pigs and rats respond to changes in both direction and intensity of the earth's magnetic field (Semm, 1983). Experimental inversion of the horizontal component of the earth's magnetic field significantly decreases synthesis and secretion of the pineal peptide hormone melatonin, which powerfully influences circadian rhythms, and also reduces activity in its synthesizing enzymes (Welker et al., 1983). This magnetic sensitivity has been traced to nerve

previously effective 15 Hz field ineffective; and doubling the geomagnetic field caused an ineffective 30 Hz signal to become effective.

3.4 Intracellular Enzymes as Molecular Markers of Transductive Coupling Through Cell Membranes.

We have identified three groups of intracellular enzymes that respond to signals initiated at cell membranes as a response to EM field exposure. These responses occur with or without concurrent interactions with humoral stimuli. These enzymes are: 1) membrane-bound adenylate cyclase involved in activation of protein kinases through conversion of ATP to cAMP, as seen in our studies with bone cells (Luben et al., 1982; Luben and Cain, 1984); 2) cAMP-independent protein kinases that perform messenger functions (Byus et al., 1984); 3) ornithine decarboxylase (ODC), essential for growth of all cells by its participation in synthesis of polyamines essential for DNA formation (Adey, 1986; Byus et al., 1987a and b). All are calcium-dependent and their actions have been reviewed in detail elsewhere (Adey, 1986; see also Proceedings of Conference I of this series).

4. INTERCELLULAR COMMUNICATION AND CANCER PROMOTION; ENZYMATIC MARKERS OF FIELD INTERACTIONS WITH CHEMICAL CANCER PROMOTERS AT CELL MEMBRANES.

Tumor formation as a manifestation of abnormal control of cell growth is now widely modeled as a multistep process, based on animal tumor models. These models of carcinogenesis envisage initial damage to the DNA genome within the cell nucleus. This stage of initiation involves actions of mutagenic substances or agents such as ionizing radiation. Initiated or transformed cells may remain indefinitely in this condition without tumor formation. Tumor formation occurs as a second step requiring promotion by agents that are not mutagenic and thus not cancer initiators by an action on DNA in the nucleus.

Tumor promoters include insecticides such as DDT, polychlorbiphenyls

carcinomas is inhibited (Newmark, 1987). We shall review evidence implicating modified intercellular communication through distorted inward and outward signal streams at cell membranes in cancer promotion.

4.1. Inward Signals at Cell Membranes from Cancer-Promoting Phorbol Esters; Enzyme Activities of Protein Kinases and Ornithine Decarboxylase (ODC).

As noted above, an important cAMP-independent protein kinase that functions both as a receptor and as an enzyme occurs widely in cell membranes and is in highest concentrations in brain tissue (Nishizuka 1983,1984). This enzyme, phosphatidyl serine protein kinase (kinase C), is Ca^{2+}-dependent and normally activated by diacylglycerol formed from inositol phospholipids by the action of cell surface stimuli. Following activation of one molecule of kinase C by a single molecule of diacyl glycerol, Nishizuka has described a spreading domino effect that activates all kinase C molecules around the whole membrane surface. Kinase C is irreversibly activated by phorbol esters. In invertebrate neurons, its activation or injection of the pure enzyme enhances inward Ca^{2+} currents (DeRiemer et al., 1984). Exhaustion of kinase C occurs with continued stimulation by phorbol esters and is associated with loss of cell division. This response is restored by intracellular injection of kinase C (Pasti et al., 1987), thus linking it to paths that signal from cell membranes to nuclear mechanisms mediating cell division. Protein kinase C belongs to a group of cAMP-independent protein kinases which we have identified as sensitive to EM fields (Byus et al., 1984).

We have also shown that induction of ornithine decarboxylase (ODC) follows stimulation of liver and ovary cells with phorbol esters (Fig.2) and that this response is sharply enhanced by 450 MHz fields (1.0 mW/cm^2), sinusoidally amplitude-modulated at 16 Hz (Byus et al., 1987a). Similar enhancement of ODC activity was noted with 60 Hz EM fields at fluid gradients of 0.1-10 mV/cm (Byus et al., 1987b), of the same order

as those induced by high voltage power line exposure. ODC activity in synthesis of polyamines is an essential step in DNA synthesis. Clinically, ODC activity in cultures of suspected cancer cells (for example, human prostatic cancer), has proved a useful index of malignancy. All agents that stimulate ODC are not cancer-promoting, but all cancer promoters stimulate ODC. Although its activation pathways are not well defined, binding of phorbol esters at membrane receptors induces ODC, and ODC activity is increased by 50 percent in a 3h test period following a 1h exposure to the same 450 MHz fields tested above (Byus et al., 1987a). Also, phorbol ester treatment of embryonic fibroblasts previously irradiated with X-rays and microwaves increases transformation frequencies above rates in cells previously irradiated only with X-rays (Balcer-Kubiczek and Harrison, 1985). The findings are consistent with persistent membrane effects from prolonged microwave exposure, and these may enhance promoter action.

4.2. Outward Signals through Gap Junctions; Disruption of Intercellular Communication by Phorbol Esters and Microwaves in Tumor Promotion.

We have noted above that phorbol esters and other chemical cancer promoters disrupt transfer of chemical signals between cells (Yotti et al., 1979). Trosko has further hypothesized that two major types of intercellular communication help to maintain "normal orchestration" of proliferation and differentiation during development and between quiescent stem-progenitor and differentiated cells in the adult (Trosko and Chang,1986): one involving transfer of molecular signals from cells of one differentiation or tissue type to another over an extracellular space and distance (for example, in the action of hormones, growth factors and neurotransmitters); and the other mediated by the transfer of relatively small molecular weight molecules and ions through neighboring cells via gap junctions. In Trosko's model, gap-junctional communication would

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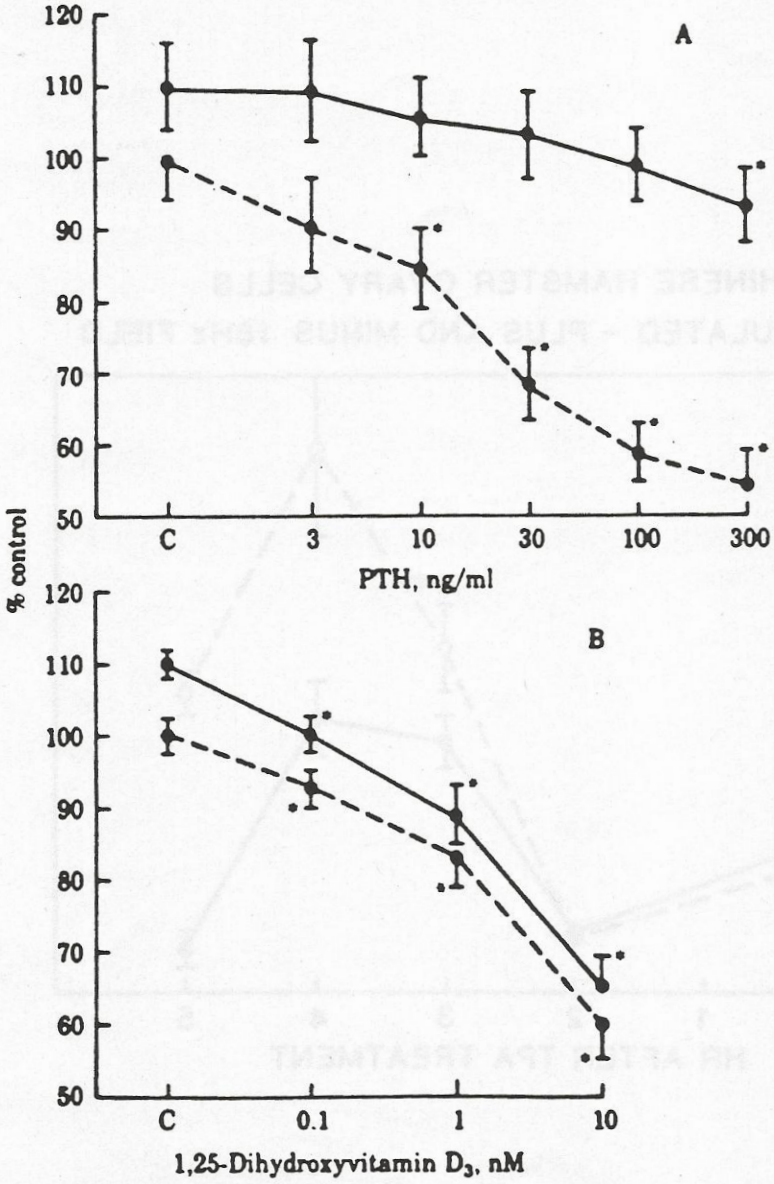
A
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110
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90
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PTH, ng/ml
B
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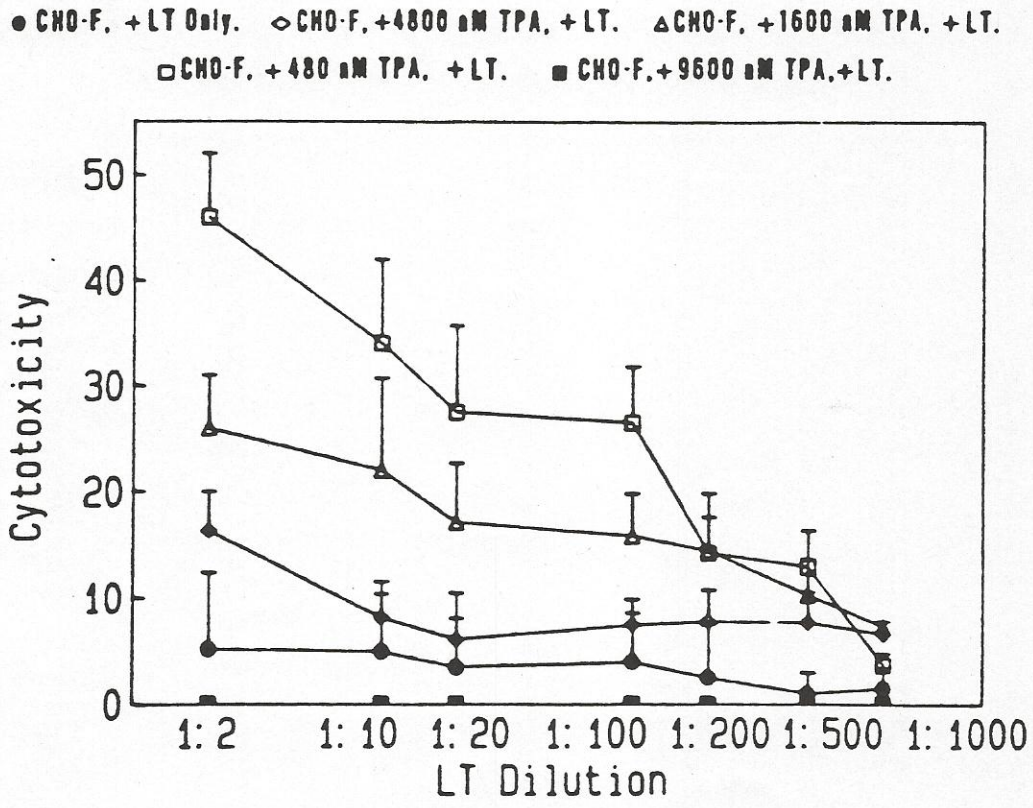
1,25-Dihydroxyvitamin D_3, nM

FIG. 1


● CHO-F. + LT Only. ◇ CHO-F. + 4800 nM TPA. + LT. △ CHO-F. + 1600 nM TPA. + LT.
□ CHO-F. + 480 nM TPA. + LT. ■ CHO-F. + 9600 nM TPA. + LT.
Cytotoxicity
50
40
30
20
10
0
1: 2
1: 10
1: 20
1: 100
1: 200
1: 500
1: 1000
LT Dilution