COMMENTARY

Ion regulation, cell injury and carcinogenesis

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Introduction
This commentary concerns ion regulation, toxicity and carcinogenesis, summarizes current advances, considers new research findings and extends our own previous hypothesis on the role of ion regulation, cell injury and differentiation (1–4). It has become increasingly evident in recent years that there is an intimate relationship between the cellular events involved in cell division, cell death, cell differentiation and carcinogenesis. Virtually all complete carcinogens are acutely toxic at carcinogenic doses and chronic toxicity typically continues during the evaluation of the subsequent neoplasia; for example, wounding in the skin is well established as a promoter, epidemiologic data strongly indicate that the major effect of tobacco smoke is chronic, repeated injurious insults, and there are increasing instances where non-mutagenic, non-clastogenic compounds such as NT A, saccharin and unleaded gasoline that produce initial and chronic toxicity result ultimately in carcinogenesis. It is, therefore, reasonable to hypothesize that a common factor or factors exist in these several seemingly disparate states. In this paper, we will develop the hypothesis that deregulation of intracellular ionized calcium ([Ca\(^{2+}\)]\(_i\)) constitutes such a factor.

Cellular calcium regulation

[Ca\(^{2+}\)]\(_i\) is regulated within narrow limits to very low levels in the cytosol at ~10\(^{-7}\) – 10\(^{-8}\) M. This regulation is in the presence of high concentrations of extracellular ionized calcium ([Ca\(^{2+}\)]\(_e\)) at ~10\(^{-3}\) M. [Ca\(^{2+}\)]\(_i\), therefore, leaks down its gradient into the cytosol through a variety of channels. Three principal buffer systems regulating [Ca\(^{2+}\)]\(_i\) are known to exist in virtually all cells: the plasma membrane, the mitochondria and the endoplasmic reticulum (ER). All require ATP directly or indirectly and all may involve Ca\(^{2+}\)-ATPases. In addition, the plasma membrane may regulate calcium through a Na\(^{+}\)–Ca\(^{2+}\) exchange mechanism which is active in excitable cells and is probably important in other transporting epithelium. At the plasma membrane, therefore, [Ca\(^{2+}\)]\(_i\) regulation is also modulated by the Na\(^{+}\)–K\(^{+}\)-ATPase. Deregulation of the plasma membrane buffer system can occur following ATP depletion, oxidative stress to the membrane from a variety of xenobiotic chemicals and some metabolites of the arachidonic acid (AA) pathway. Calcium ionophores, such as A23187 or ionomycin, can rapidly equilibrate Ca\(^{2+}\) across the cell membrane in the presence of normal [Ca\(^{2+}\)]\(_e\). Release of Ca\(^{2+}\) from the ER is facilitated by the messenger ionositol trisphosphate (IP\(_3\)), one of the main effectors of the phosphatidyl inositol (PI) pathway. In some cells, it also appears that depletion of cellular sulfhydryl groups can be associated with calcium mobilization from the ER. Release of Ca\(^{2+}\) from mitochondria can presumably be accomplished by uncoupling agents, such as halogenated phenols, and by some hydroperoxides of the prostanoic pathway.

Na\(^{+}\)/H\(^{+}\) exchange

Entry of Na\(^{+}\) into the cytosol is commonly accompanied by an increased water influx and a proton efflux. When activated, this Na\(^{+}\)/H\(^{+}\) exchange mechanism results in increased cell volume and alkalinization of the cytoplasm. The Na\(^{+}\)/H\(^{+}\) exchange mechanism is inhibited by amiloride and stimulated by monensin. The Na\(^{+}\)/H\(^{+}\) carrier is also activated at least in some cells, by protein kinase C which, in turn, can be activated by phorbol esters and related compounds or diacylglycerol (DAG) (5). Alkalinization of the cytoplasm is expected to up-regulate calcium-mediated events which are generally pH-dependent.

Ion regulation

Acute cell injury

Acute cell injury, leading to cell death, and after cell division in intact cells at the border of the necrotic zone, is characterized by marked alterations of intracellular ion content in both the pre-lethal or reversible phase as well as in the events that lead to cell death. Such alterations can be summarized as including rapid increases in [Na\(^{+}\)], and [Cl\(^{-}\)], usually accompanied by increased cell water, decreased [H\(^{+}\)], especially in certain types of injury, and early increases of [Ca\(^{2+}\)]. These changes occur in acute cell injury induced by a variety of classes of toxic compounds or conditions including anoxia or ischemia, heavy metals, chlorinated hydrocarbons and polyaromatic hydrocarbons. It is emphasized that the early increase in [Ca\(^{2+}\)] is often followed by an increase in total cell calcium, an increase of which shows a good correlation with cell death. The latter, therefore, seems to be a secondary event, while the former appears to be involved in the initiation of the events leading to cell death. In the case of total cell calcium, large increases are often correlated with massive precipitation of calcium hydroxyapatite within the mitochondria. At earlier stages, sequestration of Ca\(^{2+}\) may also occur in other compartments, including the ER and the mitochondria.

Tumor cells

It has been known for some time that neoplastic epithelial cells have a different ion composition from their normal counterparts (6). This difference is characterized by increased concentrations of Na\(^{+}\) and Cl\(^{-}\), a decreased concentration of K\(^{+}\) and, though few data are currently available, apparently increased [Ca\(^{2+}\)], (7). Although of great potential importance (see below), we currently have little information on any steady state or transient alteration of [H\(^{+}\)], in neoplastic cells (8). While similar changes in Na\(^{+}\), K\(^{+}\) and Cl\(^{-}\) occur in regenerating epithelia, some comparisons have indicated that the tumour cell changes are significantly greater.
Cell division

The best evidence for a role of ion shifts in cell division comes from studies of fertilization in which an abrupt Ca$^{2+}$ influx is followed quickly by an influx of Na$^+$ and efflux of H$^+$, with alkalinization of the cytoplasm followed by DNA synthesis and cell division (9,10). Hesketh et al. (11) found a similar sequence following stimulation of mitosis in mouse thymocytes and 3T3 fibroblasts, but in this and other systems, the mechanism by which alkalinization induces the events leading to cell division is uncertain. It is, however, clear that in cultured mammalian cells, alkalinization can often stimulate cell division, simulating the action of growth factors (12). Interestingly, an abrupt rise in [Ca$^{2+}$], occurs in PtK cells shortly before anaphase (13), calcium ionophores stimulate mitosis in Funaria (14) and calmodulin antagonists can block cell division in some systems (15).

Differentiation

In the epidermis, terminal differentiation of the epithelium is characterized by massive accumulation of keratin filaments and formation of cross-linked envelopes which are associated with a marked increase in total cell calcium. Addition of calcium ionophores, such as A23187, can rapidly induce the formation of cross-linked envelopes in epithelial cells, which have keratin filaments and the calcium-dependent, cross-linking protein, involucrin (16). When murine epidermal cells are cultured in vitro, the concentration of [Ca$^{2+}$], exerts a marked effect on differentiation (17). In the presence of 1 mM [Ca$^{2+}$], these cells cease to divide and undergo terminal differentiation with keratinization. On the other hand, when the cells are plated in low Ca$^{2+}$ media, cell division continues and little or no differentiation occurs. A similar situation exists in normal human bronchial epithelium where the calcium effect is potentiated by serum, the active principle of which has recently been shown to be transforming growth factor-3 (TGF-3) (18). Similar effects of [Ca$^{2+}$], on proliferation have been noted in other epithelia in vitro and have also been suggested for colon epithelium in vivo (19). In the skin in vitro, treatment of cells with carcinogenic agents such as DMBA is followed by a change in the response to high Ca$^{2+}$ medium — namely proliferation continues to occur. At the present time, very little is known concerning the effects of modifying the concentration of [Ca$^{2+}$], on [Ca$^{2+}$], or on [H$^+$], and fibroblasts may respond quite differently to [Ca$^{2+}$], (20). Not all epithelia respond in the same fashion. Grisham et al. (21), for example, observed that hepatocytes may also respond differently from epidermal cells.

Calcium and the cytoskeleton

As cytoskeletal function is closely linked to a number of cell functions including motility, shape, endocytosis, secretion and division, its regulation is evidently important in carcinogenesis. Many functions of the cytoskeleton are closely related to [Ca$^{2+}$]i, e.g. calcium—calmodulin complexes are involved in the depolymerization of microtubules and the contraction of actin, and the polymerization of keratin. Accordingly, rearrangements of both [Ca$^{2+}$], and calmodulin seen during the mitotic cycle may well relate to the shortening of the mitotic spindle during anaphase. Also, the blebs seen during early cell injury can be reproduced by inhibitors of microtubules, such as cytochalasin or vinblastine. Asbestos is known to interfere with chromosomal arrangement in that it is associated with rapid clastogenic effects (22). Treatment of normal human bronchial epithelium in vitro with amosite asbestos has been found to induce microtubule disruption and it is reasonable to propose that this action of asbestos may, in turn, relate to ion deregulation (unpublished results). Asbestos fibers are often found in the cytosol, having evidently directly pierced the plasma membrane or the membrane of endocytic vacuoles. Such mechanical damage to the cell membrane would be expected to result in ion deregulation.

Cell—cell communication and growth regulation

The importance of ion regulation in cell junction integrity is exemplified by the cell—cell dissociation that occurs in Ca$^{2+}$, free media. Such dissociation involves all types of cell junctions (23). In contrast, only gap junction function is known to be modified when [Ca$^{2+}$], levels are increased. Trosko and colleagues (24) have developed an in vitro assay for tumor promoters based on various techniques of measuring cell—cell communications, and most promoters tested blocked cell—cell communication in vitro. This can be correlated with decreased gap junction areas as measured by electron microscopy. Similar decreases in gap junctions occur in hepatic regeneration, as reviewed by Peracchia (25).

Growth factors and oncogene products in relation to calcium regulation

Several growth factors and other oncogene products can affect calcium regulation directly or indirectly. In A431 cells, epidermal growth factor (EGF) modifies calcium channels independently of phospholipid hydrolysis. On the other hand, platelet-derived growth factor (PDGF) is associated with phospholipid hydrolysis and mobilization of calcium through release of IP$_3$. Phospholipid hydrolysis is also apparently modified by src and ras products resulting in the formation of IP$_3$ and DAG. Ras products may represent a G protein which may be important in PDGF and other growth factor activities.

Using digital imaging microscopy and the fluorescent probe Fura 2, Tucker and Loats (26) measured changes in [Ca$^{2+}$], after stimulation of fibroblasts with EGF. Their data showed that EGF-induced increases in [Ca$^{2+}$], were not homogeneous in the cytosol but that they proceeded initially in localized regions of the cytoplasm, indicating that gradients of [Ca$^{2+}$], can exist. Furthermore, Williams et al. (27), studying excitation—contraction coupling in smooth muscle, recently noted that the nuclear envelope may be able to regulate [Ca$^{2+}$], in the nucleoplasm at levels different from those in the cytosol. These new imaging technologies promise to significantly augment our ability to further study the regulation of [Ca$^{2+}$], in much greater detail.

Hypothesis

Based on these considerations, we have formulated a hypothesis (Figure 1) to explain and to predict the relationships between cell injury and carcinogenesis, emphasizing the role of ion deregulation. The focus of the hypothesis is the regulation of [Ca$^{2+}$].

Cell membrane

The Ca$^{2+}$-ATPase and Na$^+/Ca^{2+}$ exchange at the plasma membrane can be modulated by a variety of toxic and other substances. These include ATP deficiency, essential for both Na$^+/K^+$, and Ca$^{2+}$-ATPases, and events that modify Ca$^{2+}$ entry, such as EGF, calcium ionophores, oxidative stress, or Na$^+$ entry, such as sodium ionophores including monensin and complement activation. The induction of oxidative stress by a variety of events, including prostaglandin metabolites, TPA and many other events following metabolism of xenobiotics can rapidly modify

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Permeability. It is less clear in this case whether or not the specificity of the reactions can distinguish between ions; however, perturbation of the phospholipid bilayer or of protein-lipid interaction is evidently associated with significant ion leakiness of the plasma membrane. The effects of such interactions on injury and differentiation need more study, although it is clear that the early events of cell injury, such as increased Na⁺, decreased K⁺ and increased ionized Ca²⁺, resemble the events following fertilization, which ultimately initiate cell division. In some cases, modification of regulation can modify responses. For example, it has been shown that A23187 is a first stage promoter and that amiloride, blocker of the Na⁺/H⁺ exchange mechanism, caused significant lowering of [Na⁺]j in hepatoma cells which correlates with decreased proliferation rates. The increased Na⁺ may trigger alkalinization of the cytosol as well as increased [Ca²⁺]j through diminished Na⁺/Ca²⁺ exchange.

Mitochondria
The active uptake and release system of mitochondria for [Ca²⁺]j has been well characterized. At the present time, however, little is known about the possible role for this system in tumorigenesis, though it is known that uncouplers of oxidative phosphorylation and decreased levels of NADPH resulting from oxidative stress can be associated with Ca²⁺ release to the cytosol. It has also been proposed that hydroperoxides resulting from prostanoid metabolism and, therefore, to the action of phospholipase A₂, can also induce mitochondrial Ca²⁺ release. Some data indicate that anti-prostaglandin or anti-inflammatory agents are also anti-promoters. Prostaglandin-mediated stimulation of mitosis in mouse epidermis in vivo is stimulated by Ca²⁺ ionophore or TPA (28). The compound anthralin, a promoter in mouse skin, has also been suggested to act on the mitochondrial release mechanism or at least on oxidative phosphorylation. Oxidative stress and diminished levels of NADPH are common to a variety of tumor promoting agents, including both receptor and non-receptor classes (29).

The endoplasmic reticulum
The ER constitutes an effective Ca²⁺ buffer system, especially in excitable cells, but may play a hitherto unknown role in epithelia, e.g. renal tubular or hepatic parenchymal cells. Work on the PI pathway has indicated that one of the significant signals for Ca²⁺ release from the ER is the metabolite IP₃, released from PIP₂ by phospholipase C which is activated by [Ca²⁺]j, providing a positive feedback mechanism. Furthermore, accumulation of PIP₃ is stimulated by inhibitors of its hydrolysis.
such as lithium or the ras gene product and inhibited by another feedback mechanism involving protein kinase C. The concentration of IP3, therefore, appears to be an important control point which can exert significant positive or negative feedback interactions on [Ca2+]. Recent progress in the PI pathway, involving the role of G proteins, involving the ras product and stimulated by oncogene products, including PDGF and part of the EGF receptor also may positively trigger this pathway. However, the role of the ER in non-excitatory cells, such as epithelia, needs much more attention. Kaibuchi et al. (30) suggested a role of kinase C and [Ca2+]i in growth factor induced myc expression in 3T3 cells.

The PI pathway

The PI pathway appears to be an important control mechanism related to [Ca2+]i. (31). The bifurcation resulting from phosphodiesterase-induced formation of IP3 and DAG has important implications beyond those of intracellular ion regulation, in that DAG activates protein kinase C. It is already clear in some cells that through membrane receptors, both the phorbol ester class of promoters as well as negative growth factors such as TGFβ activate kinase C. Though [Ca2+]i is permissive for protein kinase C, a major question remains as to the role of [Ca2+]i in the activation of the amiloride-sensitive Na+/H+ carrier. Activation of this Na+/H+ proton carrier often stimulates a rise in pH and an increase in [Na+]i, which can be directly fostered by monensin. In some cell types, alkalization of the cytoplasm results in division, while in others it may result in terminal differentiation. The nature of the switch and whether or not it is [Ca2+]i-dependent needs further characterization.

Consequences of deregulation of [Ca2+]i

It is hypothesized that increased [Ca2+]i will have a number of important effects on the cell relevant to cell injury, tumor promotion, and carcinogenesis. These effects include activation of [Ca2+]i-dependent enzymes e.g. phospholipases, proteases, and nucleases, formation of cross-linked envelopes, activation of genes such as myc possibly resulting in cell division, depolymerization of microtubules, and decreased cell/cell communication. Furthermore, enzymes resulting in DNA hydrolysis, such as some endonucleases are [Ca2+]i-dependent and, if increased, may result in single-strand breaks and genetic rearrangements.

Activation of phospholipase A2 results in the simultaneous activation of the prostaglandin pathway and of phospholipid degradation leading to membrane damage. If continued, membrane damage results in cell death, while the arachidonic acid metabolites may recycle, fostering release of Ca2+ from mitochondria, activation of oxidative stress at the cell membrane and release of putative calcium ionophores such as phosphatidic acid. The formation of cross-linked envelopes in growth factor- or promoter-induced terminal differentiation is [Ca2+]i-dependent as are other cytoskeletal alterations, including actin contraction and depolymerization of microtubules. Micro-environmental redistributions of [Ca2+]i may occur during cell injury and induction of division as evidenced by the wave of ionized Ca seen in fertilization in marine eggs. Increase in [Ca2+]i modifies cell/cell communication through gap junctions and such diminished communication has been proposed by Trosko (24) as an important indicator of tumor promoting activity. In summary, neoplasia is a complex process involving acute and chronic cell injury in a setting that modifies genes and/or gene expression. We propose that ion deregulation, especially that of [Ca2+]i, is a major mediator in the process and, therefore, provides a critical link between acute cell injury, tumor promotion, and carcinogenesis.

Acknowledgements

This work was supported by NIH Grants AM15440 and N01-CP-15738. This is Contribution no. 2360 from the Pathobiology Laboratory.

References


Received on May 8, 1987; accepted on May 28, 1987