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Forum Review

Activation of the Calcium/Calmodulin-Dependent Protein Kinases as a Consequence of Oxidative Stress

RICHARD A. FRANKLIN,[1,2] OSWALDO G. RODRIGUEZ–MORA,[3]
MICHELLE M. LaHAIR,[1] and JAMES A. McCUBREY[1,2]

ABSTRACT

Oxygen radicals have diverse effects on cells. In many cases, exposure to reactive oxygen intermediates (ROI) can induce cell death. Conversely, there is also evidence that suggests oxygen radicals can activate signaling pathways that are thought to prevent cell death. In this review, the authors discuss the finding that hydrogen peroxide and ROI-generating treatments trigger the activation of the calcium/calmodulin-dependent kinases (CaM-kinases), and the potential role this activation has in preventing apoptosis. Evidence is presented that CaM-kinase activation occurs by both calcium dependent- and independent-pathways in response to ROIs. In addition, the idea is discussed that ROIs have the potential to lead to the phosphorylation of calmodulin and through this mechanism potentiate the activation of the CaM-kinases. The concept that inhibition of the CaM-kinases as a mechanism to sensitize cells to the damaging effects of ROIs is also presented. Contrasting these studies, evidence is presented that exposure of the CaM-kinases directly to hydrogen peroxide also has the apparent ability to inhibit their activity. *Antioxid. Redox Signal.* 8, 1807–1817.

INTRODUCTION

CELLS CAN BE EXPOSED to oxygen radicals intrinsically by way of electrons escaping from the mitochondrial electron transport chain and passing directly to oxygen forming O_2^-. O_2^- can be spontaneously dismutated, or acted on by superoxide dismutase, giving rise to hydrogen peroxide. Extrinsic exposure to oxygen radicals can occur in inflammatory environments where both superoxide and hydrogen peroxide are produced. Furthermore, triggering of certain surface receptors leads to the intrinsic generation of hydrogen peroxide, which is thought to influence cellular signaling. For example, triggering of the T cell receptor leads to the production of hydrogen peroxide and this production is reported to have a role in the activation of extracellular signal regulated kinase (ERK) (51). Stimulation of the epidermal growth factor (EGF) receptor on cells also leads to hydrogen peroxide production (5), and hydrogen peroxide plays a role in both insulin (55) and vascular endothelial growth factor (VEGF) signaling (17). Reactive oxygen intermediates (ROIs) are also generated in response to cytokines such as tumor necrosis factor alpha (28) and interleukin 1β (62). In addition, many cancer treatments will induce the generation of ROIs. In this review we will discuss the ability of certain ROIs to activate a family of protein kinases known as the calcium/calmodulin-dependent kinases (CaM-kinases).

THE CaM-KINASES

The CaM-kinases are a family of proteins that share broadly similar structure and mechanisms of activation. The CaM-kinase family consists of CaM-kinase I, II, III, and IV, myosin light chain kinase, and phosphorylase kinase (37, 56). Many individual members of this kinase family appear to have diverse roles and distribution. They reside in both

[1]Department of Microbiology and Immunology, and [2]the Leo W. Jenkins Cancer Center, Brody School of Medicine at East Carolina University, Greenville, North Carolina.
[3]Corporacion Ecuatoriana de Biotecnologia, Quito, Ecuador.

the cytoplasm and nucleus of the cell (49, 80). Often the role that these kinases have in the cell is determined by the tissues in which they are expressed. For example, in neuronal tissue, certain CaM-kinases (CaM KII) are thought to have a role in memory (58). In T lymphocytes, CaM KII may have an important role in regulation of CD8 T cell proliferation, cytotoxic effector function, and the response to restimulation (53). CaM-kinases I, II, and IV all demonstrate broad substrate specificity. The other CaM-kinase family members show greater limitation in the number of different substrates they are capable of phosphorylating (56).

All the members of this kinase family are structurally similar, with an N-terminal kinase domain and an autoinhibitory domain (Figs. 1 and 2). The autoinhibitory domain also contains an overlapping calmodulin-binding domain. A C-terminal association domain responsible for multimerization is a feature shared by phosphorylase kinase and CaM-KII (Fig. 1), but not the other family members (18, 37). When calmodulin is associated with calcium, this complex is able to bind to the different CaM-kinase family members and result in the autoinhibitory domain becoming displaced from the immediate proximity of the catalytic pocket. Figure 1 demonstrates this process for CaM KII. The movement of the autoinhibitory domain from the catalytic pocket allows substrate access (14). CaM-kinases are also phosphorylated following calcium/calmodulin binding (Figs. 1 and 2) and this phosphorylation is reported to both prolong and further increase kinase activity (69).

Four different forms of CaM-KII are encoded by four separate genes (α, β, δ, and γ) . The RNA produced from these genes can be alternatively spliced, leading to the production of at least 24 different isoforms of CaM-kinase II *in vivo* (10, 11, 70, 71). Every cell type has at least one isoform of CaM KII, and some cell types will express multiple isoforms (37). The expression of CaM KIV is much less diverse. CaM KIV expression occurs in greatest abundance in the testes and T lymphocytes. CaM KI is reported to be expressed at the highest quantities in the brain, testes, ovaries, and adrenal gland (73). There are two forms of CaM-kinase kinase (CaM-KK), α and β. CaM-KK β exhibits the highest expression in the brain (73).

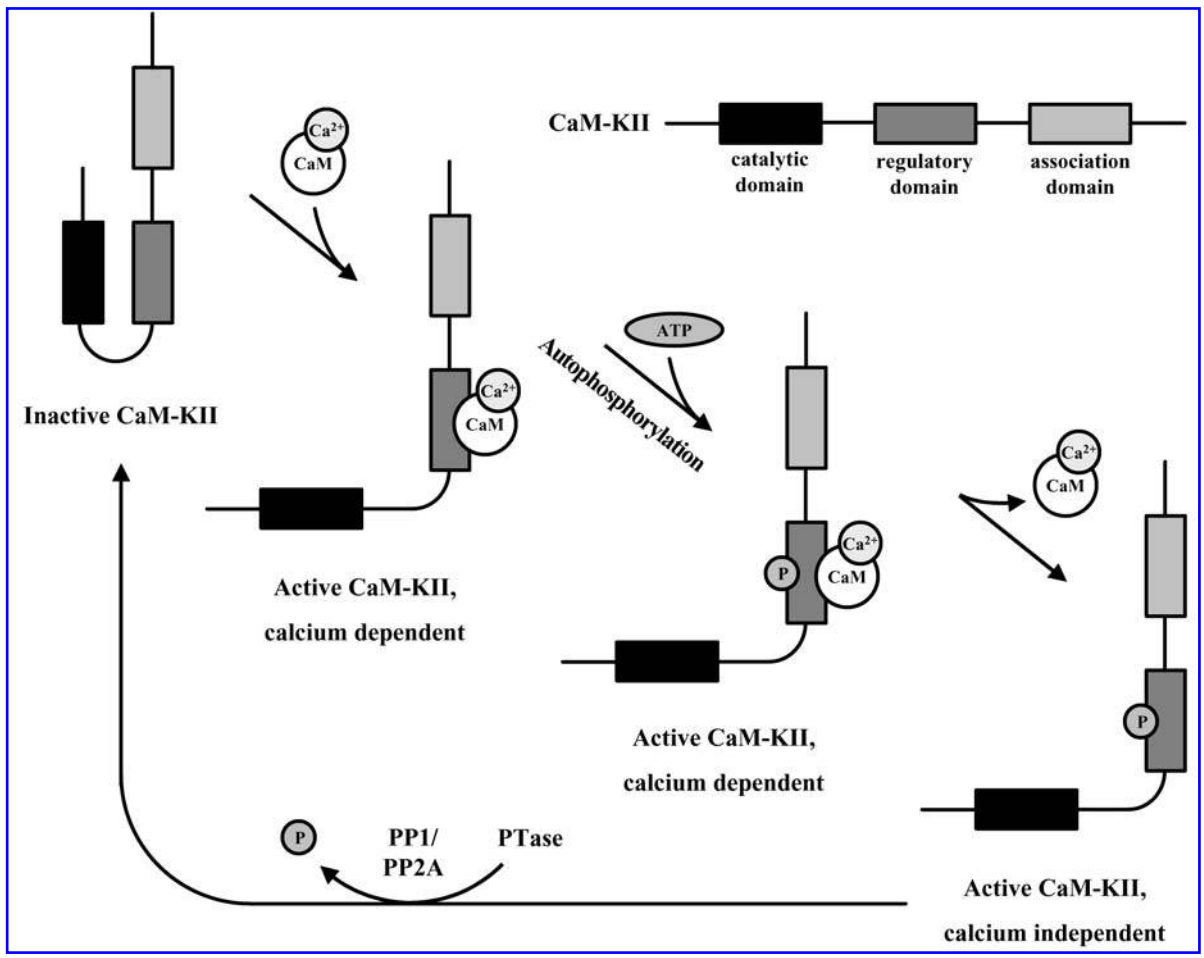

FIG. 1. Structure and regulation of CaM-kinase II. The general structure of CaM-KII includes an N-terminal kinase domain (catalytic), followed by an autoinhibitory domain (regulatory), and a C-terminal association domain that is responsible for multimerization. The activity of CaM-KII is regulated initially by binding of calcium/calmodulin complexes to the enzymes and subsequently by phosphorylation. Dephosphorylation by a regulatory phosphatase is required for inactivation.

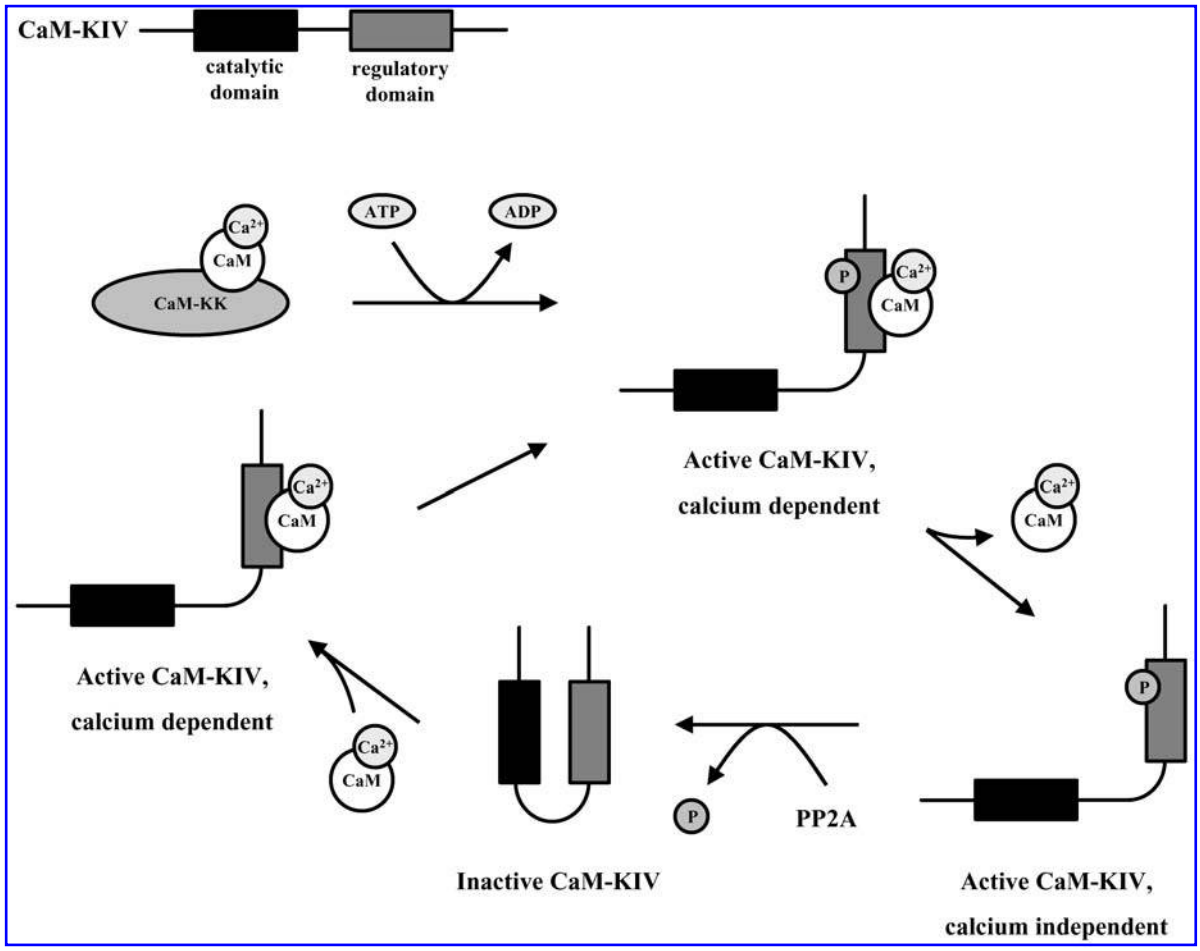

FIG. 2. Structure and activation of CaM-kinase IV. The general structure of all CaM-KIV includes an N-terminal kinase domain (catalytic), followed by an autoinhibitory domain (regulatory), and an overlapping CaM-binding domain (association). CaM-kinase IV requires both binding by Ca^{2+}/CaM as well as phosphorylation by an upstream kinase CaM-KK. CaM-KK is also activated by Ca^{2+}/CaM. Like CaM-kinase II, CaM-kinase IV retains activity as long as it is phosphorylated. The phosphatase PP2A can dephosphorylate and inactivate CaM-kinase IV.

CONVENTIONAL ACTIVATION OF THE CaM-KINASES

The activation of CaM-kinases by conventional means requires binding of calcium/calmodulin to the enzyme to initiate the process. In resting cells, the levels of cytoplasmic and nuclear calcium are low (approximately 100 n*M*) when compared to the extracellular and endoplasmic reticulum concentrations. Upon stimulation of the cell, cytoplasmic/nuclear levels of calcium can change dramatically due to the opening of calcium channels in the extracellular membrane and/or inositol trisphosphate-gated calcium channels in the endoplasmic reticulum. This increase in nuclear/cytoplasmic calcium results in an increase in the amount of calcium/calmodulin complexes and activation of calcium/calmodulin enzymes. The levels of nuclear/cytoplasmic calcium can change in the absence of any total change in the level of intracellular calcium due to release from the endoplasmic reticulum. For the purpose of this review, the increases in nuclear/cytoplasmic calcium will be referred to as increases in cytoplasmic calcium.

CaM KII forms into a multimeric enzyme that is typically composed of 10–15 catalytic subunits (7, 47). Upon calcium/calmodulin binding, adjacent catalytic subunits phosphorylate each other in the pseudosubstrate domain on Threonine 286 in the autoinhibitory domain (Fig. 3). Both the phosphorylating subunit and the subunit that is phosphorylated must be bound to calcium/calmodulin in order for this phosphorylation to occur (Fig. 3) (34, 59). Following phosphorylation, CaM KII activity becomes independent of calcium/calmodulin binding, and dephosphorylation of CaM KII must occur to return to an inactive state (18, 19, 25, 26). It is thought that this manner of phosphorylation and dephosphorylation of CaM-KII can lead to long-term changes in the activation of the CaM KII such as those required for memory (58). It is predicated that enzymes composed of 15 subunits of CaM-KII can have a stable persistent activation span of a few years to a human lifetime (58). This is because if the kinase rate within the enzyme remains high, in comparison to the phosphatase rate, the enzyme can continually reactivate by rephosphorylation by adjacent subunits (58). Both protein

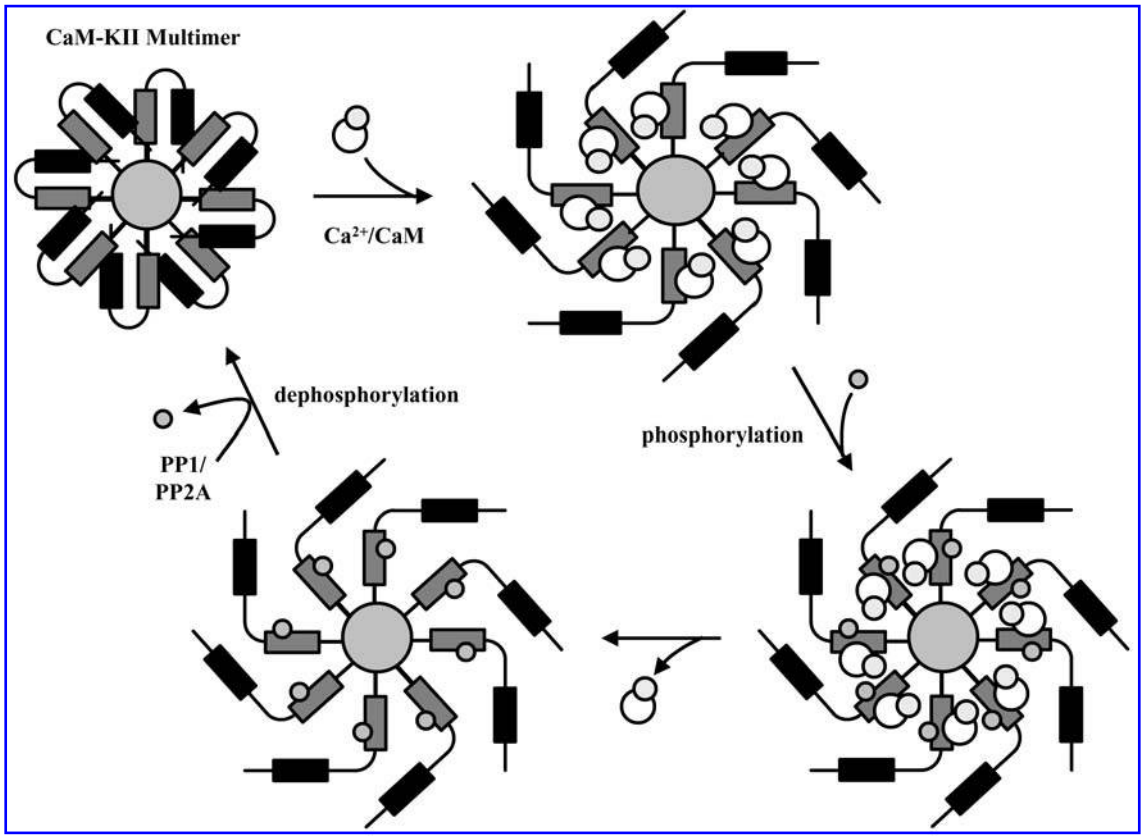

FIG. 3. Activation of CaM-kinase II. An increase of cytoplasmic calcium bound to calmodulin results in a conformational change that allows calmodulin to bind to CaM-kinase II. This binding activates CaM-kinase II, causing each subunit in the multimer to phosphorylate its neighbor. When calcium levels decrease in the cell, as long as CaM-kinase II is phosphorylated, it retains activity. Dephosphorylation by a regulatory phosphatase is required for inactivation.

phosphatases 1 and 2A have important physiological roles in the dephosphorylation of CaM KII (72). Treatment of cells with agents that increase cytoplasmic calcium, such as calcium ionophores, result in the activation of CaM KII (78).

CaM KIV is partially activated by the binding of calcium/calmodulin. Full activation of CaM KIV also requires phosphorylation. The phosphorylation of CaM KIV occurs within the activation loop on Threonine 200. This phosphorylation does not occur via autophosphorylation, but instead is mediated by another CaM-kinase, CaM-KK, which becomes active following calcium/calmodulin binding (23, 67). Similar to the other CaM-kinases, CaM-KK is activated by calcium/calmodulin complexes; however, the potential for other means of regulation has been suggested (2, 23, 35). Similar to CaM KII, phosphorylation of the critical threonine residue on CaM KIV by CaM-KK results in calcium-independent CaM KIV kinase activity. Phosphorylation of CaM KIV also increases the range of substrates that CaM KIV can phosphorylate (36). Also similar to CaM KII, removal of the phosphate group is required to shut down the kinase activity of the CaM KIV and the phosphatase PP2A is also thought to have a role in this inactivation (61, 77). Treatment of cells with agents that increase cytoplasmic calcium also results in the activation of CaM KIV similar to CaM KII (62, 76).

OXIDATIVE STRESS AND INCREASES IN CYTOPLASMIC CALCIUM

Oxidative stress is reported to induce increases in intracellular cytoplasmic calcium in a variety of cells. The laboratory of Sten Orrenius was one of the first groups to demonstrate that *t*-butyl hydroperoxide could increase cytoplasmic calcium in hepatocytes (6). This was an important and novel finding that was subsequently replicated by others also using hepatocytes (65, 66). These were important findings because they were the first to demonstrate the potential for ROIs to increase intracellular calcium in certain cells. Since this initial discovery in hepatocytes by Bellomo *et al*. (6), *t*-butyl hydroperoxide has also been shown to induce cytoplasmic calcium increases in other tissues as well. Ikeda *et al*. (43) reported that *t*-butyl peroxide induced a calcium flux in neurons and that this response also occurred in organotypic cultures of preoptic/anterior hypothalamus.

The release of calcium from intracellular stores by peroxide-containing compounds has been suggested to come from at least two different sources. In neurons, the increase in cytoplasmic intracellular calcium induced by *t*-butyl peroxide appears to be from the endoplasmic intracellular stores, as thapsigargin pretreatment inhibits intracellular calcium cyto-

plasmic increases by *t*-butyl peroxide (54). This does not appear to be the only store that can give rise to increases of calcium in the cytosol. *t*-Butyl hydroperoxide treatment of hepatocytes results in release of calcium from both mitochondrial and extramitochondrial compartments (6). Release of calcium from the endoplasmic reticulum has been proposed to occur by at least two mechanisms. One is via *t*-butyl peroxide increasing the sensitivity of the 1,4,5-trisphosphate receptor (8). The other mechanism may be mediated in part by the redox state of the cells as oxidized glutathione can mimic some of these same effects as *t*-butyl hydroperoxide (65).

Hydrogen peroxide also induces increases in cytoplasmic calcium in a number of cell types. This finding was first reported by Hyslop *et al.* (42). These authors found that hydrogen peroxide treatment of the P388D1 mouse macrophage cell line, at micromolar concentrations, resulted in increased cytoplasmic calcium concentrations. Sen *et al.* (68) later demonstrated that hydrogen peroxide treatment of both Jurkat and Wurzburg cells induced increases in intracellular calcium and that this increase in intracellular calcium had a role in NF-kappaB activation. Although these studies used endogenously added hydrogen peroxide, they were important in that they indicated that a reactive oxygen species that can be generated through normal cellular processes was capable of inducing changes in intracellular calcium. Our laboratory also reported that hydrogen peroxide caused an increase in cytoplasmic calcium in T lymphocytes, confirming the results of Sen *et al.,* and found that this increase was similar to what could be seen following stimulation via the T cell receptor (38). Hydrogen peroxide also induces calcium fluxes in cells of the vascular system (44, 57). The increase in cytoplasmic calcium, induced by hydrogen peroxide in endothelial cells, appears to be derived entirely from intracellular sources, since BAPTA-AM could block this increase (12). The ability of hydrogen peroxide to flux calcium in endothelial cells can be influenced by other signaling pathways. Mergler *et al.* (57) demonstrated that epidermal growth factor treatment of endothelial cells inhibited the increase in cytoplasmic calcium concentrations induced by hydrogen peroxide. Treatment of cells with xanthine oxidase/hypoxanthine results in the generation of superoxide anion. Treatment of smooth muscle (50), endothelial (45), and neuronal cells (75) with xanthine oxidase/hypoxanthine induces increases in cytoplasmic calcium. At least in some cases the effects of superoxide anion may be mediated via its dismutation to hydrogen peroxide as catalase has been shown to prevent increases in intracellular calcium in response to xanthine oxidase/hypoxanthine (4).

A number of reports that used a variety of cell types demonstrated that increases in cytoplasmic calcium trigger activation of the CaM-kinases. This can best be shown using calcium ionophores such as ionomycin and A23187. When cells are treated with these agents, CaM-kinase activation can be observed. Given the data using calcium ionophores, the increase in cytoplasmic calcium mediated by ROIs would be expected to promote the formation of calcium/calmodulin complexes and the subsequent activation of the CaM-kinases. This appears to be the case, since hydrogen peroxide is reported to induce CaM-kinase activation in endothelial cells (12), T lymphocytes (39), and breast epithelial cells (unpublished observation). The concept that ROIs can induce increases in cytoplasmic calcium in cells and the subsequent activation of the CaM-kinases is illustrated in Fig. 4. While a good number of articles have appeared demonstrating the overall effect of ROIs in increasing cytoplasmic calcium, very little is known on the potential role of the CaM-kinases in reactive oxygen intermediate-induced responses (12, 38, 39).

REDOX ACTIVATION OF THE CaM-KINASES IN THE ABSENCE OF A CALCIUM FLUX

Hughes *et al.* reported that inhibitors of calmodulin binding prevented PMA-induced NF-κB activation in Jurkat T lymphocytes (40). More recently, this same group demonstrated that PMA was able to induce I-κB kinase (IκK) activation (41). IκK has a critical role in NF-κB activation as it phosphorylates I-κB and targets I-κB for degradation. The degradation of I-κB results in NF-κB being able to move to the nucleus where it is able to act as a transcription factor. In this recent publication, the authors further demonstrated that both a calmodulin inhibitor and a CaM-kinase inhibitor (KN-93) were capable of inhibiting PMA-induced I-κB phosphorylation (41). Furthermore, these investigators demonstrated that transfection of these cells with a constitutively active CaM-kinase resulted in I-κB phosphorylation (41). These results strongly suggest that PMA results in the activation of the CaM-kinases. However; these results neither demonstrate the mechanism by which this occurs, nor do the results directly demonstrate CaM-kinase activation by these agents. PMA is not known to induce a calcium flux in T lymphocytes, but it is known to result in the generation of oxygen radicals by the cell (64).

A number of investigators have demonstrated that hydrogen peroxide can induce NF-κB activation. In Wurzburg T lymphocytes, hydrogen peroxide induces a calcium flux and results in NF-κB activation (68). We recently reported that treatment of Jurkat T lymphocytes with PMA results in I-κB degradation (38). Treatment of Jurkat T lymphocytes with hydrogen peroxide resulted in I-κB phosphorylation; it did not result in I-κB degradation (38). In our experiments, inhibition of the CaM-kinases downregulated I-κB phosphorylation in response to hydrogen peroxide and I-κB degradation in response to PMA. Since PMA results in the formation of oxygen radicals by cells, it is possible that PMA is mediating its effects via the generation of oxygen radicals and subsequent activation of the CaM-kinase and not via a calcium flux (39). Howe *et al.* reported that blocking increases in cytoplasmic calcium using EGTA did not prevent I-κB phosphorylation or degradation in response to hydrogen peroxide treatment (38). These studies, although suggestive of CaM-kinase activation in the absence of increases in cytoplasmic calcium, neither directly addressed the activation of the CaM-kinases nor did they determine the mechanism by which the CaM-kinases were potentially activated by redox stress.

Another recent report from our laboratory indicated that treatment of cells with agents that result in an oxidative stress induced the activation of the CaM-kinases (39). In

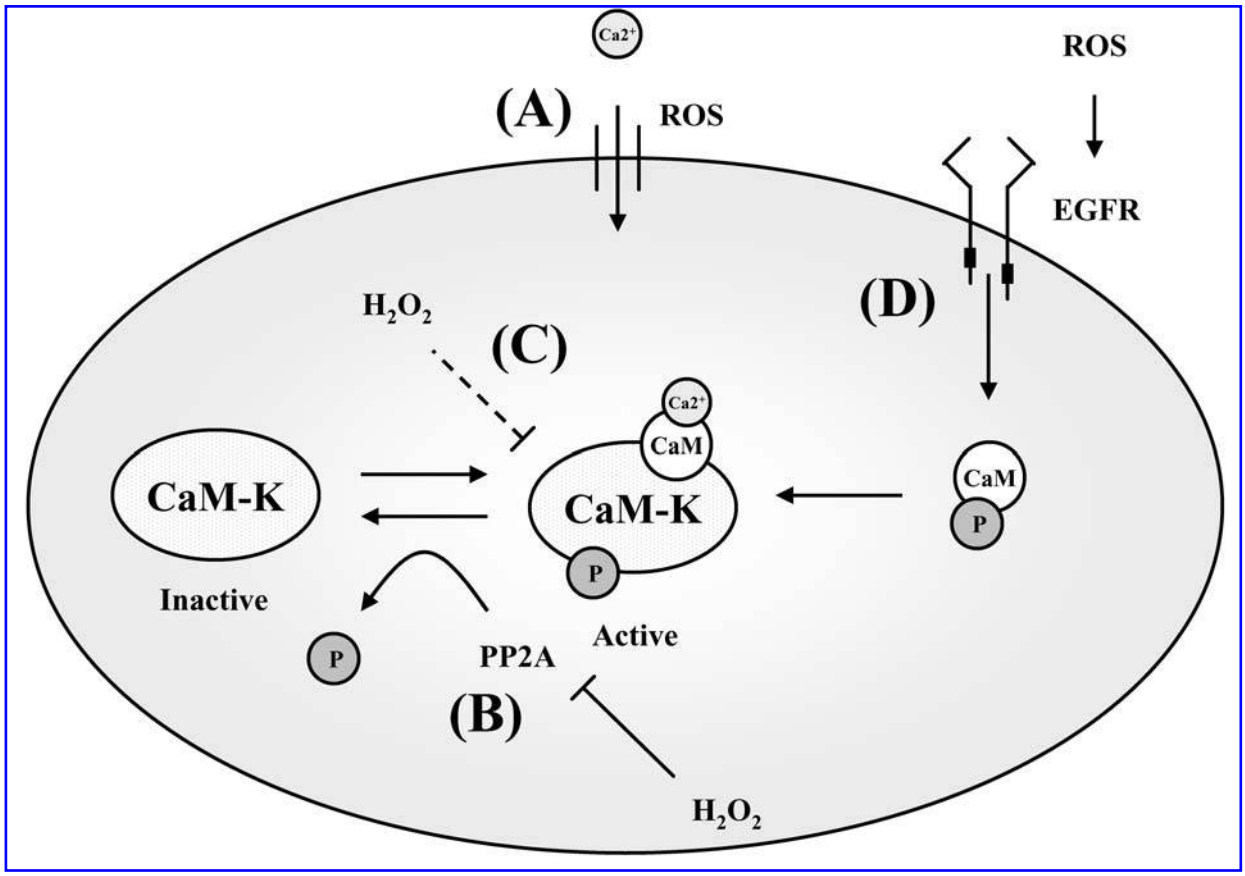

FIG. 4. Potential mechanisms by which oxidative stress can influence the activity of the CaM-kinases. (**A**) Oxidative stress can induce an increase in intracellular calcium leading to the activation of the CaM-kinases; (**B**) oxidative stress can lead to the activation of the CaM-kinases via phosphatase inhibition; (**C**) oxidants can directly inhibit the activity of the CaM-kinases; and (**D**) oxidants can potentially influence the phosphorylation of calmodulin leading to enhanced affinity for the CaM-kinases.

these studies, cells were treated with hydrogen peroxide, PMA, or glucose oxidase; and the ability of immunoprecipitates of CaM-kinase II and IV from these cells to phosphorylate the CaM-kinase specific substrate Syntide-2 was measured. These experiments were carried out in the presence and absence of EGTA in the culture media during stimulation with these agents. The presence of EGTA in the cultures during stimulation elucidated the role of calcium in the activation process of these kinases. It should be noted the presence of EGTA in cultures during stimulation was sufficient to block any increase in cytoplasmic calcium concentrations occurring due to these treatments. We found that CaM-KII and CaM-KIV activity was increased following stimulation with hydrogen peroxide, PMA, or glucose oxidase, regardless of whether increases in cytoplasmic calcium were prevented using EGTA (39). CaM-kinase activity was also increased with ionomycin, a positive control, and this activation was blocked by treatment with EGTA (39). Catalase prevented PMA-induced, but not ionomycin-induced, activation of CaM-KII and IV, indicating a role for reactive oxygen intermediates in the PMA-induced response. These results were the first to indicate that ROIs can induce CaM-kinase activity in the absence of increased concentrations of cytoplasmic calcium. It should be noted that the levels of hydrogen peroxide used in these experiments were likely above physiological levels or possibly even the levels found in the microenvironment of the cell. It is also important to note that the levels of hydrogen peroxide used to activate the CaM-kinases by Howe *et al.* did cause some toxicity at 24 h (38, 39). The experiments using PMA and catalase, however, suggest that physiologic levels of hydrogen peroxide may also be capable of causing similar activation without the associated toxicity. Future experiments should focus on determining the levels of hydrogen peroxide capable of inducing calcium-independent activation of the CaM-kinases in a variety of cell types. Although the levels of hydrogen peroxide used in these experiments did induce some toxicity, they are important findings in that they indicate that the cells are activating this novel anti-apoptotic signaling pathway to protect from oxidant-induced cell death.

One potential mechanism by which ROIs could increase CaM-kinase activity is via the inactivation of regulatory phosphatases that are sensitive to oxidation. Similar to ROIs, phosphatase inhibitors result in CaM-kinase activation (39). In support of this hypothesis; PP2A activity, associated with CaM-KIV, was inhibited in the presence of hydrogen peroxide, and underwent oxidation at cysteine residues following treatment with hydrogen peroxide (39). The findings that ox-

idative stress can modulate CaM-kinase activity by the inhibition of phosphatases is presented in Fig. 4.

OXIDATIVE STRESS-INDUCED PHOSPHORYLATION OF CALMODULIN

We and others (38, 40) reported, using antagonists of calmodulin/enzyme binding, that binding of calmodulin is required for oxidative stress-induced CaM-kinase activation. These results were surprising as they indicate that, although increases in intracellular calcium are not required for hydrogen peroxide-induced CaM-kinase activation, calmodulin is. There are several possible roles that calmodulin may play in this system even in the absence of calcium mobilization. It is reported that calmodulin weakly associates with the calmodulin-binding domain on the kinases even before binding to calcium (14). Other cellular processes have been reported to be regulated by calcium-free calmodulin (apo-CaM) (29, 30, 33). These results suggest that potentially calmodulin is able to affect the CaM-kinases when not bound to calcium. It is also possible that calmodulin undergoes some other post-stimulatory modification(s) that induces the binding of calmodulin to the CaM-kinases. Oxidative processes may modify calmodulin to allow it to bind with higher affinity to either Ca^{2+} or to the CaM-kinases. Calmodulin has been reported to be phosphorylated, and this modification appears to alter the affinity calmodulin has for either existing Ca^{2+} or for other proteins (20, 63). It has been reported that tyrosine phosphorylation of calmodulin caused a decrease in the concentration of calmodulin required for half maximal activation of CaM-KII (20). Since many kinases are activated by hydrogen peroxide, calmodulin may well be a target of one of these kinases. There is also evidence that indicates calmodulin can interact with the EGF-receptor and be phosphorylated on certain residues following stimulation with EGF (20, 52, 63). Since hydrogen peroxide can induce EGF-receptor signaling, it is quite possible that this ROI could induce the EGF-receptor dependent phosphorylation of calmodulin and in turn result in the increased ability of calmodulin to activate its target kinases. The proposed scheme that hydrogen peroxide induces calmodulin phosphorylation and the subsequent activation of the CaM-kinases needs to be investigated further. The potential for this to occur is indicated in Fig. 4.

INHIBITION OF THE CaM-KINASES BY DIRECT OXIDATION

Direct thiol oxidation or glutathionylation of certain ERK family members and other signaling proteins results in the modulation of the activity of these proteins [(21, 22) and reviewed elsewhere this issue]. In general, the direct effect of thiol oxidation or glutathionylation is dependent on the type of pathways these proteins function in with pro-apoptotic proteins demonstrating an augmentation of activity and anti-apoptotic proteins demonstrating an inhibition of activity. Our laboratory wanted to determine the direct effects of oxidative stress on the activity of the CaM-kinases. To obtain the total CaM-KII activity in cells, one can immunoprecipitate the CaM-kinases and then add calcium and calmodulin. If these immunoprecipitates are treated with hydrogen peroxide prior to the addition of calcium and calmodulin, a reduction in activity can be noted when compared to immunoprecipitates that were not treated with hydrogen peroxide (39). These results demonstrate that not all of the effects of hydrogen peroxide on the CaM-kinases are activating. At this time, the mechanism by which this is occurring is undetermined, but it is unlikely to be due to glutathionylation as the results were obtained using immunoprecipitates of the CaM-kinases. However, it is possible that glutathionylation of the CaM-kinases occurs in intact cells. Figure 4 demonstrates how hydrogen peroxide can directly influence the CaM-kinase activity in a negative manner.

FUNCTIONAL CONSEQUENCES OF REDOX ACTIVATION OF THE CaM-KINASES

Recent evidence suggests that the CaM-kinases are involved in crosstalk with the anti-apoptotic PI3K pathway (38, 69, 79). The PI3K pathway is known to mediate many of its effects via the protein kinase Akt. Threonine 308 of Akt is phosphorylated by both CaM-KII and CaM-KK *in vitro* (60, 69, 79). Akt requires phosphorylation on both threonine 308 (PDK1) and serine 473 (PDK2) to achieve full activation. However, there is evidence that partial activation of Akt can be achieved via threonine 308 phosphorylation alone (1). In intact cells, incubation with PI3K inhibitors prevents phosphorylation of Akt on threonine 308 and serine 473 to most stimuli. Reactive oxygen intermediates are reported to lead to phosphorylation of both threonine 308 and serine 473 in multiple cell types. We found using Jurkat T lymphocytes that inhibitors of PI3K block hydrogen peroxide-induced serine 473 phosphorylation but not threonine 308 phosphorylation (LaHair *et al.* this issue). Further, we found that the CaM-kinase inhibitor KN-93, but not its inactive analog, blocked threonine 308 and serine 473 phosphorylation (38). These results indicated that in response to hydrogen peroxide, CaM-kinases were involved in PI3K activation and that the CaM-kinases are able to induce the phosphorylation of threonine 308 in the absence of PI3K activity. Given the *in vitro* studies that demonstrate that CaM-kinases can directly phosphorylate Akt on threonine 308, it is possible that the CaM-kinases are directly phosphorylating threonine 308 in response to hydrogen peroxide-induced oxidative stress. These results further suggest that in response to oxidative stress, signaling pathways are turned on to protect the cell from undergoing apoptosis and that the CaM-kinases may have a role in this anti-apoptotic pathway.

NF-κB is a transcription factor that can mediate transcription of anti-apoptotic genes such as Bcl-2. Reactive oxygen intermediates are very well known to induce I-κB phosphorylation and NF-κB activation in T lymphocytes, as well as in a variety of other cell types (46, 48, 68). Multiple publications exist that suggest the CaM-kinases can phosphorylate IκK (16, 41, 74, 79). Hughes *et al.* found that transfection of cells

with a constitutively active CaM-KII resulted in I-κB phosphorylation (41). These reports all strongly suggest that the CaM-kinases can have a pivotal role leading to the phosphorylation of I-κB. As stated earlier in this review, we reported that oxidative stress of T lymphocytes resulted in I-κB phosphorylation and degradation (38). Furthermore, inhibition of the CaM-kinases using KN-93 resulted in the suppression of oxidative stress-induced IκK and I-κB phosphorylation (38). Recent evidence has suggested that CaM-KIV is directly able to phosphorylate the p65 component of NF-κB and that this phosphorylation leads to an increase in transcription of anti-apoptotic genes by NF-κB, as well as enhanced cell survival (13). These results also indicate that, in response to oxidative stress, signaling pathways are turned on to protect the cell from undergoing apoptosis and that the CaM-kinases may have a role in this anti-apoptotic pathway.

The CaM-kinases may also have a role in the activation of ERK. Transfection with constitutively active CaM-KII results in ERK activation in endothelial cells (9). In support of this finding, treatment with CaM-kinase inhibitors results in decreased calcium-induced proline-rich tyrosine kinase 2 (Pyk2) and ERK phosphorylation in smooth muscle cells (31, 32). In neurons, both CaM-KII and IV have a role in ERK activation (15, 24). We reported that CaM-kinases are involved in calcium-induced activation of ERK1 and 2 in T lymphocytes (3, 27). Furthermore, we have unpublished results that indicate that the CaM-kinases have a role in hydrogen peroxide-induced ERK activation in MCF-7 cells (unpublished results). Thus, similar to Akt and NF-κB, it would appear that oxidative stress can induce activation of ERK. We speculate that the cell may be using CaM-kinase–mediated anti-apoptotic pathways to counteract the apoptotic/toxic effects of oxidative stress. This leads to the intriguing idea that the CaM-kinases could be used as targets to sensitize cells to the killing effects of oxidative stress.

Because many cancer treatments kill cells by generating ROIs, the CaM-kinases may represent a target that can be used to sensitize cancer cells. We have evidence that inhibiting the CaM-kinases can augment apoptosis in response to ROI-inducing cancer treatments (manuscript submitted). It will be important to determine which CaM-kinases are involved in mediating the different anti-anti-apoptotic pathways in response to oxidative stress.

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ABBREVIATIONS

CaM-K, calcium/calmodulin-dependent kinase; CaM, calmodulin; EGTA, ethyleneglycol-bis-(beta-aminoethyl-ether)tetraacetate; PMA, phorbol myristate acetate; VEGF, vascular endothelial growth factor.

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Address reprint requests to:
Richard A. Franklin
Department of Microbiology and Immunology
Brody School of Medicine at East Carolina University
Brody Building
Greenville, NC 27834

E-mail: franklinr@ecu.edu

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