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Biological actions and mechanism of action of calbindin in the process of apoptosis☆

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Abstract

Although it was originally proposed that the major role of calbindin is to facilitate the vitamin D dependent movement of calcium through the cytosolic compartment of the intestinal or renal cell, we found that calbindin also has a major role in different cell types in protecting against apoptotic cell death. Calbindin, which buffers calcium, can inhibit apoptosis induced by different proapoptotic stimuli. Expression of calbindin-D_{28k} in neural cell suppressed the proapoptotic actions of presenilin-1, which is causally linked to familial Alzheimer's disease, by preventing calcium mediated mitochondrial damage and the subsequent release of cytochrome c. Calbindin, by buffering intracellular calcium can also protect HEK 293 kidney cells from parathyroid hormone induced apoptosis that was found to be mediated by a phospholipase C dependent increase in intracellular calcium. In addition, cytokine mediated destruction of pancreatic β cells can be prevented by calbindin. Induction by cytokines of nitric oxide, peroxynitrite and lipid hydroperoxide production was significantly decreased in calbindin expressing β cells. Thus, calbindin-D_{28k}, by inhibiting free radical formation, can protect islet β cells from autoimmune destruction in type 1 diabetes. Calbindin-D_{28k} can also protect against apoptosis in bone cells. Calbindin was found to block apoptosis in osteocytic and osteoblastic cells. Our findings suggest that calbindin is capable of directly inhibiting the activity of caspase-3, a common downstream effector of multiple apoptotic signaling pathways, and that this inhibition results in an inhibition of tumor necrosis factor (TNFα) and glucocorticoid induced apoptosis in bone cells. Thus, while part of calbindin's protective effect may result from buffering rises in intracellular calcium, other mechanisms of action, such as inhibition of caspase activity, also play a significant role in the prevention of apoptosis by calbindin-D_{28k}. These findings have implications for the prevention of degeneration in different cell types and therefore could prove important for the therapeutic intervention of many diseases, including diabetes and osteoporosis.

Keywords: Calbindin-D_{28k}; Caspase-3; Apoptosis; Epithelial calcium channel

1. Introduction

Calbindin-D_{28k} was initially identified in avian intestine and in avian and mammalian kidney as a major target of 1,25dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) action. It had been proposed that the major role of calbindin is to facilitate $1,25(OH)_2D_3$ dependent transcellular calcium transport [1,2]. The three steps crucial to transcellular calcium transport are apical uptake, intracellular movement and extrusion from the cell. It had been suggested that calbindin has a role in the second process. Apical calcium entry had received the least attention and the existence of a calcium entry channel had been controversial. Only recently has an apical calcium channel been identified, suggesting a mechanism for apical calcium entry [3–5]. Understanding the relationship of calbindin to the epithelial calcium channel in the absorptive cells of the intestine and in the distal nephron should result in new insight into the mechanism of calbindin's action in the calcium transport process.

Besides the suggested role of calbindin as a facilitator of the vitamin D dependent movement of calcium through the intestinal or renal cell, we found that calbindin also has a major role in different cell types in protecting against apoptotic cell death. This article focuses on our findings indicating that calbindin can protect against cell death in neuronal cells, in HEK renal cells, in pancreatic β cells as well as in bone cells and the mechanisms involved. Our findings have implications for the prevention of degeneration in different cell types and therefore could prove important for the therapeutic intervention of many diseases including diabetes and osteoporosis.

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2. Materials and methods

Cells undergoing apoptosis were measured by a nuclear fragmentation assay as previously described [6] or by the TUNEL method (apotag assay kit from Oncor, Gaithersburg, MD). Caspase 3 activity was measured in a cell free assay by determining the degradation of the colorimetric substrate DEVD-paranitroanilide (DEVD-pNA) that contains the amino acid sequence of the caspase 3 cleavage site in poly(ADP-ribose) polymerase (Biomol Research Laboratories Inc., Plymouth Meeting, PA). Caspase 3 activity in vivo was quantified by analyzing the subcellular localization of a caspase 3 sensor (YFP-caspase 3, Clonetech, Palo Alto, CA). Expression plasmids were designated pBSRα-calbindin-D_{28k} or pREP-4 calbindin-D_{28k} as described previously [7]. CaT1 mRNA was measured using real time PCR [8].

3. Results and discussion

3.1. Calbindin and epithelial calcium channels

It has been suggested that the role of calbindin is to facilitate the transcellular movement of calcium (Fig. 1; [9]). Only recently has an apical calcium channel been identified in 1,25$(OH)_2D_3$ responsive epithelia (proximal intestine and distal nephron; ECaC a.k.a CaT2; TRPV5 in kidney and CaT1 a.k.a ECaC2; TRPV6 in both kidney and intestine) [3–5]. The expression of these epithelial calcium channels coincides with the expression of calbindin [10]. In an effort to understand the relationship between calbindin and the epithelial calcium channels we investigated the regulation of these channels in intestine and kidney and compared their regulation to the regulation of calbindin. Shown in

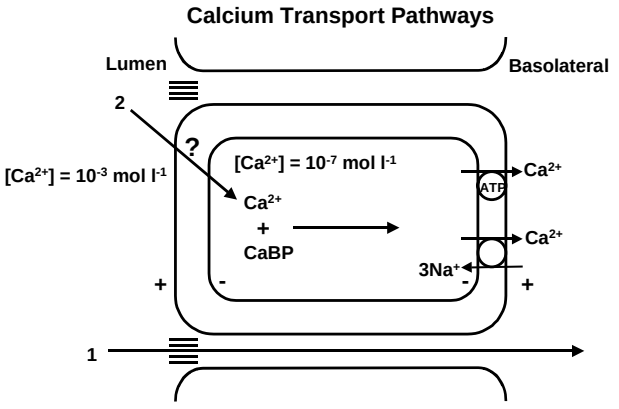

Fig. 1. Calcium transport pathways 1. Paracellular route 2. Transcellular calcium transport consists of influx through an apical calcium cannel, binding to calbindin (CaBP), diffusion through the cytosol and active extrusion at the basolateral membrane. An apical calcium channel (noted in the figure by a question mark) has now been identified in 1,25$(OH)_2D_3$ responsive epithelia [3–5]. Reproduced with permission [9].

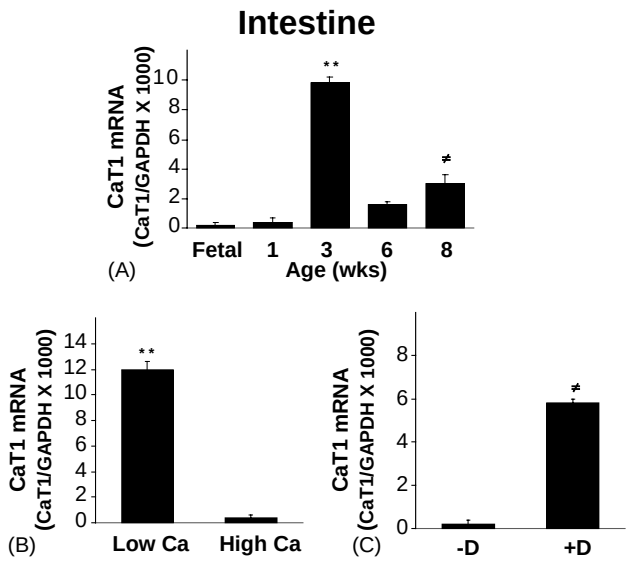

Fig. 2. Real time PCR analysis of CaT1 mRNA in mouse intestine (A). Developmental changes. (B) Under conditions of high (1%) or low (0.02%) dietary calcium. (C) Effect of 1,25$(OH)_2D_3$ (3 injections (100 ng/100 g bw) 48, 24 and 6 h prior to termination). A similar pattern of response was observed for calbindin-D_{9k} mRNA (not shown). RT PCR was done in collaboration with Dr. M.A. Hediger.

Fig. 2 is the regulation of CaT1 mRNA in mouse intestine during development, under conditions of low dietary calcium and under vitamin D deficient and vitamin D replete conditions. The marked increase in CaT1 mRNA at 3 weeks of age, when there is an onset of active intestinal calcium absorption and intestinal responsiveness to 1,25$(OH)_2D_3$ [11], as well as the induction under low calcium and under Vitamin D replete conditions reflects the pattern observed for calbindin-D_{9k} mRNA (not shown). A similar regulation suggests an interrelationship between these two proteins. A key mode of action of calbindin may be to affect the activity of the apical calcium channel. The identification of these channels makes possible for the first time studies using calbindin which should significantly advance our understanding of calbindin's role in the calcium transport process.

4. Role of calbindin in protection against cell death

Besides its suggested role in intestinal and renal calcium transport, calbindin has a major role in protecting against cellular degeneration in different cell types. Our early work in neurons indicated a direct relationship between calbindin-D_{28k} immunoreactivity, the ability of the neuron to reduce intracellular calcium levels and resistance to cellular toxicity [12]. These studies provided correlative evidence for a role for calbindin in protecting against cytotoxicity. Direct evidence for a protective role of calbindin-D_{28k} has been demonstrated in studies in cells in which the calbindin

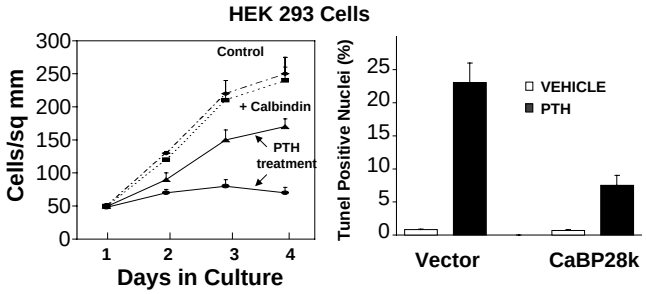

Fig. 3. Effect of PTH (1 μM) on cell number (left panel) and apoptosis (right panel) of HEK 293 cells expressing PTHR in the presence or absence of calbindin-D_{28k} ($CaBP_{28k}$). Left panel: control cells not treated with PTH and transfected with vector alone (dashed, upper line); control cells not treated with PTH and transfected with calbindin-D_{28k} expression vector (dashed, lower line); cells treated with PTH and transfected with calbindin expression vector (triangles and solid line); cells treated with PTH and transfected with vector alone (oval and solid line).

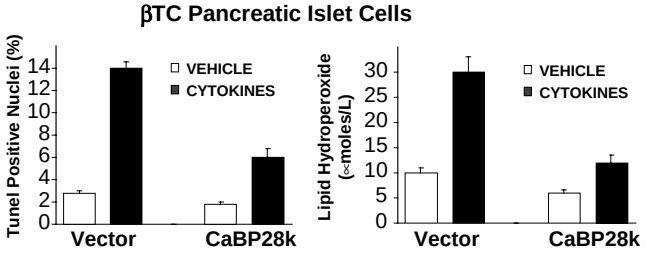

Fig. 4. Calbindin-D_{28k} overexpression protects against cytokine induced apoptosis of βTC-3 cells (left panel) and inhibits the production of free radials measured as an increase in lipid hydroperoxide (right panel).

gene has been transfected. We found that overexpression of calbindin in neural cells results in resistance to degeneration induced by mutant presenilin (PS-1) (which is causally linked to familial Alzheimer's disease) [13]. Overexpression of calbindin suppressed the proapoptotic actions of mutant PS-1, attenuated the increase in intracellular calcium and prevented impairment of mitochondrial function [13]. Further evidence for a protective role for calbindin is shown in studies in human embryonic kidney cells (HEK 293) stably expressing parathyroid hormone receptor (PTHR). PTH treatment markedly reduced the number of cells and this effect was associated with a marked increase in apoptosis. Overexpression of calbindin partially protected these cells from the reduction in cell number as well as from the induction of apoptosis in response to PTH (Fig. 3). The PTH induced apoptosis was suggested to be mediated by a phospholipase C dependent increase in intracellular calcium which would activate calcium dependent proteases such as calpain or protein kinases which promote the apoptotic signal [14]. Thus calbindin could rescue the HEK cells from apoptosis by buffering calcium.

In addition, we have noted that cytokine mediated destruction of pancreatic β cells, a cause of insulin dependent diabetes, can be inhibited by calbindin-D_{28k}. Using the β cell line βTC, calbindin-D_{28k} expression inhibited apoptotic cell death induced by the cytotoxic combination of cytokines [IL-1β (30 U/ml), TNFα (10^3 U/ml) and IFNγ (10^3 U/ml)] (Fig. 4). In order to determine the mechanism by which calbindin-D_{28k} prevents apoptosis, the effect of calbindin on cytokine induced reactive oxygen species was examined. The cytokine combination significantly induced lipid peroxidation and this effect in βTC cells was significantly decreased in the calbindin expressing cells (Fig. 4, right panel). The presence of calbindin also resulted in a significant reduction in cytokine induced nitric oxide production [15]. Similar results were observed using other β cell lines (RIN and βHC) [15]. Calbindin may protect against cytokine induced β cell death by buffering calcium, preventing mitochondrial damage and consequent generation of oxygen free radicals.

In recent studies we found that calbindin-D_{28k} can also protect against apoptosis of bone cells. Tumor necrosis factor (TNF), which inhibits bone formation, has been reported to have proapoptotic effects in osteoblasts. We found that stable transfection of calbindin in MC3T3-E1 osteoblastic cells blocked TNFα (1 nM for 16 h) induced apoptosis. Since caspase 3 is a common downstream effector of multiple apoptotic signaling pathways in response to different signals, including cytokines, we asked whether the antiapoptotic effect of calbindin may involve inhibition of caspase 3. We found that calbindin is capable of directly inhibiting caspase 3 in a cell free system [6]. In addition we found that cell extracts from TNF treated MC3T3-E1 cells expressing calbindin had significantly decreased caspase 3 activity compared to cell extracts from TNF treated vector transfected cells [6]. GST pull down assays indicated that calbindin-D_{28k} can bind directly to caspase 3. EGTA and other calcium binding proteins such as calmodulin and S100 did not inhibit caspase 3 activity [6]. Besides the inhibitor of apoptotic proteins [16], calbindin is the only other natural, non oncogenic inhibitor of caspase 3. Thus calbindin can protect against cell death induced by calcium independent as well as calcium dependent pathways.

Besides the inflammatory cytokine TNF, glucocorticoids can also reduce bone formation. The deleterious effects of glucocorticoids involve increased apoptosis of osteoblasts and osteocytes [17]. We recently found that apoptosis induced by the addition of dexamethasone (dex; 10^{-6} M) for 6 h to MLO-Y4 osteocytic cells was completely attenuated in cells transfected with calbindin-D_{28k}. Similar results were observed in osteoblastic cells. We found that dex induced apoptosis in bone cells was accompanied by an increase in caspase 3 activity. The increase in caspase 3 was markedly attenuated in the presence of calbindin (Fig. 5). Although calbindin is phosphorylated by protein kinase C, we found that phosphorylation of calbindin, unlike the phosphorylation of Bcl-2 protein family, does not affect calbindin's ability to inhibit apoptosis. However, studies in our laboratory have shown an association of calbindin's antiapoptotic effect in response to dex and ERK1/2 activation (not shown).

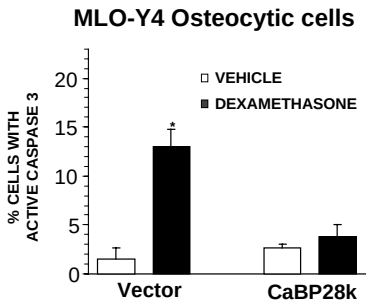

Fig. 5. Dexamethasone induces caspase 3 activity in MLO-Y4 osteocytic cells. This increase in caspase 3 activity is inhibited in the presence of calbindin-D_{28k}.

In summary, these findings indicate that calbindin has a major role in different cell types in protecting against apoptosis and therefore calbindin, a natural, non-oncogenic protein, could be an important target in the therapeutic intervention of many diseases including diabetes and osteoporosis.

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