Implication of Akt and 14-3-3 in Parathyroid Hormone-induced apoptosis in Caco-2 cells.

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PTH functions as an essential regulator of calcium homeostasis. This hormone can also promote or suppress apoptosis depending on the cellular context. In previous works we found that stimulation of human colonic Caco-2 cells with PTH (10^{-8}M) results in dephosphorylation and translocation of pro-apoptotic protein Bad from the cytosol to mitochondria. The objective of this study was to further delineate the molecular mechanisms involved in PTH-induced apoptosis in these cells. Akt controls the balance between cell survival and apoptosis and induces the phosphorylation of Bad. Western blot analysis showed that PTH (48 hrs) caused a decrease in Akt basal phosphorylation at Ser-473 and this effect was reverted by okadaic acid (10 nM) suggesting that PTH modulates Akt via the serine /threonine phosphatase PP2A. By contrast, there was no change in the total amounts of Akt. We previously reported that PTH treatment (5 days) diminishes the number of viable Caco-2 cells. Therefore, we evaluated the potential role of PTH on p53 and 14-3-3 expression, two proteins involved in cell cycle regulation. Western blot analysis and immunocitochemistry revealed that PTH (72 hrs) induced the expression of 14-3-3 and its cytosolic localization. However, the amount of total p53 was not different in the absence or presence of PTH (2-6 days). Co-immunoprecipitation assays under native conditions revealed that the association of 14-3-3 and PTH type 1 receptor was not detectable under basal conditions or after PTH exposure (72 hrs), suggesting that 14-3-3 could not regulate the subcellular localization of PTHR1. However, PTH (48 hrs) diminished the basal association of 14-3-3 and Bad, in correlation with PTH-induced Bad dephosphorylation. The present study suggests that the inhibition of PI3K-Akt pathway and 14-3-3 are involved in PTH promotion of apoptosis in Caco-2 cells.
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