Ouabain-triggered calcium oscillations; a death signal in cancer cells?

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Historically, a number of geographically distinct cultures have exploited cardiac glycoside-containing plant extracts for the treatment of cancer. Empirical data indicates that patients with breast cancer and on cardiac glycosides (digitalis) demonstrate more benign characteristics than control patients not receiving cardiac glycosides. Five-year follow-up after mastectomy: the recurrence rate was 9.6 times among patients not receiving digitalis. On the other hand, elevated serum ouabain level was observed in neonatal period and following unilateral nephrectomy, suggesting that ouabain promotes cell proliferation. We hypothesize that there is a cell type specific response to ouabain resulting in cell proliferation or cell death (apoptosis).

Our group reported that ouabain, a specific ligand of Na,K-ATPase, in low doses, induces intracellular calcium (Ca^{2+}) oscillations in rat proximal tubule (RPT) cells, causing NF-κB activation that is involved in cell proliferation. When RPT cells were exposed to a low dose of ouabain, cell proliferation, measured by ^{3}H-thymidine incorporation, was promoted. Low dose ouabain-induced intracellular Ca^{2+} oscillations were also observed in a renal cancer cell line (A498) derived from human proximal tubular cells. Apoptosis was demonstrated in A498 cells as a downstream effect of ouabain-induced intracellular Ca^{2+} oscillations. No promotion of cell proliferation was observed in A498 cells. Following incubation with 1 μM ouabain, a dose that had little effect on Na,K-ATPase activity, highly regular Ca^{2+} oscillations with a periodicity of approximately 7 min were recorded in more than 70% of A498 cells. After twelve hours of low dose ouabain exposure, cytochrome *c* release and caspase 3 degradation were observed. By 48 hours, the majority of A498 cells had undergone apoptosis. The intracellular Ca^{2+} chelator, Bapta, and 2-APB, an inhibitor of inositol 1,4,5-trisphosphate ($InsP_3$) receptors and store operated Ca^{2+} channels, attenuated cytochrome *c* release and DNA fragmentation implicating the role of Ca^{2+} signaling in ouabain-induced apoptosis. Since Ca^{2+} uptake in mitochondria is considered a potent apoptotic signal, we double-labeled cells with fluo-4 and rhod-2 for recording the cytosolic and mitochondrial Ca^{2+} concentration, respectively. The results of these studies demonstrate that increases in cytosolic Ca^{2+} were accompanied by increases in mitochondrial Ca^{2+}. Although ouabain triggers Ca^{2+} oscillations in RPT cells, no apoptotic response was observed when these cells were challenged with ouabain. Our data define a role for the ouabain/Na,K-ATPase complex as an inducer of an apoptotic cascade specifically in cancer cells and may offer an explanation to the empirical finding that ouabain has beneficial effects in kidney cancer.