

(P11) Ouabain-induced Ca²⁺ oscillation in MDCK cells and astrocytes

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Recently, novel roles for the ubiquitous ion pump, Na-K-ATPase (NKA), have been noted, because of the finding that ouabain, a steroid hormone and also an endogenous ligand of NKA, can trigger intracellular Ca²⁺ oscillation (Aizman et al., 2001). More recently, it has been shown, in the renal proximal tubule (RPT) cells, that this Ca²⁺ oscillation is due to Ca²⁺ release from the internal Ca²⁺ store by the activation of IP3 receptor (IP3R) resulting from direct interaction of NKA and IP3R (Mayakawa-Naito et al.). One interesting question concerning this action of ouabain is whether the effect is specific to the renal proximal tubes, or the effect is more general and ouabain is effective in other kinds of cells. In order to address this question, we used two kinds of cells with different origins to test the ability of ouabain to induce Ca²⁺ oscillation: MDCK cells, a cell line derived from the canine kidney, and astrocytes, a population of glial cells in the central nervous system (CNS). These cells were loaded with the Ca²⁺ indicator, fura-2, and subjected to the digital imaging using fluorescence microscopy to monitor [Ca²⁺]_i changes. Following are the results obtained.

- 1) Ouabain could induce Ca²⁺ oscillation in MDCK cells, indicating that this cell line can be used as a model system to investigate the mechanism of ouabain action.
- 2) Ouabain could induce robust Ca²⁺ oscillation also in primary cultured astrocytes, suggesting that ouabain exerts its effect in the CNS.
- 3) Ouabain-induced Ca²⁺ oscillation in astrocytes was not blocked by a PLC inhibitor, U73122, suggesting that the Ca²⁺ oscillation was not mediated by production of IP3.
- 4) Ouabain-induced Ca²⁺ oscillation in astrocytes was effectively blocked by an IP3R blocker, 2-APB. This result, together with the result with U73122, suggests that the Ca²⁺ oscillation was due to Ca²⁺ release by the activation of IP3R resulting from direct interaction between NKA and IP3R, as shown by Miyakawa-Naito et al.
- 5) Extracellular Ca²⁺ was required for ouabain-induced Ca²⁺ oscillation: ouabain failed to induce marked [Ca²⁺]_i changes in the absence of extracellular Ca²⁺. It is interesting, but has not been clear so far, how extracellular Ca²⁺ is involved in the induction of Ca²⁺ oscillation by ouabain.

These results reproduced most of the results obtained in RPT cells by Miyakawa-Naito et al. As shown in RPT cells, it is likely that ouabain induces the changes in gene expression such as NF-κB and CREB by evoking Ca²⁺ oscillation in astrocytes. In addition, since accumulating evidence has shown that astrocytes regulate neuronal activities, it is suggested that ouabain may modulate neuronal functions through Ca²⁺-dependent release of bioactive substances. In conclusion, the results demonstrate that the effect of ouabain to induce Ca²⁺-oscillation is not specific to the RPT cells but is generally seen in a variety of cells or tissues including the CNS, suggesting a physiological significance of ouabain.

Publications:

Aizman et al., Proc. Natl. Acad. Sci., 98, 13420-13424, 2001

Miyakawa-Naito et al., J. Biol. Chem., in press 2003