

The osmolarity of the electroporation medium affects the transient expression of genes

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During transfections on 5123 and FT02B hepatoma cells and NIH 3T3 fibroblast cells by electroporation, it was observed that the expression of the reporter gene and the viability of the cells was very sensitive to varying amounts of DNA solution (DNA in bidest) added, despite a constant setting of voltage and capacity during electroporation. As few quantitative data are available that detail the extent to which the efficiency of electroporation is dependent on medium osmolarity (1), the following experiment was performed according to Potter *et al.* (2), except that the complete procedure was done at room temperature which resulted in an increase of the specific activity of CAT by approximately 25%. For each transfection 10^7 subconfluent cells were suspended in DMEM/F12 (Gibco; number 074-02400P), and mixed with 50 μg of a DNA construct containing the Rouse Sarcoma Virus (RSV) promoter in front of the bacterial chloramphenicol acetyl transferase (CAT) gene. To create electroporation media with different osmolarities, various amounts of twice concentrated DMEM/F12 (650 mOsm) or H_2O were added. The actual osmolarity of the electroporation medium was determined with a Knauer Halbmicro-Osmometer. The cells were electrotransfected in Bio-Rad 0.2 cm Gene Pulser cuvettes with a Bio-Rad Gene Pulser apparatus and Capacitance Extender at a setting of 300 V and 500 μF for 5123 cells, 300 V and 960 μF for FT02B and 350 V and 960 μF for NIH 3T3 cells. 48 hours after electrotransfection the cells were harvested in 200 μl 200 mM Tris (pH = 8), 0.1% Triton X-100 and the expression of CAT was determined according to Seed and Sheen (3). In figure 1 the specific activity of CAT for 5123 cells and the protein concentration of the respective lysates is shown versus the osmolarity of the electroporation medium. Virtually identical results were obtained for FT02B and NIH 3T3 cells.

These experiments show that a defined optimum of medium osmolarity is found for the expression of a reporter gene. Based on data obtained with electric treatment of erythrocytes (4), this optimum is possibly the result of two opposing processes during electroporation. The first, lowering the medium osmolarity during electroporation widens the membrane pores which facilitates the uptake of the DNA. On the other hand, resealing of the larger pores is more difficult, which results in cell death due to swelling and eventually lysis of the cells as a result of the low medium osmolarity.

In any case the data clearly indicate that the use of hypotonic medium during electroporation has to be avoided.

REFERENCES

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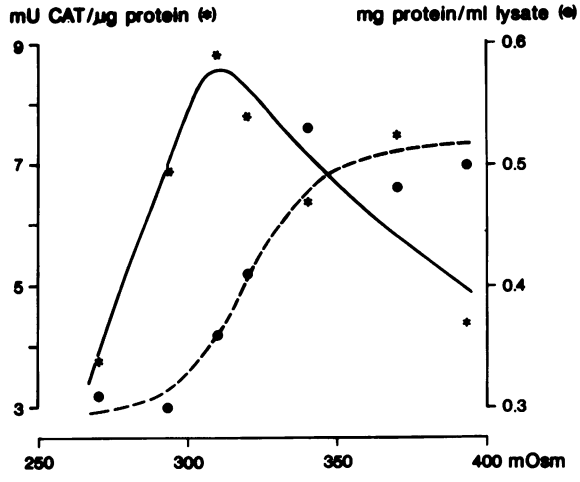

Figure 1. The expression of the CAT gene and the cell survival at different osmolarities of the electroporation medium. Cells seeded without electric treatment grew to 0.72 mg protein/ml lysate. In mock transfected cells (340 mOsm) no CAT activity and 0.50 mg protein/ml lysate was found. The medium osmolarity of normal DMEM/F12 is 300 mOsm.