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微弱電流処理は細胞取込みと細胞質輸送を促進する

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Effective delivery of extraneous molecules into the cytoplasm of the target cells is important for several drug therapies but difficult to achieve. Previously, we showed effective in vivo transdermal delivery of naked siRNA induced by faint electric treatment (ET) iontophoresis, and significant suppression of target mRNA levels (Kigasawa et al., IJP., 2010). In the present study, we analyzed the intracellular delivery of naked anti-luciferase siRNA by faint ET, and found that luciferase activity of cells expressing luciferase was reduced by in vitro ET like in vivo iontophoresis. As the results, we found that cellular uptake of hydrophilic fluorescent calcein was increased by ET, while low temperature exposure significantly prevent calcein uptake and the clathrin-mediated endocytosis inhibitor sucrose slightly reduced uptake of calcein. These results indicate that the cellular uptake mechanism involved energy-dependent pathways. In addition, voltage sensitive fluorescent dye $DiBAC_4$ (3) uptake was increased by ET, and the transient receptor potential channel inhibitor SKF96365 slightly reduced calcein uptake, suggesting that faint ET altered membrane potentials by changing intracellular ion levels. Moreover, to analyze cytoplasmic delivery, we used in-stem molecular beacon (ISMB), which fluoresces upon binding to target mRNA in the cytoplasm. Surprisingly, cytoplasmic ISMB fluorescence appeared immediately after ET, indicating that cytoplasmic delivery is markedly enhanced by ET. In conclusion, we demonstrated for the first time that faint ET can enhance cellular uptake and intracellular delivery into the cytoplasm.