FUNCTIONAL CHARACTERIZATION OF THE CARRIER INVOLVED IN CLONIDINE UPTAKE BY HACAT CELLS
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It has recently been suggested that carrier-mediated transport is involved in the uptake of clonidine, a cationic drug with a tertiary amine structure, by HaCaT, a cell line of human keratinocyte, although this drug has been believed to be passively transported because of its lipophilic nature. The carrier involved in clonidine uptake may be one of those which play important roles in disposition of cationic or tertiary amine drugs, but it has not been identified yet, only being suggested to be distinct from the OCT family of organic cation transporters. We therefore examined clonidine uptake in HaCaT cells to characterize the clonidine carrier in detail. HaCaT cells were seeded in 24-well plates at the density of 2×10⁵ cells/ml/well and cultured in F-12 medium for 2 days and then in DMEM for 2 days, before being used for uptake experiments (37°C and pH 7.4, unless otherwise indicated) using [³H]clonidine. The uptake of clonidine in HaCaT cells was highly temperature-dependent and concentration-dependent (saturable), suggesting the involvement of carrier-mediated transport. According to kinetic analysis, the Michaelis constant was 0.17mM and the maximum uptake rate was 7.3 nmol/min/mg protein. Clonidine uptake was further found to increase with an increase in extracellular pH and when intracellularly acidified via NH₄Cl prepulse, while it was not dependent on Na⁺. However, clonidine uptake was not inhibited by tetrathylammonium and cimetidine, which are typical substrates of renal organic cation/H⁺ antiporters. These results suggest that the clonidine carrier is an antipoter driven by a H⁺ gradient, but distinct from renal organic cation/H⁺ antiporters, including recently cloned MATE1 (multidrug and toxin extrusion protein 1).