Monitoring of the Mitochondrial and Plasma Membrane Potentials in Human Fibroblasts by Tetraphenylphosphonium Ion Distribution

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Abstract

The lipophilic cation tetraphenylphosphonium (TPP\(^+\)) is accumulated by human skin fibroblasts across both the plasma and mitochondrial membranes. We show here that TPP\(^+\) uptake is indeed greatly decreased under conditions leading to de-energization of mitochondria. The TPP\(^+\) accumulation in the presence of the proton ionophore FCCP has been used for determination of the plasma membrane potential across the plasma membrane, after correction for potential-independent binding of TPP\(^+\) to cellular components. Following this procedure, a value of 75 mV has been obtained. Through the amount of TPP\(^+\) released by FCCP treatment, an estimate of the in situ mitochondrial membrane potential has been made. Furthermore, we report that the mitochondrial component of TPP\(^+\) accumulation decreases with aging of fibroblast cultures.

Key Words: Human fibroblasts; plasma membrane potential; mitochondrial membrane potential; tetraphenylphosphonium ion.

Introduction

Human skin fibroblasts represent a cellular system in which many biochemical processes have been thoroughly investigated. Owing to this and to the fact that fibroblasts can be easily obtained from biopsies and maintained in...
culture, they have been widely utilized to investigate the molecular defects associated with several human genetic disorders (Seegmiller, 1976; Neufeld et al., 1975; Brown and Goldstein, 1976; Rodemann and Bayreuther, 1986; Rugolo et al., 1986b).

In this study we describe a method, based on distribution of the lipophilic cation tetraphenylphosphonium (TPP⁺) between cells and medium, for determination of the membrane potentials across both the plasma and inner mitochondrial membranes in human fibroblasts. Indeed, fibroblasts are rather small cells, and the direct determination of the plasma membrane potential by means of microelectrodes, although technically feasible (Swift and Todaro, 1968; Villereal and Cook, 1978; Moolenaar et al., 1982), is a rather difficult approach to be used as a routine method.

Lipophilic cations have previously been used for the estimation of changes in Δψᵣ in neuroblastoma–glioma hybrid cells (Lichtshtein et al., 1979), lymphocytes (Kiefer et al., 1980), murine epidermal cell lines, and 3T3 fibroblasts (Seemann et al., 1983). However, in all these reports, the Δψᵣ was estimated by means of the TPP⁺ distribution ratio obtained from total TPP⁺ accumulation in low [K⁺] medium, after subtraction of the cation accumulation measured in a high [K⁺] medium. This quantitative approach contains two rather inappropriate assumptions, namely: (1) that Δψᵣ is zero in high [K⁺] medium, and this is not true for Δψᵣ with a significant Cl⁻ component (Felber and Brand, 1982), and (2) that the amount of TPP⁺ taken up into mitochondria remains constant even when the plasma membrane is depolarized by high K⁺ concentration. However, Δψᵣ depolarization causes cytosolic [TPP⁺], and therefore mitochondrial [TPP⁺], to drop by an order of magnitude, even if Δψᵣ remains unchanged (Felber and Brand, 1982). For this reason, assumptions (1) and (2) give an erroneous evaluation of Δψᵣ and do not allow one to establish whether variations in TPP⁺ accumulation are functional changes in either Δψᵣ and/or Δψᵣ. In the present paper we show that indeed the lipophilic cation TPP⁺ is accumulated in cultured human fibroblasts across both the plasma and mitochondrial membranes, since the TPP⁺ accumulation ratio is greatly decreased under conditions leading to de-energization of mitochondria. We conclude that the TPP⁺ mitochondrial uptake must be taken into consideration for any quantitative evaluation of the plasma membrane potential by means of lipophilic ion distribution. This conclusion appears also to be in accordance with previous considerations concerning the use of lipophilic ions for Δψᵣ measurements in mitochondria both isolated and in situ (Nicholls, 1974; Hock et al., 1980; Scott and Nicholls, 1980; Davis et al., 1981; Gallo et al., 1984).