ABSTRACT

The computerized device for membrane potential measurement using the tetraphenylphosphonium-selective electrode was constructed. Signal acquisition, processing and data storage were realized by MATLAB/Simulink software. The selective membrane was optimized incorporating TPP^+TPB^- precipitate into the membrane. The electrode had a Nernstian response from 3.10^{-6} M TPP^+. The TPP^+TPB^- precipitate was later replaced by sodium tetrakis[3,5-bis(1,1,1,3,3,3-hexafluoro-2-methoxy-2-propyl)phenyl]borate (NaHFPB). The electrode with incorporated NaHFPB had a Nernstian response from 1.10^{-6} M TPP^+ and had better sensitivity than commercially available electrodes. The values of selectivity coefficients for K^+, Na^+, Ca^{2+} and Mg^{2+} were calculated.

The device was used for mitochondrial membrane potential measurement of isolated mitochondria and for evaluating the respiratory chain function of digitonin-permeabilized cells (hepatocytes, HeLa G, BSC-40 and control transmitochondrial cybrids). This method was used also for monitoring the mitochondrial permeability transition pore (MPTP) function of isolated mitochondria and permeabilized hepatocytes. MPTP opening was induced by high calcium concentration and the action of calcium was enhanced by pro-oxidant *tert*-butyl hydroperoxide (*t*-BHP). This process was inhibited by cyclosporin A.

We also found that *t*-BHP caused $\Delta\psi_m$ dissipation in permeabilized hepatocytes which could be caused by complex I or mitochondrial aconitase inhibition. In addition, *t*-BHP brought about MPTP opening.

Our data indicate that the constructed device can be successfully used for studies of many aspects of mitochondrial bioenergetics, for evaluation of hepatotoxic action of various agents and as a diagnostic tool for mitochondrial oxidative phosphorylation disorders.