

# Effect of Peripheral Benzodiazepine Receptor (PBR/TSPO) Ligands on Opening of $\text{Ca}^{2+}$ -Induced Pore and Phosphorylation of 3.5-kDa Polypeptide in Rat Brain Mitochondria

O. V. Krestinina<sup>1</sup>, D. E. Grachev<sup>1</sup>, I. V. Odinokova<sup>1</sup>,  
G. Reiser<sup>2</sup>, Yu. V. Evtodienko<sup>1</sup>, and T. S. Azarashvili<sup>1\*</sup>

<sup>1</sup>*Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia; fax: (4967) 33-0553; E-mail: tazarash@rambler.ru*

<sup>2</sup>*Institute of Neurobiochemistry, Otto-von-Guericke University, Department of Medicine, 39120 Magdeburg, Germany; fax: (49391) 67-13097*

Received November 1, 2007

Revision received May 8, 2008

**Abstract**—The effect of nanomolar concentrations of PBR/TSPO ligands—Ro 5-4864, PK11195, and PPIX—on  $\text{Ca}^{2+}$ -induced permeability transition pore (PTP) opening in isolated rat brain mitochondria was investigated. PBR/TSPO agonist Ro 5-4864 (100 nM) and endogenous ligand PPIX (1  $\mu\text{M}$ ) were shown to stimulate PTP opening, while antagonist PK11195 (100 nM) suppressed this process. Correlation between PBR ligand action on PTP opening and phosphorylation of a 3.5 kDa polypeptide was investigated. In intact brain mitochondria, incorporation of  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$  into 3.5 kDa peptide was decreased in the presence of Ro 5-4864 and PPIX and increased in the presence of PK11195. At threshold  $\text{Ca}^{2+}$  concentrations leading to PTP opening, PBR/TSPO ligands were found to stimulate dephosphorylation of the 3.5 kDa peptide. Specific anti-PBR/TSPO antibody prevented both PTP opening and dephosphorylation of the 3.5-kDa peptide. The peptide was identified as subunit *c* of  $\text{F}_0\text{F}_1\text{-ATPase}$  by Western blot using specific anti-subunit *c* antibody. The results suggest that subunit *c* of  $\text{F}_0\text{F}_1\text{-ATPase}$  could be an additional target for PBR/TSPO ligands action, is subjected to  $\text{Ca}^{2+}$ - and TSPO-dependent phosphorylation/dephosphorylation, and is involved in PTP operation in mitochondria.

**DOI:** 10.1134/S0006297909040105

**Key words:** brain mitochondria, peripheral benzodiazepine receptor, permeability transition pore,  $\text{F}_0\text{F}_1\text{-ATPase}$  subunit *c*, PBR/TSPO