Naunyn-Schmied Arch Pharmacol (2007) 375:291
DOI 10.1007/s00210-007-0161-y

ABSTRACT

Additional abstract to the supplement of the 48th Spring Meeting, 13–15 March 2007 in Mainz, Germany

ESTABLISHMENT OF AN AEQUORIN LUMINESCENCE-BASED CALCIUM ASSAY IN A 96-WELL FORMAT FOR THE CHARACTERIZATION OF 5-HT$_3$ RECEPTORS

J Walstab, S Combrink, M Brüss, M Göthert, B Niesler* and H Bönisch

Agonist-induced activation of the ligand-gated 5-HT$_3$ receptor results in the opening of its cation selective channel that is among others permeable for Ca^{2+} ions. Thus, receptor function can be monitored by a luminescence technique using the Ca^{2+}-sensitive photoprotein aequorin. We have developed a new functional assay for measuring 5-HT$_3$ receptor responses in a 96-well format based on aequorin bioluminescence upon Ca^{2+} influx. The cell suspension-based assay was established for the pharmacological characterization of human embryonic kidney (HEK) 293 cells transiently expressing apoaequorin and human 5-HT$_{3A}$ receptors as well as 5-HT$_3$ receptors composed of varying subunits (5-HT$_{3A,B,C,D,E}$). Therefore, parameters such as the apoaequorin:receptor cDNA ratio used for transfection and the cell loading procedure with the chromophore cofactor coelenterazine *h* were optimized. The potencies of two tested agonists and antagonists at the 5-HT$_{3A}$ receptor were in the range of those obtained with other functional methods. To determine, whether the assay is suitable to detect pharmacological differences between 5-HT$_3$ receptors composed of different subunits we investigated the pharmacological properties of serotonin at the previously characterized heteromeric 5-HT$_{3A/B}$ receptor. Co-expression of the 5-HT$_{3B}$ subunit led to a decrease of pEC_{50} value and Hill slope as reported previously (Davies et al. [1999] Nature 397:359–363) indicating a reduced affinity and cooperativity for serotonin. The maximum response to serotonin was higher at the heteromeric 5-HT$_{3A/B}$ than at the homopentameric 5-HT$_{3A}$ receptor confirming a higher single channel conductance of the 5-HT$_{3A/B}$ receptor. Thus, we conclude that the aequorin assay provides a convenient and highly sensitive method for reporting 5-HT$_3$ receptor function and the comparison of 5-HT$_3$ receptors composed of different subunits. This assay is well suited for high-throughput screening and can be applied to other Ca^{2+}-permeable ligand-gated ion channels.

This work was supported by the DFG.

Institute of Pharmacology and Toxicology, University of Bonn, Reuterstraße 2b, 53113 Bonn, Germany
*Department of Human Molecular Genetics, University of Heidelberg, Im Neuenheimer Feld 366, 69120 Heidelberg, Germany