BACMAM SYSTEM FOR FRET BASED cAMP SENSOR EXPRESSION IN STUDIES OF G-PROTEIN COUPLED RECEPTORS

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Cyclic adenosine monophosphate (cAMP) is a second messenger of many G-protein coupled receptors (GPCRs) and is thus a useful readout molecule to estimate the biological activity of various GPCR-specific agents.

Here we report the development and use of baculovirus-based BacMam transduction system for expression of a FRET biosensor for cAMP (Epac2-camps) [1,2]. The new viral transduction system is an easy and robust tool for ligand screening at second messenger level in a variety of mammalian cell lines, whereas the level of protein expression is adjustable in a dose-dependent manner depending on the viral multiplicity of infection of cells.

The functional assays were performed on B16F10 murine melanoma cell line endogenously expressing melanocortin-1 receptor (MC_1R). The activation profile of the receptor was characterized by a set of full and partial agonists of MC_1R.

The bivalent ions Ca^{2+} as well as Mg^{2+} modulated potencies of ligands, this effect was ligand and ion-specific.

Table: The effect of bivalent cations on MC_1R activation by its agonists.

Agonist	EDTA treatment, pEC_{50} ± S.E.		
	DPBS	**1 mM Ca^{2+}**	**1 mM Mg^{2+}**
α-MSH	6.66 ± 0.09	10.05 ± 0.06	8.10 ± 0.07
NDP-α-MSH	8.84 ± 0.08	9.78 ± 0.09	9.58 ± 0.06
MS05	5.53 ± 0.61	8.02 ± 0.09	5.69 ± 0.07
β-MSH	N.D.	8.62 ± 0.09	7.00 ± 0.17
SHU-9119	N.D.	9.05 ± 0.21	7.00 ± 0.39

Cells were transduced with BacMam-Epac2-camps virus for 3 h and further incubated for 21 h in complete growth medium supplemented with 10 mM sodium butyrate. Cells were washed with 1 mM EDTA before the experiment. Chelating agent weas removed and cells were assayed in DPBS (with or without 1 mM Ca^{2+} or Mg^{2+}) upon 10 min treatment with MC_1R ligand. Responses were measured using Epac2-camp sensor FRET change. pEC_{50} ± standard error values are calculated from a selected representative experiment measured in duplicates with comparable results obtained from two independent replicate experiments. N.D.: not detectable.

Our results obtained for MC_1R indicate that BacMam-Epac2-camps system may also be applicable for characterization of activation of other GPCRs and can be implemented for routine analysis and high throughput screening ($Z' > 0,6$).

The work was funded by Estonian Ministry of Education and Science (SF0180032s12) and by the European Union through the European Regional Development Fund (TK114, 30020).

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