Abstract

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The human MRGPRD (Mas-related G protein-coupled receptor D) belongs to the big family of GPCRs (G Protein-coupled receptors). Signaling pathways mediated by GPCRs regulate a high number of vital body functions and approximately 30 % of all modern clinical drugs target GPCRs (Overington *et al*., 2006). The MRGPR subfamily was discovered 10 years ago and still remains mainly unexplored and considered "orphan" (Solinski *et al*., 2014).

Several ligands such as β-alanine, GABA, β-aminobutyric acid (Shinohara *et al*., 2004; Ajit *et al*., 2010; Uno *et al*., 2012), angiotensin and alamandine (Gembardt *et al*., 2008; Lautner *et al*., 2013) are able to bind to the human MRGPRD.

The hMRGPRD is specifically expressed in the primary sensory trigeminal ganglia neurons. Activation of the receptor by β-alanine has shown to elicit pruritogenic sensation and to contribute to normal mechanical and thermal pain thresholds. The restricted expression pattern suggests that the hMRGPRD could be a new specific target for the development of antinociceptive drugs.

In the present study, I tested 16 compounds (including β-alanine) to find out whether they can activate the hMRGPRD receptor. The strategy involved searching for ligands by monitoring signaling transduction pathways of the hMRGPRD, such as changes in cellular cAMP production by the activation of Gi-proteins or Gq-mediated changes in Ca^{2+} - related signaling in CHO Flp-In cells stably expressing the recombinant hMRGPRD receptor. As a positive control, β-alanine was used to activate the receptor protein.

In experiments, measuring Gi-mediated changes in cellular cAMP formation, β-alanine caused an inhibition of forskolin-induced cAMP production by 36.63 %. 10 of the 16 tested compounds showed no effect, but (*R)*-3-amino-2-methylpropionic acid and also its diastereomer *(RS)*-3-amino-2-methylpropionic acid caused an inhibition of forskolin induced cAMP production by 41. 31 % and 43.11 %, respectively. In contrast, 3-aminopropan-1-ol increased intracellular cAMP levels (56.1 %) and showed the same effect also in nontransfected CHO Flp-In cells, suggesting this effect to be nonspecific. 1 mM concentration of 1-(3-piperidinopropyl)piperazin also caused a nonspecific 34.4 % increase of forskolin-induced cAMP production.

For the measurement of Gq-mediated effects, the Nuclear Factor of Activated T-cells (NFAT)-driven luciferase reporter gene assay was used. β-alanine was again used as a positive control in these experiments, causing an increase of the luciferase activity by 28.00 %. The addition of 1-(3-piperidinnopropyl)piperazin caused an increase in luciferase activity by 99.40 %. Fenpropidin [100 μM] caused 57.6 % increase in RLU and 1 mM concentration of this compound showed an inhibition of 56.1 %. (*R)*-3-amino-2-methylpropionic acid was also tested in this assay and increased the luciferase activity by 29.40 %.