Spatio-temporal dynamics of GPCR-mediated signals

M.J. Lohse, M. Bünemann, M. Castro, C. Hoffmann, C. Krasel, V.O. Nikolaev, J.P. Vilardaga
Institute of Pharmacology and Toxicology, University of Würzburg

Signaling via G-protein-coupled receptors presumably occurs in a temporally and spatially organized manner that is – with the exception of rhodopsin – largely unknown because of a lack of suitable techniques that can sufficiently resolve such signals in space and time. Over the past years, we have set out to develop fluorescent methods that permit the analysis of ligand binding, receptor activation, G-protein activation, and the generation of the second messengers cAMP and cGMP. Our data show that ligand binding, studied with PTH as an example, occurs in a potential process and that the second phase coincides with a conformational change in the receptor. This conformational change, presumably reflecting transition into an active signaling conformation, is much faster than previously thought, requiring switch times in the millisecond to second range. It depends both on the nature of the receptor and on the type of ligand; for example, at the alpha2A-adrenergic receptor, switching by full agonists is much faster (∼30-50 ms) than by partial agonists or inverse agonists (∼1000 ms). G-protein activation is significantly slower than receptor activation and appears to be temporally tightly linked to effector activation such as opening of the GIRK K-channel. Increases in second messenger concentrations are again much slower, occurring over seconds to minutes, and there are complex interactions between the levels of cAMP, cGMP and calcium.

References