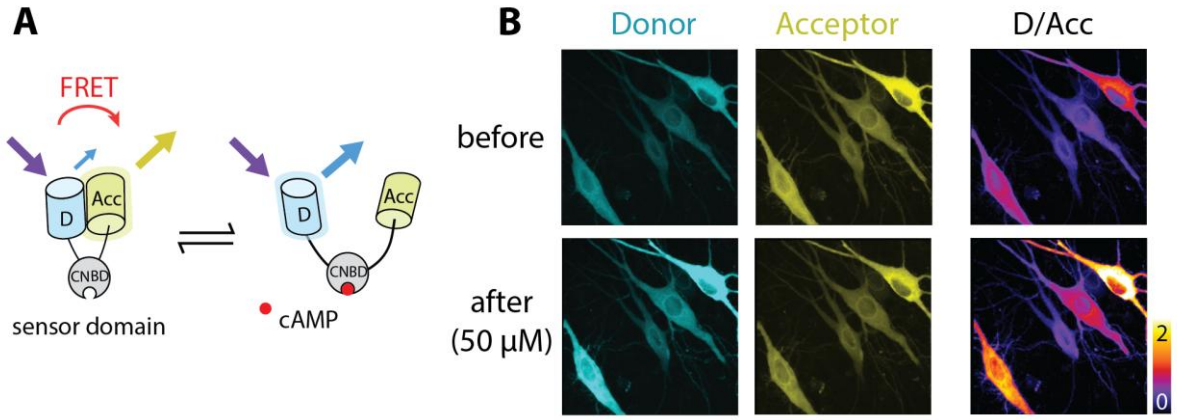
Lipohilic, Bioreversibly Masked Cyclic Nucleoside Monophosphate Derivatives-Tools for Non-Invasive Cell Assays

Dominik Werz[1], Alexandra Ruthenbeck[1], Oana Constantin[2], Elisa Marangoni[2], Björn-Ph Diercks[2], Aileen Krüger[2], Alexander Froese[2], Nadja I Bork[2], Viachselav O Nikolaev[2], Andreas H Guse[2], Chris Gee[2], Chris Meier[1]

[1]Organic Chemistry, Deparment of Chemistry, Faculty of Science, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg.
[2]University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany

The second messenger cAMP is ubiquitously present in cells and is involved in various signalling processes. To transduce a signal, cAMP can either bind to distinctive cyclic nucleotide-gated (CNG) ion channels or activate protein kinase A. However, the role and mechanism of action of different cNMPs in T cell regulation are not yet fully elucidated. A better understanding could enable the identification of novel targets for the treatment of autoimmune diseases. In cell studies with cAMP or other cNMPs, their high polarity makes passive membrane transport difficult. Membrane-permeable cNMPs would enable such studies. Starting from the nucleosides, we synthesized different acyloxybenzyl (AB)-masked cNMP derivatives via a short and flexible chemical route using phosphoramidite chemistry. Hydrolysis tests showed high chemical stability of the AB-cNMPs, which was significantly higher than the rate of enzymatic activation. We evaluated the ability of AB-cNMPs to cross cell membranes and deliver the AB-ester intracellularly. A FRET-based cell assay in cardiomyocytes and hippocampal neurons proved the cellular uptake of AB-cAMP or AB-cGMP and their esterase-catalyzed activation. In a second setup, Jurkat T cells were loaded with a Ca^{2+}-selective fluorescent dye and incubated with AB-cAMP derivatives. This assay confirmed the rapid uptake of AB-cAMP into cells and the almost immediate cellular response. Thus, we have developed novel membrane-permeable delivery systems for several highly polar, phosphorylated nucleoside second messengers, enabling non-invasive studies of intracellular signalling.



Selected references

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