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Oral presentation

Specificity of commonly used cyclic nucleotides with PKA, PKG and Epac-implementing microcalorimetry to determine PDE activities

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Background

Two main structural classes of cyclic nucleotide receptors exist in mammalian cells. The first class contains a conserved cyclic nucleotide binding (CNB) domain, found in the regulatory regions of PKG and PKA in cyclic nucleotide regulated ion channels and in Epac. The second class contains a GAF domain that can bind cyclic nucleotides but is structurally unrelated to CNB domains. GAF domains are present in some phosphodiesterases (PDEs) allosterically regulated by cyclic nucleotides. In addition, cGMP and cAMP are bound and hydrolysed by PDEs at their catalytic site.

Materials and methods

The important feature of this work was to measure 12 of the most commonly used cyclic nucleotide analogues on protein kinase A I and II (PKA I and II), protein kinase G Iα, Iβ and II (PKG Iα, Iβ and II), and the guanine nucleotide exchange factor Epac I. The study is extended to eight cyclic nucleotide hydrolysing phosphodiesterases (PDE IA, IB, IC, II, IV, V, VI and X), thereby introducing microcalorimetry to overcome radioactive labeling of the tested analogs and allowing analysis of the kinetic parameters K_m, K_i and V_{max} [1].

Results and discussion

We successfully used isothermal titration microcalorimetry (ITC) for analysing the kinetic parameters of eight PDEs by various cAMP- and cGMP-analogs. The measured data reflect very well the reported literature and the technique offers significant advantages over traditional microbiological methods. The measuring of both, k_{cat} and K_m, provide additional insight into protein active sites by determining competitive, non-competitive and linear-mixed inhibition of the PDEs by the cyclic nucleotides.

To our surprise, some accepted hydrolysis-stable analogs became degraded by the newer phosphodiesterases or show high inhibitory effects. As an example, we show side effects of an Epac specific activator on platelet signalling pathways.

The defined specificity profiles of the tested cyclic nucleotides are presented in clearly arranged tables and will help to interpret *in vivo* data in the light of the cyclic nucleotide receptor proteins present in a given cell.

An extended overview of the data will be accessible to the scientific community at: http://www.cyclic-nucleotides.net after publication.

References

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