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Activation profile of cGMP-dependent protein kinase Iα

Sabine Wolter[1*], Marina Golombek[1], Frank Schwede[2], Hans-Gottfried Genieser[2], Stefan Dove[3], Roland Seifert[1]

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Background

cGMP-dependent protein kinase (PKG) is a serine/threonine kinase which is potently activated by cGMP [1]. PKG is encoded by two genes, forming two different proteins, PKGI and PKGII. The two isoforms of PKGI, PKGIα and PKGIβ, differ in the N-terminal amino acid sequences. PKGI isozymes are homodimers with two identical subunits possessing a catalytic and a regulatory domain each. The regulatory domain contains two non-identical binding sites for cyclic nucleotides (cNMPs), i.e., a slowly exchanging and a rapidly exchanging site. The activation constant (K_a) of PKGIα for cGMP is about 3-fold lower than the corresponding K_a of PKGIβ suggesting distinct physiological roles of the isoforms. In addition to cGMP, other cNMPs and also cNMP analogues activate or inhibit PKG [2-4]. While many investigations focussed on discrimination between the cNMP binding sites by employing cGMP and cAMP analogues, little is known about interaction of PKGIα with cCMP analogues or with *Rp*- and *Sp*- diastereomers of cCMP phosphorothioates.

As was shown by Desch et al. [5], the membrane-permeable cCMP analogue dibutyryl-cCMP (DB-cCMP) induces smooth muscle relaxation and activates PKGI in aortic tissue lysates. Therefore, we have studied 4-MB-cCMP, the resulting active metabolite after cleavage of DB-cCMP by esterases, and also corresponding substances from cAMP and cGMP, on purified PKGIα.

Materials and methods

PKG kinase activity was measured *in-vitro* by a radiometric kinase assay in the presence of cGMP or different cNMP analogues. pEC_{50} values, K_a, Hill slopes and E_{max} values were calculated using GraphPad Prism software. E_{max} values were related to E_{max} values of the activation of PKGIα by cGMP, which was set to 1.00.

Results and discussion

Besides the known activator cGMP, many other cNMPs and cNMP analogues are activators of PKGIα, with distinct activation constants (pEC_{50}), specific Hill slopes and different maximal effects (E_{max}) (Table 1). The most potent and effective activator for PKGIα was cGMP. The active metabolite of DB-cGMP, 2-MB-cGMP was less potent and effective.

cAMP and 6-MB-cAMP showed similar potency, but 6-MB-cAMP had a higher efficacy than cAMP. 4-MB-cCMP was a more effective activator than cCMP, but showed a reduced potency.

Table 1 pEC_{50}, K_a, Hill slopes and E_{max} for the activation of PKGIα by cNMPs.

cNMP	pEC_{50}	K_a (μM)	Hill slope	E_{max}
cGMP**	6.98 ± 0.04	0.11	1.71 ± 0.36	1.00 ± 0.04
2-MB-cGMP	5.84 ± 0.13	1.45	1.12 ± 0.34	0.66 ± 0.03
cAMP**	4.82 ± 0.11	15.13	1.28 ± 0.39	0.59 ± 0.05
6-MB-cAMP	4.67 ± 0.06	21.38	1.35 ± 0.19	0.81 ± 0.03
cCMP**	**4.58 ± 0.14**	**26.30**	**1.84 ± 0.53**	**0.55 ± 0.04**
4-MB-cCMP	**4.05 ± 0.10**	**89.13**	**1.10 ± 0.23**	**0.71 ± 0.06**
Sp-cAMPS	3.72 ± 0.61	190.55	1.38 ± 2.86	0.16 ± 0.01*
Rp-cAMPS	n.d.	n.d.	n.d.	n.d.
Sp-cCMPS	3.53 ± 0.97	295.12	1.11 ± 1.34	0.17 ± 0.02*
Rp-cCMPS	n.d.	n.d.	n.d.	n.d.
cIMP**	4.72 ± 0.04	19.05	1.54 ± 0.16	0.87 ± 0.02
cUMP**	4.15 ± 0.04	70.79	0.85 ± 0.21	0.72 ± 0.03
cXMP**	3.98 ± 0.04	104.71	2.06 ± 1.93	0.69 ± 0.03

*: value shows the maximum activation with 3 mM cNMP without saturation of the concentration/response curve

**: data from Wolter et al, Biochem Biophys Res Commun. 2011, 415: 563-566.

n.d.: not determinable because of lack of saturation of the concentration/response curve.

* Correspondence: wolter.sabine@mh-hannover.de
[1]Institute of Pharmacology, Hannover Medical School, Hannover, Germany
Full list of author information is available at the end of the article

The cNMP analogues activated PKGIα in the order of potency cGMP > 2-MB-cGMP > cAMP > 6-MB-cAMP > cCMP > 4-MB-cCMP and in the order of efficacy cGMP > 6-MB-cAMP > 4-MB-cCMP > 2-MB-cGMP > cAMP > cCMP.

Rp-cAMPS and *Rp*-cCMPS did not activate PKGIα. The stable phosphorothioates *Sp*-cAMPS and *Sp*-cCMPS activated PKGIα only at high concentrations in the order of potency and efficacy cGMP > cAMP > cCMP > *Sp*-cAMPS ~ *Sp*-cCMPS.

Furthermore, we illustrate binding of cNMPs for PKG based on existing crystal structures and discuss current problems with respect to molecular modelling approaches. In conclusion, 4-MB-cCMP is a more effective PKG activator than cCMP and, therefore, a valuable tool for analysing the second messenger role of cCMP [6].

Authors' details
[1]Institute of Pharmacology, Hannover Medical School, Hannover, Germany. [2]Biolog Life Science Institute, Bremen, Germany. [3]Department of Pharmaceutical/Medicinal Chemistry II, University of Regensburg, Regensburg, Germany.

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