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cAMP Signalling via Epac:

Spotlight on a New Signal Transduction System and its Role in Neuronal Function

Epac proteins: Novel cAMP Targets

The abbreviation Epac stands for **e**xchange **p**rotein directly **a**ctivated by **c**yclic AMP (cAMP) and describes a new receptor for cAMP, discovered a few years ago. Up to now two different proteins, Epac1 and Epac2, have been identified although to date their functions and importance are not fully understood. In contrast to the protein kinase A (PKA) isozymes, Epac does not phosphorylate target proteins, but acts as a guanine nucleotide exchange factor (GEF) for the Ras-like small GTPases Rap1 and Rap2. Epac is also known as cAMP-GEF.

Epac is not just another rare exception from PKA signalling, but is present in most tissues

Epac is expressed in many tissues, such as the brain, blood vessels, kidney, adrenal gland, and the pancreas. Furthermore, in a large number of corresponding studies Epac was identified to play a role in diverse biological functions and processes, ranging from neuronal signalling to vascular permeability, cell proliferation, and insulin secretion. Since research into this relatively new field has only recently begun, many unforeseen results are still to be expected, allowing for a much more detailed insight into the already quite complex cAMP signalling systems.

Numerous "classical" cAMP/PKA systems need to be re-investigated regarding Epac

The discovery of Epac argues that all well-established cAMP systems should be re-investigated either reconfirming PKA as the only target, or to discover a more complex regulation system involving Epac alone or as a parallel pathway to PKA. Already there have been numerous surprising results and publications in this new field. Also, cases where a certain complexity of the cAMP signal transduction systems had been recognized but could not be further resolved are now becoming more transparent and better understood.

cAMP analogues with a methylated 2'-OH group can discriminate between Epac and PKA

In close collaboration with laboratories in Bergen and Utrecht, BIOLOG has developed a new class of Epac-specific compounds. These compounds are still analogues of cAMP, however, their ribose 2'-hydroxy group has been methylated. Interestingly, this modification prevents these compounds from being recognized and accepted by PKA, but they are well tolerated by Epac. In the case of the specific Epac activator 8-pCPT-2'-O-Me-cAMP (Cat. No. C 041, Figure 1) the resulting discrimination between both receptors is three orders of magnitude. The involvement of Epac in a number of biological systems has been shown using this membrane-permeant analogue.

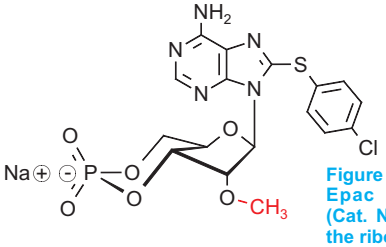

Figure 1: Chemical structure of the specific Epac activator 8-pCPT-2'-O-Me-cAMP (Cat. No. C 041). The 2'-hydroxy group of the ribose has been methylated (red).

N^6-modified cAMP analogues are specific PKA agonists and can be used as Epac-negative controls

Conversely specific and potent activators of PKA that have no effect on Epac are needed. Most cAMP analogues tested so far do not discriminate well between both receptors. Only structures that carry a modified 6 position at the adenine nucleobase were shown to be unable to activate Epac. Thus, analogues such as 6-Bnz-cAMP (Cat. No. B 009, Figure 2) have now attracted new attention as PKA-specific agonists.

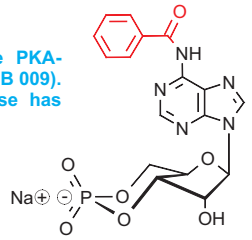

Figure 2: Chemical structure of the PKA-specific agonist 6-Bnz-cAMP (Cat. No. B 009). Position 6 of the adenine nucleobase has been benzoylated (red).

Similar to N^6-modified cAMP analogues, cyclic GMP (cGMP) and its analogues do not activate Epac. Due to the oxygen modification at position 6 of the purine ring system, Epac does not tolerate the guanine nucleobase of cGMP. Thus, all established agonists and antagonists for protein kinase G, such as 8-Br-cGMP or Rp-8-Br-PET-cGMPS are expected to have no effect on Epac signalling.

Epac plays an important role in regulating diverse neuronal functions

Epac proteins are present in most parts of the nervous system, although their expression patterns seem to be subject to alteration during development or under pathophysiological circumstances, e.g. in brain regions associated with Alzheimer's disease. Consequently, Epac has been found to play a role in the regulation of many neuronal functions and processes. For example, Epac activation influences neurotransmitter release in glutamatergic synapses and is involved in the modulation of neuronal excitability. Also, Epac may play a critical role in cognition and learning and memory based on the observation that Epac activation exerts beneficial effects on memory retrieval in mice. Epac is involved in the regulation of axon regeneration and neuronal differentiation, and has been reported to be a component of the circadian pacemaker as well as a key element in a cAMP-protein kinase C (PKC) signalling pathway in inflammatory pain.

Although recent research has shown Epac to participate in the regulation and modulation of diverse neuronal functions, the mechanisms of Epac-dependent cAMP signalling in the nervous system are not yet fully understood. The interaction with PKA-dependent signalling pathways as well as the spatio-temporal dynamics of Epac signalling are only two of the aspects that need to be investigated further.

LIT: J. de Rooij et al., *Nature*, **396**, 474-477 (1998): "Epac is a Rap1 Guanine-Nucleotide-Exchange Factor Directly Activated by cyclic AMP" • A.E. Christensen et al., *J. Biol. Chem.*, **278**, 35394-35402 (2003): "cAMP Analog Mapping of Epac1 and cAMP-Kinase. Discriminating Analogs Demonstrate that Epac and cAMP-Kinase Act Synergistically to Promote PC-12 Cell Neurite Extension" • N. Zhong & R.S. Zucker, *J. Neurosci.*, **25**, 208-214 (2005): "cAMP Acts on Exchange Protein Activated by cAMP/cAMP-Regulated Guanine Nucleotide Exchange Protein to Regulate Transmitter Release at the Crayfish Neuromuscular Junction" • T.B. Hucho et al., *J. Neurosci.*, **25**, 6119-6126 (2005): "Epac Mediates a cAMP-to-PKC Signaling in Inflammatory Pain: An Isolectin B4(+) Neuron-Specific Mechanism" • M. Ouyang et al., *Proc. Natl. Acad. Sci. USA*, **105**, 11993-11997 (2008): "Epac Signaling is Required for Hippocampus-dependent Memory Retrieval" • A. Ostroveanu et al., *Hippocampus*, Epub ahead of print (2009): "Exchange Protein Activated by cyclic AMP 2 (Epac2) Plays a Specific and Time-Limited Role in Memory Retrieval" • M. Gloerich & J.L. Bos, *Annu. Rev. Pharmacol. Toxicol.*, **50**, 355-375 (2010): "Epac: Defining a New Mechanism for cAMP Action" • M. Grandoch et al., *Br. J. Pharmacol.*, **159**, 265-284 (2010): "The Role of Epac Proteins, Novel cAMP Mediators, in the Regulation of Immune, Lung and Neuronal Function"

See back page for selected products for Epac and PKA signalling research.

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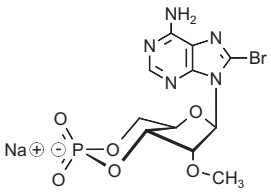
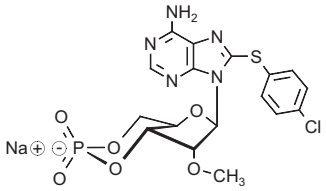
Specific Epac Activators

Specific activator of Epac, suitable for direct comparison with common 8-Bromo-cAMP, which activates both, PKA and Epac.



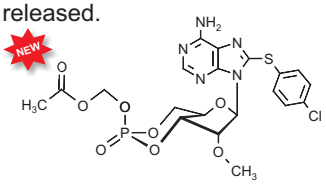
8-Br-2'-O-Me-cAMP
B 022-05, 5 µmol/~2.2 mg
B 022-25, 5 x 5 µmol

Specific activator of Epac with high lipophilicity and membrane permeability, as well as increased phosphodiesterase stability.



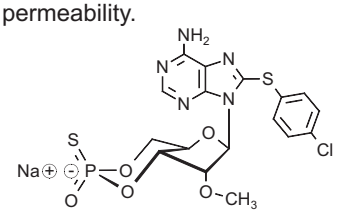
8-pCPT-2'-O-Me-cAMP
C 041-05, 5 µmol/~2.5 mg
C 041-25, 5 x 5 µmol

Extremely membrane-permeant precursor of the specific Epac activator 8-pCPT-2'-O-Me-cAMP (Cat. No. C 041). After cell permeation and metabolic activation by esterases the active compound is released.



8-pCPT-2'-O-Me-cAMP-AM
C 051-01, 1 µmol/~0.6 mg
C 051-05, 5 x 1 µmol

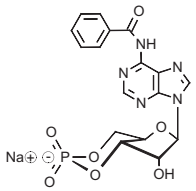
Hydrolysis-resistant form of the Epac activator 8-pCPT-2'-O-Me-cAMP (Cat. No. C 041) with high lipophilicity and membrane permeability.



Sp-8-pCPT-2'-O-Me-cAMPS
C 052-01, 1 µmol/~0.5 mg
C 052-05, 5 x 1 µmol

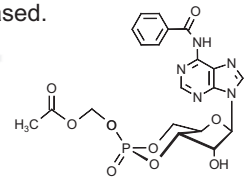
Epac-negative Controls

Site-selective and membrane-permeant activator of PKA which does not activate Epac and thus can be used as an Epac-negative control.



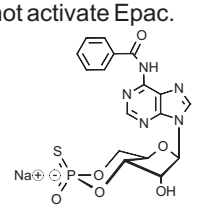
6-Bnz-cAMP
B 009-10, 10 µmol/~4.6 mg
B 009-50, 5 x 10 µmol

Extremely membrane-permeant precursor of the PKA activator and Epac-negative control 6-Bnz-cAMP (Cat. No. B 009). After cell permeation and metabolic activation by endogenous esterases the active compound is released.



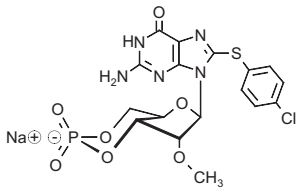
6-Bnz-cAMP-AM
B 079-01, 1 µmol/~0.5 mg
B 079-05, 5 x 1 µmol

Hydrolysis-resistant form of the Epac-negative control 6-Bnz-cAMP (Cat. No. B 009). Sp-6-Bnz-cAMPS is a site-selective, PDE-resistant and membrane-permeant activator of PKA which does not activate Epac.



Sp-6-Bnz-cAMPS
B 040-05, 5 µmol/~2.4 mg
B 040-25, 5 x 5 µmol

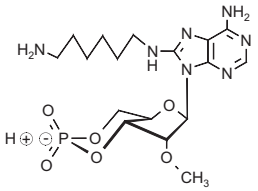
cGMP-based Epac-inactive analogue with structural similarity to the Epac activator 8-pCPT-2'-O-Me-cAMP (Cat. No. C 041).



8-pCPT-2'-O-Me-cGMP
C 048-05, 5 µmol/~2.6 mg
C 048-25, 5 x 5 µmol

Epac-directed Ligand

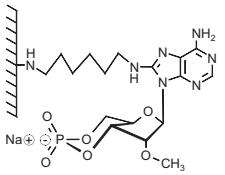
Precursor for labelling with dyes or other markers. Also suitable as a ligand in affinity chromatography of cAMP binding proteins that do not require an intact 2'-OH group, such as Epac and certain phosphodiesterases.



8-AHA-2'-O-Me-cAMP
A 099-05, 5 µmol/~2.3 mg
A 099-25, 5 x 5 µmol

Epac Affinity Gel

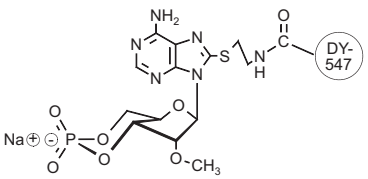
The Epac agonist 8-AHA-2'-O-Me-cAMP (Cat. No. A 099) immobilized on agarose. This gel is suitable for affinity chromatography of various cAMP-responsive proteins, especially those which tolerate modification of the ribose 2'-hydroxy group, such as Epac. Available in pre-packed columns or as free beads.



8-AHA-2'-O-Me-cAMP-Agarose
A 057-06, 0.6 mL
A 057-25, 2.5 mL
A 057-60, 6 mL

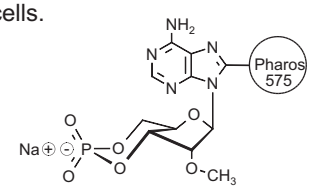
Fluorescent Epac Activators

Fluorescent Epac activator in which the dye is connected to position 8 of the adenine nucleobase via a 5-atom spacer. λ_{exc} 557 nm, λ_{em} 574 nm.



8-[DY-547]-AET-2'-O-Me-cAMP
D 089-001, 0.1 µmol/~0.1 mg
D 089-005, 5 x 0.1 µmol

Fluorescent and membrane-permeant Epac activator. The relatively small Pharos 575 fluorophore (λ_{exc} 577 nm, λ_{em} 605 nm) can be excited e.g. by a Kr/Ar laser. Due to its high lipophilicity and bright red fluorescence 8-[Pharos-575]-cAMP is especially suitable for studies with intact cells.



8-[Pharos-575]-2'-O-Me-cAMP
P 021-001, 0.1 µmol/~74 µg
P 021-005, 5 x 0.1 µmol

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