

Poster presentation

Cytidylyl cyclase activity of bacterial exotoxins

Martin Göttle¹, Frieder Kees¹ and Roland Seifert*²

Address: ¹Department of Pharmacology and Toxicology, University of Regensburg, Germany and ²Department of Pharmacology, Hannover Medical School, Germany

Email: Roland Seifert* - seifert.roland@mh-hannover.de

* Corresponding author

from 4th International Conference of cGMP Generators, Effectors and Therapeutic Implications Regensburg, Germany. 19–21 June 2009

Published: 11 August 2009

BMC Pharmacology 2009, 9(Suppl 1):P16 doi:10.1186/1471-2210-9-S1-P16

This abstract is available from: <http://www.biomedcentral.com/1471-2210/9/S1/P16>

© 2009 Göttle et al; licensee BioMed Central Ltd.

3',5'-Cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP) are key second messengers for a wide variety of mammalian cellular processes. The natural occurrence of a third cyclic nucleotide, 3',5'-cyclic cytidine monophosphate (cCMP) had been discussed very controversially 30–35 years ago [1-3], but later, cCMP was identified unambiguously in various mammalian tissues [4] indicating cCMP to be a novel second messenger with potential importance in regulation of cell growth, proliferation, tissue development and modulation of immune responses [5]. However, the precise identity of the cCMP-forming enzyme in mammalian cells is still unclear. Here, for the first time, we provide evidence for cytidylyl cyclase (CC) activity of purified bacterial exotoxins.

Bacillus anthracis and *Bordetella pertussis*, the causative bacteria of anthrax disease and whooping cough, respectively, secrete the adenylyl cyclase (AC) toxins edema factor (EF) and CyaA, weakening immune responses through massive cAMP production and, thereby, promoting the pathogenesis of the infections [6]. We found that both toxins also possess cytidylyl cyclase activity, resulting in the conversion of CTP to cCMP.

In our CC activity assay, the radioactively labeled substrate [α -³²P]CTP is converted to [³²P]cCMP which is quantified by liquid scintillation. Upon incubation of the toxins with [α -³²P]CTP, [³²P]cCMP is produced in a linear manner over time. As [³²P]cCMP production depends on the endogenous toxin activator protein calmodulin, as

physiological pH promotes the reaction and as potent AC inhibitors show high potency on EF and CyaA, catalysis results from specific CC activity. Michaelis-Menten kinetics of EF CC activity yielded $K_m = 13 \pm 3 \mu\text{M}$ and $V_{max} = 8 \pm 1 \text{ s}^{-1}$.

When CTP consumption and cCMP formation were monitored by HPLC, 100 μM CTP were converted to cCMP by 20 nM EF within 1 h. In the presence of heat-inactivated enzyme, no cCMP was formed. The identity of cCMP was confirmed by co-eluting standard cCMP in HPLC experiments and by mass spectrometry methods. Based on these findings and the fact that cCMP inhibits host immune responses [7,8], we propose that the bacterial exotoxins edema factor (EF) and CyaA generate cCMP, resulting in increased infection severity. The molecular targets of cCMP remain to be determined.

References

1. Bloch A, Dutschman G, Maue R: **Cytidine 3',5'-monophosphate (cyclic CMP), initiation of leukaemia L-1210 cell growth in vitro.** *Biochem Biophys Res Comm* 1974, **59**:955-959.
2. Cech SY, Ignarro LJ: **Cytidine 3',5'-monophosphate (cyclic CMP) formation by homogenates of mouse liver.** *Biochem Biophys Res Comm* 1978, **80**:119-125.
3. Gaion RM, Krishna G: **Cytidylate cyclase: The product isolated by the method of Cech and Ignarro is not cytidine 3',5'-monophosphate.** *Biochem Biophys Res Comm* 1979, **86**:105-111.
4. Newton RP, Groot N, van Geyschem J, Diffley PE, Walton TJ, Bayliss MA, Harris FM, Games DE, Brenton AG: **Estimation of cytidylyl cyclase activity and monitoring of side-product formation by fast-atom bombardment mass spectrometry.** *Rapid Commun Mass Spectrom* 1997, **11**:189-194.
5. Anderson TR: **Cyclic cytidine 3',5'-monophosphate (cCMP) in cell regulation.** *Mol Cell Endocrinol* 1982, **28**:373-385.

6. Leppla SH: **Anthrax toxin edema factor: A bacterial adenylate cyclase that increases cyclic AMP concentrations in eukaryotic cells.** *Proc Nat Acad Sci* 1982, **79**:3162-3166.
7. Elliott GR: **Dibutyryl cytidine 3':5'-cyclic monophosphate; an inhibitor of A23187-stimulated macrophage leukotriene B₄ synthesis.** *Agents and Actions* 1991, **32**:90-91.
8. Ervens J, Seifert R: **Differential modulation by dibutyryl cytidine 3',5'-cyclic monophosphate of neutrophil activation.** *Biochem Biophys Res Comm* 1991, **174**:258-267.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

