BMC Pharmacology



Poster presentation



# Sensitive assay for the detection of cyclic nucleotides by mass spectrometry

Corinna M Spangler*, Bettina Wilhelm, Heike Burhenne, Kerstin Rauch, Roland Seifert and Volkhard Kaever

Address: Institute of Pharmacology, Hannover Medical School, Hannover, Germany

Email: Corinna M Spangler* - spangler.corinna@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):P66 doi:10.1186/1471-2210-9-S1-P66

This abstract is available from: http://www.biomedcentral.com/1471-2210/9/S1/P66



## Background

cAMP and cGMP have been known for a long time to be important second messengers in living cells, whereas other cyclic nucleotides like cCMP or cUMP have only been on the fringes of scientific interest. Recently, our group has discovered that bacterial adenylyl cyclase toxins produce a number of different cyclic nucleoside 5'-monophosphates (cNMPs) apart from cAMP, and the interest in specific and sensitive detection methods rises.

Common approaches for the identification and quantitation of cNMPs are based on recognition of nucleotides by antibodies in case of ELISA or RIA. Furthermore, nucleoside 5'-triphosphates (NTPs) labelled with [$^{32}$P] at the α-position and read-out of the formed amount of radioactively labelled cNMPs are routinely used [1]. In addition, fluorescent assays have been recently developed either depending on fluorescently labelled NTPs or on luminescent probes like terbium(III) norfloxacin reporting the turnover of ATP or GTP. However, all of these methods show drawbacks like use of radioactivity, high costs, non-availability of radioactive substrates or cross-reactivity of antibodies with other nucleotides. Figure 1.

Our aim was to develop a universal method to detect all cNMPs in one single measurement which is sensitive and specific, and can be used for determination of cNMP concentrations in cell and tissue extracts, membrane preparations or with purified enzymes.

## Methods and results

We have used a liquid chromatography (LC) coupled tandem MS (MS/MS) system to separate cNMPs on a reversed-phase column and to identify the specific mass transitions by MS/MS (see Fig 1). Hence, we are able to detect levels of cNMPs as low as approximately 3 nmol/L.

We used the bacterial adenylyl cyclase toxins edema factor (EF) from *Bacillus anthracis* and CyaA from *Bordetella per-*

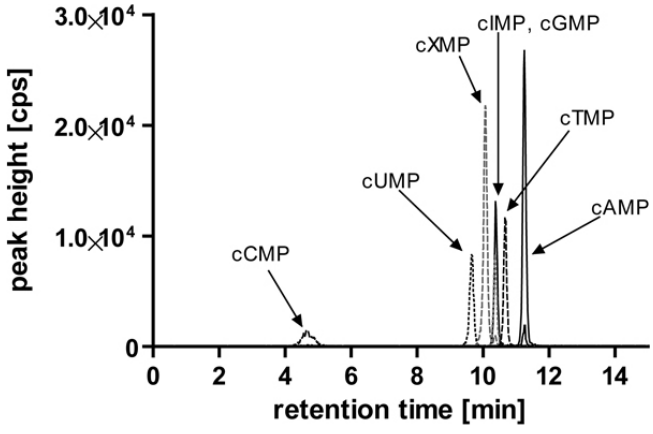


**Figure 1**
**Separation of cNMPs on a reversed-phase column and identification by MS/MS at a concentration of 128 ng/mL.**

*tussis* to record Michaelis-Menten-kinetics with ATP and GTP as substrate. In contrast to generally held belief, both enzymes showed turnover of GTP to cGMP, although with low velocities.

## Conclusion

We have developed a specific and sensitive assay for identification and quantitation of a number of cyclic nucleotides and have applied this method to determine the formation of cNMPs at very low concentrations.

## References

1. Schmidt PM: **Biochemical Detection of cGMP From Past to Present: An Overview.** In *cGMP: Generators, Effectors and Therapeutic Implications Handbook of Experimental Pharmacology 191* Edited by: *Schmidt HHHW, Hofman F, Stasch J-P. Berlin Heidelberg, Springer-Verlag*; 2009:195-228.