

POSTER PRESENTATION

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# Quantification of cAMP and cGMP analogs in intact cells: pitfalls in enzyme immunoassays for cyclic nucleotides

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From 5th International Conference on cGMP: Generators, Effectors and Therapeutic Implications  
Halle, Germany. 24-26 June 2011

## Background

The present work evaluates the cross-reactivity of commercially available cyclic nucleotide analogs with cAMP- and cGMP-immunoassays from Cayman, IBL (both IBL International, Hamburg, Germany) and ENZO Life Sciences (Loerrach, Germany).

## Results and conclusion

Most of the tested cyclic nucleotide analogs showed low degree competition with the antibodies; however, with Rp-cAMPS, 8-Br-cGMP and 8-pCPT-cGMP a strong cross-reactivity with the ENZO cAMP- respectively cGMP-EIA and the IBL cGMP-RIA was observed (Table

**Table 1 Lipophilicity (log  $K_w$ ), cell permeability and EIA/RIA specificity of selected cyclic Nucleotide analogs.**

| Analog                              | Log $K_w$   | Permeability | Specificity ENZO cAMP-EIA | Specificity ENZO cAMP-EIA | Specificity IBL cGMP-RIA | Specificity Cayman cGMP-EIA |
|-------------------------------------|-------------|--------------|---------------------------|---------------------------|--------------------------|-----------------------------|
| 2'-dcGMP                            | 0.65        | 0%           |                           | 5.21%                     |                          |                             |
| <b>cGMP</b>                         | <b>0.77</b> |              |                           | <b>100%</b>               | <b>100%</b>              | <b>100%</b>                 |
| Rp-cGMPS                            | 0.89        |              |                           | 0.27%                     | 10.6%                    |                             |
| 2'-dcAMP                            |             | 0%           | 2.4%                      |                           |                          |                             |
| <b>cAMP</b>                         | <b>1.09</b> |              | <b>100%</b>               |                           |                          |                             |
| 8-Br-cGMP                           | 1.17        | 12.1%        |                           | 490%                      | 20%                      | 0.5%                        |
| Rp-cAMPS                            | 1.21        | 12.2%        | 68%                       |                           |                          |                             |
| 8-Br-cAMP                           | 1.35        | 8.0%         | 0.4%                      |                           |                          |                             |
| Rp-8-Br-cAMPS                       | 1.47        |              | 0.3%                      |                           |                          |                             |
| 6-MB-cAMP                           | 1.64        |              | 0.4%                      |                           |                          |                             |
| 6-Bnz-cAMP                          | 1.9         |              | 0.6%                      |                           |                          |                             |
| 8-pCPT-cGMP                         | 2.52        | 19.6%        |                           | 240%                      | 30%                      | 0.008%                      |
| 8-pCPT-cAMP                         | 2.65        | 22.0%        | 0.05%                     |                           |                          |                             |
| 8-Br-PET-cGMP                       | 2.83        | 30.9%        |                           | 10%                       | 0.15%                    | 1.6%                        |
| Rp-8-Br-PET-cGMPS                   | 2.83        |              |                           | 0.2%                      |                          |                             |
| 8-pCPT-2'-OMe-cAMP (Epac Activator) | 2.94        |              | 0.03%                     | 0.02%                     |                          |                             |
| Sp-5,6-DCI-cBIMPS                   | 2.99        |              | <0.001%                   |                           |                          |                             |

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1). As a consequence we tested these derivatives with the Cayman cGMP-EIA. This assay is less sensitive to cGMP (1.0 pmol/ml) than the ENZO cGMP-EIA (0.01 pmol/ml), however the specificity concerning cGMP-analogs is superior and therefore advantageous when measuring cGMP in the presence of 8-Br-cGMP or 8-pCPT-cGMP.

The determined EIA binding constants enabled the measurement of the intracellular cyclic nucleotide concentrations and revealed a time- and lipophilicity-dependent cell membrane permeability of the compounds in the range of 10-30 % of the extracellular applied concentration after 20 min (Table 1).

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Published: 1 August 2011

doi:10.1186/1471-2210-11-S1-P75

**Cite this article as:** Werner *et al.*: Quantification of cAMP and cGMP analogs in intact cells: pitfalls in enzyme immunoassays for cyclic nucleotides. *BMC Pharmacology* 2011 **11**(Suppl 1):P75.

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