

Poster presentation

## Binding of [<sup>3</sup>H]BAY 58-2667 to purified bovine lung soluble guanylate cyclase by scintillation proximity assay

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from 2nd International Conference of cGMP Generators, Effectors and Therapeutic Implications  
Potsdam, Germany, 10–12 June, 2005

Published: 16 June 2005

BMC Pharmacology 2005, 5(Suppl 1):P20 doi:10.1186/1471-2210-5-S1-P20

### Background

Activation of soluble guanylate cyclase (sGC) by BAY 58-2667 is independent of nitric oxide (NO) and is potentiated by oxidation or removal of the heme prosthetic group [1]. This compound will displace heme from sGC but only at concentrations several orders of magnitude higher than those which activate the enzyme [2]. Therefore the nature of the interaction of BAY 58-2667 with sGC, the mechanism of activation, and the role of heme-displacement under physiological conditions is of considerable interest.

### Materials and Methods

Scintillation proximity assay (SPA) technology avoids the need for separation of bound and free radioligand, enables the real-time study of binding kinetics, and is particularly well suited to soluble receptor proteins. Therefore we investigated the feasibility of utilizing this methodology in the characterization of binding of the heme-independent activator BAY 58-2667 to sGC.

### Results

Binding assays were configured using Protein A coated SPA beads, an anti-sGC antibody, purified bovine lung sGC and [<sup>3</sup>H]BAY 58-2667. Binding was dependent on the presence of both antibody and sGC. A number of different antibodies and sources of sGC were investigated and concentrations optimized to maximize specific binding (defined as that displaced by a 1000-fold excess of non-radioactive BAY 58-2667). Specific binding was saturable and reversible. Saturation data was best-fit with a single-site model and a K<sub>d</sub> of approx 2 nM which is significantly higher than EC<sub>50</sub> values derived from relaxation of vascular preparations in-vitro by ourselves and others

[1]. Maximum binding was approximately 1000 cpm. Kinetic experiments revealed a mono-exponential dissociation phase. No significant competition of [<sup>3</sup>H]BAY 58-2667 was observed between heme-dependent sGC activators such as YC-1 in the absence or presence of an NO-donor. Likewise, a range of guanine nucleotides were tested for their ability to influence binding of [<sup>3</sup>H]BAY 58-2667 and no effect was observed.

### Conclusion

SPA technology is a convenient method to study the interaction of sGC activators with the enzyme. Binding mirrors functional effects but appears of lower affinity than expected from functional measurements in vascular preparations. The reasons for this discrepancy will be the subject of future investigation.

### References

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