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Poster presentation

Characterisation of human a1 and b1 soluble guanylate cyclase promoter activity in human aortic smooth muscle cells

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As a principal intracellular receptor for nitric oxide (NO), soluble guanylate cyclase (sGC) plays a fundamental role in cardiovascular homeostasis and disease. However, there is a paucity of information regarding expressional regulation of sGC in the vasculature. Since the enzyme is an obligatory heterodimer composed of α and β subunits [1], we investigated the transcriptional regulation of human sGC α_1 and β_1 genes in human aortic smooth muscle cells (HASMCs).

The 5' flanking regions harbouring 0.3–3.0 kb of both α_1 and β_1 sGC genes were isolated and analysed for promoter activity by using luciferase-reporter constructs. Upstream fragments of 0.3 kb and 0.5 kb exhibited maximal promoter activity for the α_1 and β_1 sGC promoters, respectively (Figure 1). The functional significance of consensus transcriptional factor (TF) binding sites proximal to the transcriptional start site was investigated by site-specific deletions in both 0.3 kb (α_1 sGC) and 0.5 kb (β_1 sGC) promoter fragments. Putative binding sites for the α_1 (c-Myb, NFAT, GABF) and β_1 (NFκB, Sp-1, CBF) sGC promoters were studied. Preliminary data reveal that the activity of the constructs containing deletions of these TF binding sites was significantly altered in each case, suggesting that the analysed binding sites are major determinants of the α_1 and β_1 sGC promoter activity in HASMCs. Electrophoretic mobility-shift assay (EMSA) and immunoblot analyses of proteins bound to biotinylated EMSA probes will be performed to confirm the interaction of these factors with the corresponding sGC promoter.

These data therefore provide a systematic, comparative analysis of human α_1 and β_1 sGC promoter regulation in HASMCs and thereby identify potentially important factors regulating human sGC expression within a cell system relevant to cardiovascular physiology and pathology.

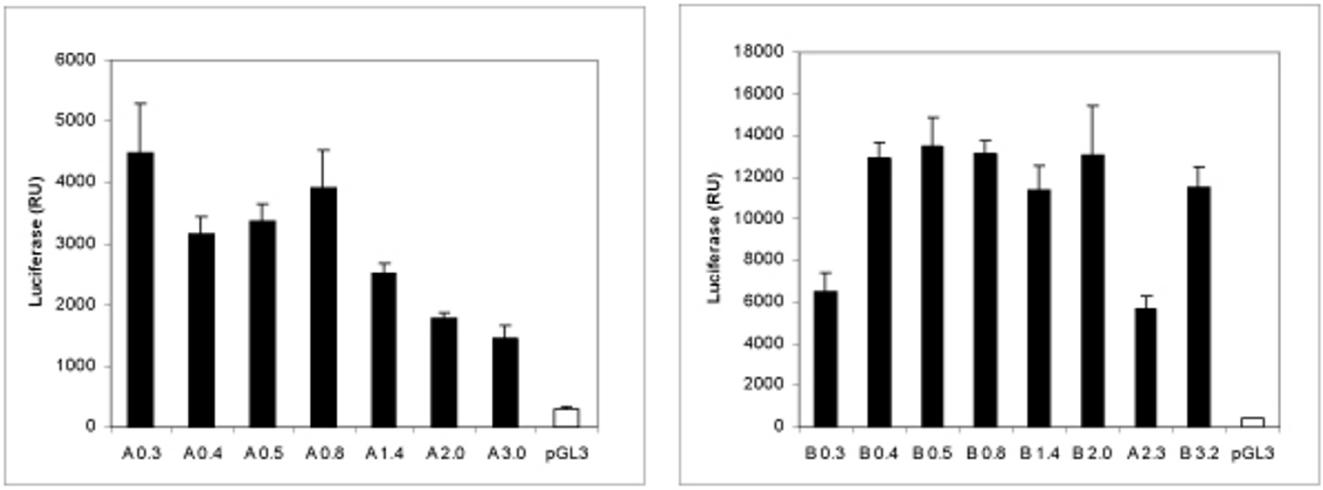

Figure 1
Promoter activity of α_1 and β_1 human sGC luciferase reporter constructs analysed in HASMCs. Promoter activity for each construct was measured in relative (firefly/renilla luciferase) light units. Results are expressed as mean ± SEM (n = 4).

References

1. Kamisaki Y, Saheki S, Nakane M, Palmieri JA, Kuno T, Chang BY, Waldman SA, Murad F: **Soluble guanylate cyclase from rat lung exists as a heterodimer.** *J Biol Chem* 1986, **261:**7236-7241.