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Adrenoceptor modulation of matrix metalloproteinase-9 (MMP-9) activity and promoter activity in murine fibroblasts (NIH3T3): not all that it seems!

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Fibroblasts are critical for the maintenance of the extracellular matrix in normal tissue, and as a mediator of inflammatory and fibrotic remodelling in disease. As part of their regulatory function, fibroblasts secrete a number of matrix metalloproteinases, including MMP-2 and MMP-9, which can degrade the extracellular matrix. MMP activity is tightly regulated at several levels including transcriptional and zymogen activation (Nagase & Woessner, 1999). We have previously shown that isoprenaline and phenylephrine stimulate MMP-9 promoter activity in ECV304 cells through a 762-bp region containing binding sites for NFκB (-600bp) and AP-1 (-533bp) (Song *et al.*, 2006). Based upon this, the present study investigates the effects of adrenoceptor stimulation on MMP-9 activation in fibroblasts and the possible involvement of NFκB.

NIH3T3 fibroblasts were grown in DMEM containing 2mM glutamine, 10% calf serum and antibiotics. Cells were transfected with MMP-9 or NFκB luciferase reporter constructs and an internal standard (pRL-CMV), and incubated in medium supplemented with either isoprenaline (ISO 1μM), phenylephrine (PE; 10, 50μM), medium (untreated control) or TNFα (0.4 or 1 ng) for 24hrs. Transcriptional activation was measured using a dual-luciferase reporter assay. Data were normalised to internal standard and expressed relative to the untreated controls. MMP activity in cell lysates was determined by gelatin zymography. All experiments were performed in phenol red free medium containing 5% charcoal stripped serum. Data were analysed by one-way ANOVA with *post hoc* analysis (Bonferroni), and expressed as mean±SEM. A value of $P<0.05$ was taken to indicate statistical significance.

ISO and PE did not alter MMP-9 promoter activity in transfected NIH3T3 fibroblasts compared to untreated controls (0.98±0.16 AU); while TNFα (1ng) elicited a 2 fold increase in promoter activity ($P<0.05$; 2.8±0.27 fold). Furthermore, ISO and PE did not affect NFκB promoter activity compared to control (0.98±0.05 and 1.22±0.33 vs 1.0±0.13 fold, respectively). TNFα increased NFκB promoter activity by 50 fold ($P<0.05$). In support of these data, neither ISO nor PE altered MMP-9 activity in cell lysates compared to untreated cells (ISO 1μM: 1.15±0.22 AU; PE 10 μM: 0.53±0.21 vs 0.9±0.19 AU), while TNFα increased MMP-9 activity by 5 fold ($P<0.05$; 6.20±0.69 vs 0.9±0.19 AU).

In conclusion, unlike in ECV304 cells adrenoceptor stimulation does not increase either MMP-9 activity, MMP-9 promoter activity or NFκB promoter activity in fibroblasts. The implications of these findings are that the anti-remodelling effects of β-adrenoceptor antagonists observed in patients with heart failure are unlikely to be mediated through direct effects on cardiac fibroblasts.

Nagase,H. & Woessner,J.F. (1999). *J. Biol. Chem.,* 274, 21491-21494.

Song,G., et al (2006). *Pharmacol. Res.,* 54, 57-64.