3. RESULTS

Part I: in vitro studies

3. 1 Basic expression of B1R and B2R mRNA in different cell lines

Basic expression of B1R and B2R mRNA by neonatal cardiomyocytes (CMC), cardiac fibroblasts (CFB), and aortic smooth muscle cells (SMC) was investigated respectively. Cells were treated with serum free medium, then total RNA was extracted from each cell line and subjected to RPA assay. The results showed that B1 and B2 receptor mRNA were detectable in CMC and SMC RNA samples, which is in agreement with previous findings (McLean et al., 2000; Christopher et al., 2001), indicating that rat CMC and SMC are sources of BK B1and B2 receptors. For B2R mRNA, using the generated anti-sense RNA probes, two splicing variants (containing part of intron3+exon4 and/or exon4 of the B2R gene), could be detected in the RPA blots. These two splicing variants however, are expressed in a similar pattern. In RPA bolts, the expression of both B1 and B2 receptor mRNA were detected higher in CMC than in SMC (approximately 2.5 fold for both). In contrast, both B1 and B2 receptor mRNA were not detected in CFB RNA samples, , although 18 μg –20 μg RNA were used in the experiments (Fig 3.1, Fig 3.2).

3. 2 Effects of IL1ß on the expression of B1R and B2R mRNA in CMC

3.2.1 12h IL1ß treatment

On the 4th day of cell culture, confluent monolayer of CMC could be observed beating synchronously at a rhythm between 110 and 170 times/min. After 12 h serum free medium treatment and subsequent 12 h co-treatment with different concentrations of IL1ß, the expression of both B1R and B2R mRNA were analysed. RPA blots showed that B1R mRNA was upregulated by IL1ß in a strong concentration dependent pattern. 40 or 400 pg/ml IL1ß mildly increased the level of B1R mRNA, but this effects were not significant, while 4000 pg/ml IL1ß caused a significant increase (20 fold) in B1R mRNA levels compared to the basal

expression (Fig 3.3). In contrast, IL1ß at different concentrations did not cause significant changes in B2R mRNA expression (Fig 3.4)

3.2.2 Time course experiment

To further extend this finding, a time course experiment was conducted. As shown in Fig 3.6 and Fig 3.8, after treating these cells with 400 pg/ml IL1ß, a dramatic time-dependent increase of both B1R and B2R expression by CMC could be detected, with a peak response at 3 h post-stimulation. IL1ß significantly increased the B1R at 3 h and this effect was maintained until 6 h post-stimulation. Also, IL1ß elicited a rapid and transient increase in the level of B2R mRNA at 3 h, but at 6 h, 9 h post-stimulation this effect was not significant anymore. Both receptor levels remained unchanged at different time points when cells were not treated with IL1ß (Fig 3.5; Fig 3.7)

3.2.3 Dose-dependency experiment

Based on the above results, in this experiment different concentrations of IL1ß were used and cells were treated for 3 hours. As shown in Fig 3.9, with increasing concentrations of IL1ß, its effect on B1R appeared stronger, 400 pg/ml IL1ß already caused an significant increase of B1R mRNA expression. A further upregulation of B1R expression was observed by a factor of 25 ($p<0.005$, T-test) when cells were treated with 4000 pg/ml IL1ß, compared to the untreated groups (Fig 3.9). In contrast to B1R, the upregulation of B2R by IL1ß was mild. However, when cells were treated with 4000 pg/ml IL1ß, there was a three to four fold increase in the expression of the B2R mRNA compared to untreated groups (Fig 3.10).

3. 3 Effects of IL1ß on the expression of B1R and B2R mRNA in CFB

Compared to CMC, CFB divided rapidly. After passaging two times, these cultures appeared morphologically homogenous. Since basic expression of both B1R and B2R mRNA were not detectable from these cells (Fig 3.1-2), different concentrations of IL1ß was used during cell culture. After 3 h treatment, total RNA was isolated from cells and RPA was performed. Results showed that neither B1R nor B2R mRNA were induced by IL1ß stimulation. In the same blot, B1R or B2R mRNA could be detected when myocardium or ileum RNA sample was used, indicating the experiment system worked in a normal way (Fig 3.11).

3. 4 Effects of IL1ß on the expression of B1R and B2R mRNA in SMC

To elucidate the influence of IL1ß on the expression of B1R and B2R mRNA in vascular cell types, time-course or dose-dependency experiments were carried out with rat aortic SMC. As shown in Fig 3.1-2, both B1R and B2R mRNA could be detected in SMC, however, IL1ß treatment at different conditions did not influence the expression of both B1and B2 receptors in SMC (Fig 3.12-15).

Part II: in vivo studies

3. 5 Influence of ICEI on the expression of B1R after MI induction

In previous work, we showed that MI induction upregulated the expression of B1R and B2R mRNA in the left ventricle. The expression of B1R as well as B2R reached a peak level at 24 h post MI induction and maintained upregulated in the myocardium for at least 3 weeks. Comparably, Regulation of IL1ß expression by MI induction in the left ventricle showed a similar time pattern, with a peaked release at 24 h post MI. In the present study, it was shown that stimulation of IL1ß dramatically upregulated both B1R and B2R mRNA expression in cultured cardiac myocytes. To elucidate the possible influence of cytokine IL1ß on the expression of both kinin receptors in the heart, rat MI model was set up. Rats were treated with ICEI (IL1ß converting enzyme inhibitors) from the first day of MI induction. 3 weeks later, the expression of both kinin receptors in the ventricular myocardium was investigated. As shown in Fig 3.16, B1R mRNA expression was detected in the left ventricle (induced by MI induction), while the administration of ICEI significantly downregulated its expression.

3. 6 Influence of ICEI on the expression of B2R after MI induction

The expression of B2R mRNA in the left ventricle after MI induction and ICEI administration was investigated using the same method. As shown in Fig 3.17, when rats were treated with ICEI, a reduced expression of B2R could be detected, but this effect was not significant.