4. DISCUSSION

4. 1 Basic expression of B1R and B2R mRNA by different cell systems

Bradykinin receptors especially B2R mediate the majority of the diverse biological actions of kinins. The B1 and B2 receptor subtypes have been previously identified on various cell types such as endothelial cells (Keravis et al., 1991), bronchial smooth muscle cells (Schmidlin et al., 1998), lung fibroblasts (Webb et al., 1994), cardiomyocytes (Yayama et al., 2000), neural-derived cells (Fujiwara et al., 1988), and deciduas-derived cells (Rehbock et al., 1999). In agreement with theses findings, data from the present study show that bradykinin B1 and B2 receptors are expressed by cardiomyocytes and aortic smooth muscle cells. 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 most of the RPA blots, basal levels of the B2R mRNA are higher than the B1R mRNA, which is in agreement with the knowledge that B2R is ubiquitously released while B1R is lack normally. The amount of both receptors were detected to be higher in cardiomyocytes than in smooth muscle cells. This may reflect a higher amount of kinin receptors on cardiomyocytes, however, cell growing conditions may also affect the expression of kinin receptors: for smooth muscle cells, experiments were performed at passage between 15-20, while cardiomyocytes were used by primary culture. In cardiac fibroblast, both kinin receptor mRNA are not detectable although 15-20 μg RNA was used for the RNase protection assay. It is known that the heart tissue consists different cell types, cell to cell interactions between identical and different cell types modulate the hypertrophic reaction. Among them, the myocytes comprise 70-80% of the heart mass but represent only 20-30% of total cardiac cells. Whereas fibroblasts, neurons, and endothelial cells account for the majority in numbers (Marcus et al., 1997). Under normal conditions, basic expression of the kinin receptors especially B2R by these cells systems has been documented. In the present study, lack of detection of the kinin receptors in cardiac fibroblasts is not due to methodological problems, since the signals are detectable in myocardium or ileum RNA samples in the same blot. In the heart, many studies suggest that ACEI treatment and activation of bradykinin are capable of modulating the process of cardiac hypertrophy and fibrosis, however, reports concerning the abundance of kinin receptors in

fibroblasts are sparse. In one *in vitro* study, the authors documented that the functional B2R was much less (100 fold) expressed on cardiac fibroblasts than on cardiomyocytes (Minshall et al., 1995). This may suggest that kinin receptors are quite less expressed in cardiac fibroblasts under normal conditions.

4. 2 Influence of IL1ß on the expression of B1R and B2R in different cell lines

It has been well known that the cytokine IL1ß can induce B1R expression in various cell types, and this effect is shown to be exerted through a transcriptional regulation, which is proposed to involve NK-$_k$B (Kessler et al., 1992). Also, IL1ß may upregulate B2R expression through a prostanoid dependent pathway that activates cAMP formation, which may stimulate B2 gene transcription (Schmidlin et al., 1998). However, these effects may vary among different cell systems. For instance, in human lung fibroblasts, it was reported that both B1 and B2 receptors were expressed (Webb et al., 1994) and could be upregulated by cytokines such as TNFα or IL1ß (Phagoo et al., 2000 *b*); While in cultivated human decidua-derived cells, although the expression of B2R protein was induced significantly up to 300% after incubation with IL1ß, the same treatment failed to induce B1R expression (Rehbock et al., 1999). The effects of cytokine IL1ß on the expression of kinin receptors seem cell type-dependent. Data from the present study showed that IL1ß dramatically affect both B1 and B2 kinin receptor expression in cardiomyocytes, but these effects were not observed in aortic smooth muscle cells.

Effects of IL1ß on the expression of B1R and B2R in CMC

Using *in vivo* and *in vitro* studies, Katsutoshi et al. (2000) demonstrated that rat cardiomyocytes expressed mRNAs for the components of the kallikrein kinin system, such as kininogens, kallikreins and B1/B2 receptors, and the only kininogen synthesised in myocardium seems to be T-kininogen, which is unique to the rat. They also found that the level of B1R in cultured cardiomyocytes was increased after treatment with LPS (lipopolysaccharide), IL1ß, or TNFα, but decreased after treatment with IL6. In contrast, these agents had no influence on B2R expression on cardiomyocytes. The authors documented that

cardiomyocytes were source of both kinin receptors and B1 (but not B2) receptor could be upregulated by cytokine IL1ß. In these experiments however, cells were treated with agents for 24 hours, the upregulating effects of cytokines on B2R expression might not be observed at this time point. In the present study, RPA blots showed that 3h IL1ß treatment caused an upregulation of B2R expression (a factor of 3 fold) in cardiomyocytes, while 12 h of the same treatment blunted this effect, suggesting both receptors could be upregulated by IL1ß but in a time-depending pattern. However, it can be observed that although IL1ß increased B2R mRNA in CMC, the effect was less pronounced than that on B1R. Under normal conditions, the B2R is believed to mediate most of the actions of kinins. However, after inflammatory insult, the response to B1R agonist can develop within hours and last for over 3 days (Davis et al., 1994). The inducible character of B1R has been a scientific interest for long time and was investigated extensively. In a rat isolated heart, it has been reported that *in vitro* perfusion with IL1ß could promote the B1 receptor mRNA expression and cause coronary vasodilation (McLean et al., 1999). As to B2R, It has also been previously reported that its expression could be upregulated by cytokines, including IL1ß and other factors such as platelet-derived growth factor (Dixon et al., 1996). In the body, cytokines may affect kinin receptors in a complex context, via autocrine-paracrine mechanisms, correlate with other effecting molecules, and lead to kinin-dependent biological effects.

IL1ß failed to induce the expression of kinin receptors in CFB

In cardiac fibroblasts, signals for both kinin receptors were not detected. Also, the addition of cytokine IL1ß did not induce their expression, further suggesting that bradykinin receptors are quite lowly expressed by fibroblasts in the heart. The fact, that cardiac fibroblasts had no dramatic B1/B2 receptor expression in response to cytokine IL1ß stimulation, may suggest that cardiac fibroblasts do not play a role in bradykinin receptor mediated effects. However, since cardiac cell cultures were performed from neonatal rat hearts, it can not be assumed that all the observed effects are relevant to adult cardiac cells. Additionally, under pathological conditions, behavior of the cells may undergo certain modulations. After MI, the heart undergoes an complementary adaptation termed heart remodelling. This complex process is not limited to the areas of infarction, the healthy cardiac myocytes undergo hypertrophy (or with a small percentage myocyte division) to compensate the heart failure. The cardiac

endothelial cells, smooth muscle cells, as well as fibroblasts are known to proliferate under these conditions, and new evidence suggests that even myocytes may also divide to compensate for the exhaustion of myocyte hypertrophy (Antonio et al., 2001). Under normal conditions, both BK receptors may be less expressed by fibroblasts, after induction of MI however, fibroblasts are converted to fibromyocytes, it can not be excluded that under these conditions, fibroblasts may express BK receptors due to cytokine stimuli, thus extending the cardioprotective roles of kinins such as vasodilation which are important for initiating subsequent repair phase. Further studies are needed to test the regulation of kinin receptors in cardiac fibroblasts during myocardial infarction.

Effects of IL1ß on the expression of B1 and B2 receptors in SMC

In several *in vitro* studies, effects of IL1ß on bradykinin receptor expression have beeen investigated on some smooth muscle cell systems. Galizzi et al. (1994) reported that in rabbit mesenteric artery smooth muscle cells, IL1ß promoted binding of the specific bradykinin B1 receptor agonist, [3H]-des-Arg^{10}-kallidin, and increased PGI_2 production. Also , it stimulated the response to B2 agonists, while LPS treatment of the cells did not affect the B1 agonist response. Schmidlin et al. (1998) showed that IL1ß induced a 5-fold increase in B2 mRNA levels and a 71.5 ± 16.1% increase in the maximal number of B2 binding sites in human bronchial smooth muscles cells, mainly through a prostanoid pathway that activates cAMP formation, leading to B2 gene transcription. In contrast to these findings, results from the present study show that the mRNA levels of both receptors are not significantly affected by IL1ß although a baseline population of B1R as well as B2R mRNA are detectable in cultured aortic smooth muscle cells. Experiments were performed at least 3 times. This may suggest a varying effects on different cell systems. However, the cell culture conditions should also be considered when evaluating the bradykinin receptor inducing effects of the IL1ß. In the present study cells were used between the 15th and 20th passages instead of by primary culture. In the RPA blots, it can be observed that the density of both B1R and B2R is lower in vascular smooth muscle cells than in cardiomyocytes, while the less prominent response to IL1ß may indicate a weaker influence on kinin receptors in these cells. Further studies are needed with primarily cultured aortic vascular smooth muscle cells.

4. 3 Effects of ICEI on the B1R and B2R expression post MI induction

During myocardial infarction, the generation and release of the cytokine IL1ß is greatly elevated. IL1ß is predominantly released from activated human monocytes. It is synthesized firstly as a cytosolic inactive precursor of 33 kD, which is released only upon cleavage to the mature 17.5 kD form. The intracellular protease that is responsible for this processing is caspase-1, also referred as interleukin-1 converting enzyme (ICE). In the present study, experimental MI rats were treated by the ICE inhibitor (ICEI) for 3 weeks, which was given to suppress the generation and release of IL1ß early after MI induction. As shown in Fig 3.16-17, after ICEI administration, the expression of both kinin receptors in the left ventricle were reduced, a significant downregulation of the B1R mRNA could be observed in the left ventricle. After ICEI administration, the known upregulation of IL1ß in the early phase of MI would be blocked. Since IL1ß induces B1R upregulation through induction of transcriptional as well as post-transcriptional mRNA stabilisation, and it also induces B2R through a prostanoid cyclic AMP-dependent pathway. It is possible that after inhibiting of the IL1ß production, the kinin receptor inducing effects would be greatly attenuated. The reduced expression of both B1 and B2 receptors in ICEI treated MI rats provides another strong evidence, that IL1ß is an important mechanism for the upregulation of both kinin receptors early after MI induction.

4. 4 Conclusion

In conclusion, cardiomyocytes and aortic vascular smooth muscle cells constitutively express both bradykinin B1 and B2 receptors, and stimulation with the cytokine IL1ß significantly upregulated both receptor mRNA expression especially in cardiomyocytes. In aortic vascular smooth muscle cells, expression of both receptor mRNA could not be influenced by IL1ß stimulation. In cardiac fibroblasts, expression of both kinin receptor mRNA could not be detected, and the addition of IL1ß failed to induce their expression. *In vivo* studies revealed that the administration of IL1ß converting enzyme inhibitor downregulated the bradykinin B1

receptor expression in the heart after MI induction, but this treatment caused no significant changes in B2R expression. It can be concluded that the elevated release of cytokine IL1ß early after myocardial infarction may, at least in part, upregulate bradykinin B1 and B2 receptor expression through a CMC-specific pathway.