

Reduced cardiac hypertrophy and altered blood pressure control in transgenic rats with the human tissue kallikrein gene¹

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SPECIFIC AIM

The aim of this study was to evaluate the cardiovascular actions of kinins, which have been implicated in the beneficial effects of angiotensin-converting enzyme (ACE) inhibitors by the generation and analysis of a transgenic rat line harboring the human tissue kallikrein gene, TGR(hKLK1).

PRINCIPAL FINDINGS

1. Transgene expression

Expression of a human tissue kallikrein transgene (hKLK1) under the control of the mouse metallothionein promoter was detected in all organs of the newly generated transgenic rat line, TGR(hKLK1) (Fig. 1). Translation of the hKLK1-mRNA was verified by the demonstration of human kallikrein in the urine of transgenic rats (700 ± 127 ng/ml).

2. Blood pressure

Mean arterial pressure determined by telemetric measurement turned out to be slightly but significantly lower in TGR(hKLK1) animals compared to Sprague Dawley (SD) control rats (110.5 ± 1.1 vs. 114.9 ± 1.0 mmHg; $P < 0.01$). In contrast, no significant difference was observed between the transgenic and SD rats with respect to heart rate or locomotor activity. The B2 antagonist icatibant increased blood pressure (BP) significantly by 2.0 ± 0.9 mmHg ($P < 0.01$) only in TGR(hKLK1).

The 24 h rhythm of mean arterial pressure in TGR(hKLK1) animals was dampened in comparison to untreated SD rats (amplitude of the dominant

24 h period in TGR(hKLK1): 1.6 ± 0.2 ; in SD: 2.7 ± 0.4 ; $P < 0.05$). However, in both strains, the acrophases of the 24 h period occurred around midnight for BP, and the rhythms of heart rate and locomotor activity were similar.

3. Reduced cardiac hypertrophy and fibrosis in TGR(hKLK1)

To study the role of kallikrein in cardiac hypertrophy and fibrosis, TGR(hKLK1) and SD control rats were treated with a suppressor dose of isoproterenol for 7 days. This treatment resulted in a marked increase in relative heart (Fig. 2A) and left ventricular weight (Fig. 2B) in both strains of rats. However, the effect was significantly less pronounced in TGR(hKLK1), indicating a protective action of transgene expression. Expression of ANP in the left ventricle, an early marker of cardiac hypertrophy, supported these findings. Whereas it was markedly induced in SD rats, no effect on ANP expression was observed in TGR(hKLK1) (Fig. 2C). Kinins and their B2 receptors obviously mediated the kallikrein action as the transgene effect on cardiac hypertrophy was abolished by the coadministration of icatibant (Fig. 2).

Moreover, interstitial fibrosis in the left ventricle, also induced by isoproterenol treatment and quantified by detecting collagen III mRNA in left ventricles, was less pronounced in TGR(hKLK1) compared to SD rats (Fig. 2D).

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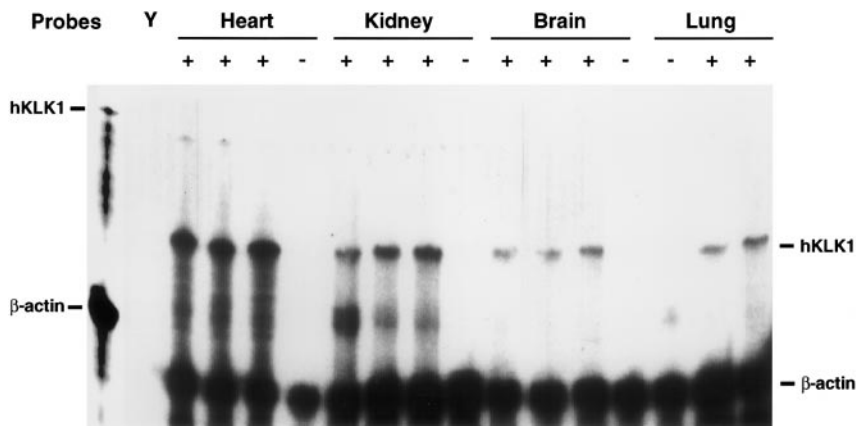


Figure 1. Ribonuclease protection assay detects expression of the hKLLK1 gene in different organs of TGR(hKLLK1) (+) but not in SD control rats (-). 20 µg of total RNA from tissues of individual animals or yeast (Y) together with hKLLK1- and β-actin-specific probes was used.

CONCLUSIONS AND SIGNIFICANCE

Transgenic rats expressing human tissue kallikrein at high levels in all organs investigated became hypotensive probably through increased generation of kinins as evidenced by a partial normalization of BP after treatment with the B2 receptor antagonist icatibant. These findings are in line with results obtained in mice expressing the same transgene and hypertensive rats after somatic gene transfer of the hKLLK1 gene.

An important novel observation in this study is the

role of the kallikrein-kinin system (KKS) in circadian fluctuations of arterial pressure. The biological mechanisms underlying regulation of circadian rhythms of cardiovascular parameters are largely unknown. In mammals, most circadian rhythms are governed by what appears to be the main internal oscillator, the suprachiasmatic nucleus of the hypothalamus (SCN), since lesions in this brain region can obliterate the rhythms of heart rate and BP. Nonetheless, little is known about how oscillations in the SCN are biochemically achieved or which neuronal or hormonal pathways are used to establish the

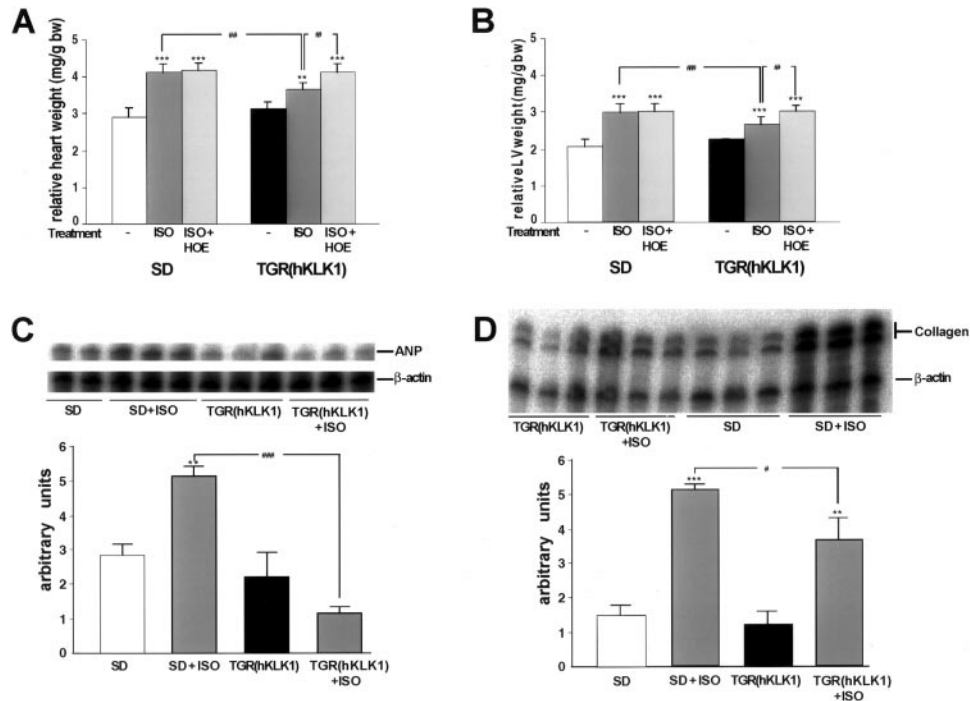
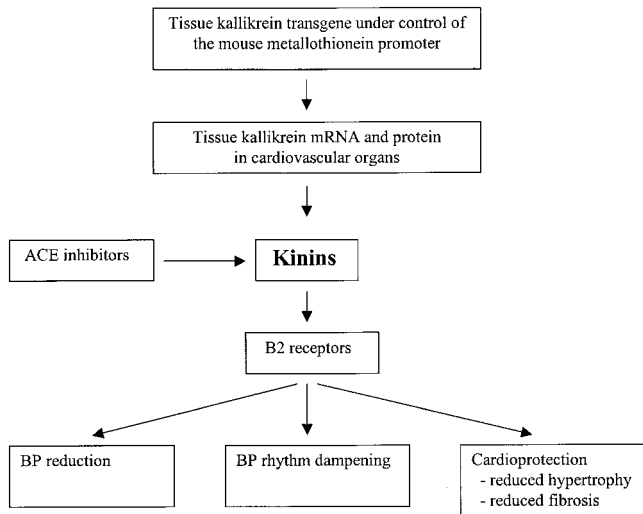


Figure 2. Cardiac hypertrophy and fibrosis after isoproterenol treatment is reduced in TGR(hKLLK1) compared to SD rats. Heart weight (A) and left ventricular weight (B) to body weight ratios were determined of untreated SD and TGR(hKLLK1) rats and of animals treated with isoproterenol (ISO) alone for 7 days ($n=10$) or with coadministration of icatibant (HOE, $n=4$). ANP (marker of hypertrophy, C) and collagen III (marker of fibrosis, D) expression were detected by ribonuclease protection assay in left ventricles of untreated and ISO-treated SD and TGR(hKLLK1) rats (upper panel). 20 µg of total RNA of left ventricles was used with probes for rat ANP, collagen III, and β-actin. The levels of ANP and collagen III mRNA relative to β-actin mRNA were quantified using the software TINA on a PhosphorImager system (lower panel). *** $P < 0.005$, ** $P < 0.01$ vs untreated rats; ### $P < 0.005$, ## $P < 0.01$, # $P < 0.05$.



Scheme 1

rhythm of the cardiovascular system. Kallikrein, kininogens, kinins, and their receptors, B1 and B2, are present in several regions of the brain, including SCN, and the central KKS is involved in BP regulation. Furthermore, a circadian rhythm of kallikrein expression has recently been reported in the rat pineal gland. Kinins may also be important in transmitting the oscillations generated in SCN and pineal gland to other brain areas or to the periphery. A peripheral action of the KKS governing diurnal BP variations is supported by the circadian rhythm of urinary kallikrein excretion reported, with highest concentrations preceding lowest BP values. Thus, the KKS may be deeply involved in the circadian regulation of BP. As the circadian rhythmicity of heart rate is unaltered in TGR(hKLK1), this study confirms earlier reports that the rhythms of BP and heart rate are differentially regulated.

The most important novel finding of this study is the prohibitive effect of tissue kallikrein in isoproterenol-induced cardiac hypertrophy and fibrosis. Reductions in hypertrophy and fibrosis were abolished by icatibant, which further supports kinins as medi-

ators of the transgene effects. Consistent with an important role of kinins, previous pharmacological studies have shown that isoproterenol-induced cardiac hypertrophy can be diminished more effectively by ACE inhibitors than by AT1 antagonists. Kinins have also been implicated in the antihypertrophic actions of ACE inhibitors in the aortic coarctation model since icatibant effectively blunted the drug-induced reduction of left ventricular hypertrophy. Furthermore, bradykinin infusion prevented development of increased cardiac mass in the same model. While kinins may act as weak growth factors on cultured cardiomyocytes and fibroblasts, they have also been shown to reduce cardiac collagen synthesis and growth via release of prostaglandins and nitric oxide. According to our results, the latter seems to be the prevailing *in vivo* effect. Adaptation of cardiac muscle to an increased work load can be achieved either by an increase in muscular mass (i.e., hypertrophy) or by an improved performance of the existing myocardium. Kinins may inhibit hypertrophy by improving the supply of cardiomyocytes with nutrients and oxygen via two independent mechanisms: increase in coronary perfusion and stimulation of myocardial glucose uptake. These kinin effects may be crucial for the prominent cardioprotective actions of ACE inhibitors. In summary, we have developed a new transgenic rat model with an overactive KKS. These rats may be applicable to the study of multiple issues of cardiovascular regulation and other physiological and pathophysiological mechanisms in which kinins might participate. **[FJ]**

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