Kallikrein gene delivery attenuates cardiac remodeling and promotes neovascularization in spontaneously hypertensive rats

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Bledsoe, Grant, Lee Chao, and Julie Chao. Kallikrein gene delivery attenuates cardiac remodeling and promotes neovascularization in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 285: H1479–H1488, 2003. First published June 19, 2003; 10.1152/ajpheart.01129.2002.—Hypertension that results in left ventricular (LV) hypertrophy and/or fibrosis can lead to cardiac dysfunction. Spontaneously hypertensive rats (SHR) develop high blood pressure and LV hypertrophy at an early age and are a popular model of human essential hypertension. To investigate the role of the tissue kallikrein-kinin system in cardiac remodeling, an adenovirus containing the human tissue kallikrein gene was injected intravenously into adult SHR and normotensive Wistar-Kyoto (WKY) rats. The blood pressure of WKY rats remained unchanged throughout the experiment. Alternatively, kallikrein gene transfer reduced blood pressure in SHR for the first 2 wk, but had no effect from 3 to 5 wk. Five weeks after kallikrein gene delivery, SHR showed significant reductions in LV-to-heart weight ratio, LV long axis, and cardiomyocyte size; however, these parameters were unaffected in WKY rats. Interestingly, cardiac collagen density was decreased in both SHR and WKY rats receiving the kallikrein gene. Kallikrein gene transfer also increased cardiac capillary density in SHR, but not in WKY rats. The morphological changes after kallikrein gene transfer were associated with decreases in JNK activation as well as transforming growth factor (TGF)-β1 and plasminogen activator inhibitor-1 levels in the heart. In addition, kallikrein gene delivery elevated LV nitric oxide and cGMP levels in both rat strains. These results indicate that kallikrein-kinin attenuates cardiac hypertrophy and fibrosis and enhances capillary growth in SHR through the suppression of JNK, TGF-β1, and plasminogen activator inhibitor-1 via the nitric oxide-cGMP pathway.

hypertension; cardiac hypertrophy; cardiac fibrosis; angiogenesis

HYPERTENSION INCREASES the risk of cardiovascular events such as coronary heart disease and heart failure. Left ventricular (LV) hypertrophy and/or fibrosis accompanied with hypertension may contribute to cardiac dysfunction. Cardiac hypertrophy is characterized not only by increased myocyte size and protein synthesis but also by alterations in the expression of cardiac-specific genes (28). Numerous studies (4, 14, 35, 38) have shown that elevated tissue kallikrein levels by gene transfer approach attenuate hypertension and cardiac hypertrophy in several hypertensive rat models. In transgenic rats harboring the human tissue kallikrein gene, isoproterenol-induced cardiac hypertrophy and fibrosis were reduced compared with control rats, and icatibant (a bradykinin B2 receptor antagonist) abolished this effect (33). In addition, bradykinin has been shown to exert antihypertrophic actions on angiotensin (ANG) II-infused isolated rat hearts via cGMP (31). Furthermore, studies (29, 30) in vitro have shown that in the presence of endothelial cells, bradykinin can prevent ANG II-induced hypertrophy in cardiomyocytes by increasing nitric oxide (NO) levels. These studies implicate a role for the kallikrein-kinin system in protection against hypertrophy.

Hypertrophy is usually, but not always, accompanied by cardiac fibrosis, the pathological accumulation of extracellular matrix (ECM) components, particularly collagens I and III and fibronectin. Cardiac fibrosis can develop not only by augmented collagen synthesis, but also insufficient collagen degradation by matrix metalloproteinases (7). Increased interstitial collagen accretion contributes to abnormal myocardial stiffness and thus diastolic dysfunction associated with progressed LV hypertrophy (36). Cardiac fibrosis and hypertrophy have been shown to develop in bradykinin B2 receptor knockout mice (8, 19). It has also been observed that kinin can reduce the expression of collagen and fibronectin in cultured cardiac fibroblasts via NO and cGMP (17). These observations provide evidence that kinins have an important role in the prevention of fibrosis.

Cardiac hypertrophy induced by hypertension is often accompanied by a decline in capillary supply (20). However, ANG-converting enzyme (ACE) inhibitor treatment of spontaneously hypertensive rats (SHR) and stroke-prone SHR was shown to increase capillary length density, allowing more oxygen and nutrients to reach hypertrophied cardiomyocytes (10, 26, 34). Bradykinin has been implicated to play a role in this effect by ACE inhibitors, as blockade of the kinin B2 receptor prevented the increase in capillary density during ACE inhibitor treatment (10). It has been previously demonstrated in cultured endothelial cells that stimulation...
of the bradykinin B₁ and/or B₂ receptors results in NO release and endothelial cell proliferation (6, 24). In fact, NO alone has shown to increase endothelial cell growth by stimulating cGMP production (40). Hence, it seems that kinin can increase the proliferation of endothelial cells through the generation of NO and cGMP. Collectively, it appears from these previous studies that kinin induces the proliferation of endothelial cells and has protective actions against cardiac hypertrophy and fibrosis. On the basis of these in vitro and in vivo observations, we therefore investigated the effect of kallikrein gene transfer in SHR on cardiac hypertrophy, fibrosis, and neovascularization.

METHODS

**Animals and treatments.** Eleven-week-old male (~250 g) SHR and Wistar-Kyoto (WKY) rats (Harlan Sprague Dawley; Indianapolis, IN) were used in this study. The rats were housed at a constant room temperature with a 12:12-h light-dark cycle and had free access to tap water and rat chow. All procedures complied with the standards for care and use of animal subjects as stated in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1985).

**Preparation of replication-deficient adenovirus vector Ad.CMV-cHK.** A human kallikrein plasmid DNA fragment and cytomegalovirus (CMV) promoter were inserted at the XhoI and BglII site of Kan-pShut plasmid. Subsequently, the 4 fl enhancer and bovine growth hormone poly(A) sequence was inserted at the XhoI site of the Kan-pShut vector. The Kan-pShut plasmid was modified from pBluescript (Stratagene; La Jolla, CA) by insertion of a kanamycin-resistant gene expression unit. The expression cassettes of human kallikrein cDNA and the kanamycin-resistant gene were released with I-Ceu I and PstI and inserted at the same sites of pAdHM4 plasmid (kindly provided by Dr. Mark A. Kay, Stanford University Medical Center), a backbone vector of E1/E3-deleted adenovirus. The expression cassettes of human kallikrein cDNA along with the E1/E3-deleted adenoviral backbone DNA were released with Pac I and transfected into 293 cells. The E1/E3-deleted adenovirus was generated and amplified through six passages. Purification of the adenovirus Ad.CMV-cHK-4F2 was performed as previously described (2).

**Intravenous delivery of Ad.CMV-cHK and blood collection.** Rats were randomly divided into two groups. The Ad.CMV-cHK-4F2 adenovirus was injected into the tail vein of one group at a dosage of 5 × 10¹¹ plaque-forming units (pfu/rat). The other group received no injection and was used for control. For blood collection, unanesthetized rats were placed in a 37°C incubator for 10–15 min. Rats were then transferred to a plastic holder, and an insulin syringe was used to withdraw blood from the tail vein. Blood (700 μl) was withdrawn from all rats in the kallikrein group and from two rats from the control group at predetermined time points for the human tissue kallikrein ELISA analysis. After collection, blood samples were centrifuged at 1,000 g for 20 min and sera were removed and stored at −20°C.

**Human tissue kallikrein RT-PCR/Southern blot and ELISA.** One week after kallikrein gene delivery, total RNA from various organs was collected and RT-PCR/Southern blot analysis for human tissue kallikrein was performed as previously described (35). The levels of immunoreactive human tissue kallikrein in rat sera were determined with the use of an ELISA specific for human tissue kallikrein as previously described (35).

**Blood pressure measurement.** Systolic blood pressure was measured with DASYlab version 5.5 software (Kent Scientific; Turrington, CT) by the tail-cuff method. Unanesthetized rats were placed in a plastic holder resting on a pad that was maintained at 37°C during the measurements. Average readings were taken for each animal after the animals became acclimated to the environment. Body weight and heart rate were recorded at the time blood pressure was monitored.

**Nitrite/nitrate assay and Western blot analysis.** One week after kallikrein gene delivery, a subset of rats was euthanized. LV tissue (0.3 g) was homogenized in 1.5 ml of lysis buffer (25 mM Tris·HCl, pH 7.4, 1% Triton X-100, 0.1% SDS, 2 mM EDTA) containing 1:100 protease inhibitor cocktail (Sigma; St. Louis, MO) and centrifuged at 14,000 revolutions/min at 4°C for 30 min. The supernatants were stored at −80°C.

LV nitrite levels were determined by fluorometric assay (22). Briefly, 5 μl of 40 μM β-NADPH (reduced form) and 5 μl of 14 mM nitrate reductase (Sigma) were added to 40 μl of LV homogenate and the mixture was allowed to stand for 5 min at room temperature. Fifty microliters of ddH₂O and 10 μl of 2,3-diaminonaphthalene (50 μg/ml in 0.62 N HCl) were then added. After a 20-min incubation period, the reaction was stopped by addition of 2 ml of 0.07 N NaOH in a cuvette. Fluorescence was measured at an excitation of 365 nm and emission at 450 nm. Sodium nitrite standard measurements, with a dilution range from 0.1 to 10 μM in 20 mM Tris·HCl, pH 7.6, were performed similarly but in the absence of β-NADPH and nitrate reductase.

LV homogenates were used for Western blot analysis with the use of specific antibodies for phospho-JNK, total JNK (Cell Signaling Technology; Beverly, MA), PAI-1, and TGF-β1 (Santa Cruz Biotechnology; Santa Cruz, CA). Protein concentrations were determined by Lowry’s method.

**Radioimmunoassays of cardiac cGMP and cAMP.** A subset of rats was euthanized 1 week after kallikrein gene delivery. The left ventricles were homogenized in 10 vol of 0.1 N HCl. The homogenates were centrifuged at 15,000 g for 30 min, and aliquots of the supernatants were used for the assay. Protein concentrations were determined by Lowry’s method. Cardiac cGMP and cAMP levels were determined by radioimmunoassay as previously described (3, 39).

**Cardiac morphological parameters and histological investigations.** Rats were euthanized 5 wk after adenovirus injection, and hearts were removed and weighed. The atria were then cut away, and the right ventricular free wall was carefully dissected from the left. The intraventricular septum was included in the LV weight. LV enlargement was determined by measuring the distance from the atrial valve to the apex; this measurement was termed LV long axis. Left ventricles were then fixed in 4% formaldehyde solution and placed in 70% ethanol for storage. After fixation, the ventricles were cut into three transversal slices (1 basal, 1 mid, and 1 apical, 2 mm thick each), which were dehydrated and embedded. One 4-μm-thick section was obtained from each slice.

For measurement of cardiomyocyte size, LV sections were stained by the Gordon and Sweet silver method for reticulin. At ×400 magnification, 100 cardiomyocytes were chosen randomly on each slide and traced with the use of NIH Image software (version 1.61). LV sections underwent Sirius red staining or collagen I immunohistochemistry for the determination of the extent of fibrosis. For each section, 10 fields were analyzed in the LV wall at ×200 magnification. Adobe PhotoShop software was used to determine the percentage of
collagen staining to total LV tissue, which was expressed as collagen density. Immunohistochemistry was performed using the Vectastain Universal Elite ABC Kit (Vector Laboratories; Burlingame, CA) and following the supplied instructions. LV sections were incubated at 4°C overnight with Factor VIII (von Willebrand factor) antibody (DAKO; Carpinteria, CA), rat endothelial cell antigen (RECA-1) antibody (Serotec; Raleigh, NC), or collagen 1 antibody (Sigma). To determine capillary density, 10 to 20 random fields of LV sections stained with Factor VIII or RECA-1 antibody were selected at ×400 magnification and immunostained capillaries were counted.

Statistical analysis. Data were analyzed with the use of standard statistical methods, ANOVA, and unpaired Student’s t-test. Group data are expressed as means ± SE. Values of all parameters were considered significantly different at a value of P < 0.05.

RESULTS

Effect of kallikrein gene delivery on blood pressure in SHR and WKY rats. The effects of adenovirus-mediated kallikrein gene transfer on systolic blood pressure were monitored in SHR and WKY rats before injection and every week afterward (Fig. 1). SHR receiving kallikrein gene transfer maintained a significantly lower blood pressure than the SHR control group for 2 wk. Before gene delivery, basal systolic blood pressure for both SHR groups was 180 ± 1 mmHg. At week 1, the blood pressure of SHR receiving kallikrein gene transfer decreased to 170 ± 4 mmHg, whereas the blood pressure of the SHR control group rose to 184 ± 2 mmHg (P < 0.01). At week 2, the blood pressure of SHR receiving Ad.CMV-cHK was still significantly lower than the SHR control group (178 ± 4 vs. 187 ± 2 mmHg; P < 0.05). However, at weeks 3 and 4 there was no significant difference between the experimental and control SHR groups. Systolic blood pressures of control WKY rats and WKY rats receiving kallikrein gene delivery were also monitored at the same time periods. Throughout the experiment, the blood pressure of WKY control rats remained stable, ranging from 126 ± 2 to 128 ± 3 mmHg, and WKY rats receiving the kallikrein gene showed no significant decrease in blood pressure.

Expression of human tissue kallikrein. Total RNA was prepared from various organs and RT-PCR/Southern blot was performed with the use of oligonucleotides specific for human tissue kallikrein. As shown in Fig. 2A, human tissue kallikrein mRNA was detected in the heart, kidney, adrenal gland, lung, and liver of SHR receiving kallikrein gene delivery, but not in control SHR. Expression of human tissue kallikrein mRNA was also detected in WKY rats after gene transfer (data not shown). Immunoreactive human tissue kallikrein levels in rat sera were measured with the use of an ELISA specific for human tissue kallikrein. Rat serum was collected at 3 days postinjection and every week for 4 wk. The peak level of immunoreactive human tissue kallikrein in the serum was at 3 days postinjection, with 6693.6 ± 1541.9 ng/ml for WKY rats and 7920.2 ± 1728.7 ng/ml for SHR, and levels declined thereafter (Fig. 2, B and C). No immunoreactive human tissue kallikrein was detected in the sera of SHR or WKY control rats.

Effect of kallikrein gene delivery on cardiac NO, cGMP, and cAMP levels. A subset of rats from each group was euthanized 1 wk after gene delivery to measure cardiac NO, cGMP, and cAMP levels. Kallikrein gene delivery in both SHR and WKY rats significantly increased NO levels over control rats, as determined by nitrate/nitrite assay (Fig. 3A). WKY rats receiving kallikrein gene transfer had higher LV nitrite levels (129.8 ± 9.3 pmol/mg protein; P < 0.05) than control WKY rats (93.1 ± 7.2 pmol/mg protein). Kallikrein gene delivery in SHR also increased LV nitrite levels (158.5 ± 8.2 pmol/mg protein; P < 0.05) compared with control SHR (113.3 ± 13.6 pmol/mg protein). WKY rats receiving kallikrein gene transfer had significantly higher cGMP levels (0.153 ± 0.007 pmol/mg protein; P < 0.01) than control WKY rats (0.101 ± 0.008 pmol/mg protein; Fig. 3B). Kallikrein gene delivery in SHR also resulted in increased cGMP levels in the LV over control SHR (0.105 ± 0.006 vs. 0.083 ± 0.005 pmol/mg protein; P < 0.05). However, cAMP levels remained unchanged after kallikrein gene delivery in both WKY rats and SHR (Fig. 3C).

Effect of kallikrein gene delivery on cardiac hypertrophy. Five weeks after gene delivery, the rats were euthanized, and the morphological parameters of the heart were measured. SHR receiving the kallikrein
gene had significantly decreased LV weight-to-heart weight ratio (0.76 ± 0.01 g/g; *P < 0.05) compared with control SHR (0.80 ± 0.01 g/g; Fig. 4A). The LV long axis, a measure of LV enlargement, was also significantly reduced in SHR receiving kallikrein gene transfer (12.4 ± 0.3 mm; *P < 0.05) compared with control SHR (13.2 ± 0.2 mm; Fig. 4B). However, these parameters were not affected in WKY rats receiving the kallikrein gene compared with WKY control rats. In addition, the LV weight-to-heart weight ratio and LV long axis were significantly higher in control SHR compared with WKY control rats. The effect of kallikrein gene transfer on cardiomyocyte size in SHR and WKY rats was determined by Gordon and Sweet silver staining of LV sections (Fig. 4C). Quantitative analysis showed that WKY rats receiving the kallikrein gene had similar average cardiomyocyte size (374.0 ± 5.6 μm²) as that of control WKY rats (366.9 ± 6.3 μm²; Fig. 4C).
In contrast, kallikrein gene delivery in SHR significantly reduced cardiomyocyte size (480.5 ± 14.8 μm²; P < 0.01) compared with control SHR (543.1 ± 15.2 μm²). It was also evident that both SHR groups had significantly larger cardiomyocytes compared with WKY rats (P < 0.01).

Effect of kallikrein gene delivery on cardiac collagen accumulation. LV sections were stained with Sirius red for determination of collagen density (Fig. 5A). Cardio-myocytes stain yellow, and collagen stains red. Morphologically, kallikrein gene delivery appeared to attenuate collagen density in both SHR and WKY rats. Quantitative analysis revealed that kallikrein gene delivery significantly reduced collagen density in both rat strains. Evaluation of Sirius red-stained LV sections showed that collagen density was reduced from 4.25 ± 1.03% to 2.29 ± 1.18% (P < 0.05) in WKY rats and from 8.58 ± 2.17% to 3.43 ± 0.77% (P < 0.0001) in SHR after kallikrein gene transfer (Fig. 5A). Interestingly, collagen density in SHR receiving kallikrein gene delivery was normalized to that of WKY control rats. To further verify the reduction on collagen density, LV sections were immunostained with collagen I antibody (Fig. 5B). Consistent with the results from Sirius red staining, it was observed that kallikrein gene delivery reduced collagen I levels in both SHR and WKY rats. Indeed, quantitative analysis showed that collagen I density was significantly reduced in both rat strains receiving the kallikrein gene compared with their controls (Fig. 5B). Kallikrein gene delivery in WKY rats decreased collagen I staining from 4.79 ± 1.25% to 1.97 ± 0.96% (P < 0.05). In SHR, kallikrein gene delivery reduced collagen I density from 8.34 ± 1.95% to 3.87 ± 0.62% (P < 0.0001). In addition, SHR control rats had significantly more collagen I staining than WKY control rats (P < 0.01).

Effect of kallikrein gene delivery on cardiac TGF-β1 and PAI-1 levels and JNK activation. Because transforming growth factor (TGF)-β1 is such a powerful regulator of extracellular matrix production (37), we evaluated the effect of kallikrein gene transfer on cardiac TGF-β1 protein expression by Western blot. As shown in Fig. 6A, kallikrein gene delivery in both SHR and WKY rats reduced TGF-β1 protein levels compared with control groups. Plasminogen activator inhibitor (PAI)-1, which is induced by TGF-β1 (37), inhibits tissue and urokinase plasminogen activators. In turn, plasminogen activators convert plasminogen to plasmin, a protease that is known to mediate matrix degradation and activate matrix metalloproteinases. We showed that kallikrein gene transfer decreased cardiac PAI-1 levels compared with control groups (Fig. 6B).

The mitogen-activated protein kinases (MAPKs) ERK and JNK are known to be involved in the regulation of cardiac hypertrophy (23). Therefore, we investigated the effect of kallikrein gene transfer on MAPK activation by Western blot in the left ventricles of SHR.
and WKY rats. The phosphorylation of JNK (p54 and p46) was decreased in rats receiving the kallikrein gene compared with controls ($P < 0.05$), as shown in Fig. 6C. It is interesting that although kallikrein gene delivery had no effect on cardiomyocyte size in WKY rats, a reduction in phospho-JNK was observed. No difference was observed for ERK1/2 in both rat strains (data not shown).

**Effect of kallikrein gene delivery on cardiac capillary density.** SHR receiving kallikrein gene transfer had significantly more capillaries immunostained by antibody to Factor VIII ($2,485 \pm 51$ cap/mm$^2$; $P < 0.01$) compared with SHR controls ($2,163 \pm 46$ cap/mm$^2$), as shown in Fig. 7A. However, Factor VIII immunostaining showed that kallikrein gene delivery in WKY rats had no effect on cardiac capillary density. It was also observed that control SHR had significantly lower capillary density compared with WKY control rats ($2,709 \pm 118$ cap/mm$^2$; $P < 0.01$). To verify the results from the Factor VIII immunostaining, LV tissue sections were immunostained with RECA-1 (Fig. 7B). Quantification of the RECA-1 staining was consistent with the Factor VIII staining. These results showed that capillary density in WKY rats was not altered after kallikrein gene delivery. On the other hand, SHR receiving the kallikrein gene had significantly higher capillary density ($2,281 \pm 136$ cap/mm$^2$; $P < 0.005$) compared with the SHR control group ($1,549 \pm 131$ cap/mm$^2$). SHR control rats also had significantly lower capillary density than WKY controls ($2,257 \pm 58$ cap/mm$^2$; $P < 0.01$).

**DISCUSSION**

The present study showed that a single intravenous injection of the human tissue kallikrein gene in an
adenoviral vector not only attenuates cardiac hypertrophy and fibrosis, but also promotes neovascularization in the heart of SHR. SHR receiving kallikrein gene delivery showed a rapid and significant decrease in systolic blood pressure in the first 2 wk after gene transfer compared with control SHR. At 3–5 wk postinjection, however, no difference was observed in the blood pressures of the two groups, suggesting that the changes in cardiac morphology may be partly independent of the temporary blood pressure reduction. This is supported by the observation that the WKY rats that received the kallikrein gene had a reduction in cardiac fibrosis despite the inability of kallikrein to significantly lower blood pressure compared with WKY control rats. Expression of human tissue kallikrein resulted in increased cardiac NO and cGMP levels in both SHR and WKY rats, but had no effect on cAMP levels. NO, which stimulates the generation of cGMP (40), would explain the increase in cardiac cGMP levels in both WKY rats and SHR over their respective controls after kallikrein gene delivery. These data suggest that adenovirus-mediated human tissue kallikrein gene transfer results in cardiac protection via the NO-cGMP pathway.

Reduced active cardiac kallikrein and kininogen levels have been reported in SHR, suggesting a possible protective role for the kallikrein-kinin system against hypertension and LV hypertrophy (32). This protective role of the kallikrein-kinin system was exemplified in SHR receiving the kallikrein gene, in that LV hypertrophy was attenuated in these rats. The regulation of cardiac hypertrophy has been shown to involve all members of the MAPK family to some extent (23). However, previous studies in stroke-prone SHR have shown that JNKs, but not ERK1/2, play an important role in the development of LV hypertrophy (12, 13). In these studies, inhibition of ANG type 1 receptor and ACE decreased JNK activities, but had no effect on ERK. Consistent with these findings, our results showed that phosphorylation of JNK (p54 and p46) decreased after kallikrein gene delivery in both SHR and WKY rat LV tissues, but phosphorylation of ERK1/2 was unaffected (data not shown). Cardiomyocyte size and other cardiac morphological parameters were unaffected in the WKY rats receiving kallikrein gene transfer, yet JNK activation was reduced. Apparently, other factors in addition to reduced JNK activities affect cardiomyocyte size.

Kallikrein gene transfer attenuated cardiac fibrosis in both SHR and WKY rats as evidenced by both Sirius red staining and collagen I immunostaining. In addition to being able to reduce collagen expression (9, 17), bradykinin is a stimulator of plasminogen activator...
release from endothelial cells (21), which would then initiate the fibrolytic cascade resulting in ECM degradation. We also observed that TGF-β1 and PAI-1 protein levels were reduced in the hearts of rats receiving kallikrein gene transfer compared with control groups. This is significant in that both TGF-β1 and PAI-1 are profibrotic factors (37). TGF-β1 is known to stimulate the production of ECM components (e.g., collagens) and their accumulation by increasing the expression of inhibitors (e.g., PAI-1) that block enzymes responsible for matrix degradation. Therefore, the combined effects of collagen gene downregulation and collagen protein degradation would result in reduced cardiac fibrosis.

We demonstrated that kallikrein gene delivery reduces collagen levels in association with upregulation of the NO-cGMP pathway. This is supported by a previous study (17) in which kinin prevented collagen expression via NO and cGMP. Furthermore, it has been shown that NO can suppress the expression of TGF-β1 and collagen in cultured mesangial cells (5). A potential mechanism for the anti-fibrotic effect of kallikrein/kinin may involve the decrease in JNK activity, which would then reduce the activity of the transcription factor activator protein-1 that regulates TGF-β1, collagen, and PAI-1 gene expression (15, 16, 18). The regulation of other factors, either directly or indirectly, by NO and/or cGMP is also possible.

In the present study, we showed that kallikrein gene delivery in SHR attenuated hypertension, LV hypertrophy, and cardiac fibrosis, which is in agreement with studies using ACE inhibitors in SHR and stroke-prone SHR (10, 26, 34). In these same studies, an increase in capillary length density (the three dimensional distribution of capillaries) was also observed, and a role for kinin was implicated in cardiac angiogenesis after ACE inhibitor treatment (10). Although we employed a different method for evaluating capillary growth (counting the number of capillaries per cardiomyocyte area), our results from both Factor VIII and RECA-1 immunostaining suggest that kallikrein gene transfer also promotes neovascularization in the hearts of SHR, leading to enhanced endothelial function. A recent study by Agata et al. (1) showed that after myocardial infarction, kallikrein gene transfer improved cardiac and endothelial function, which was associated with an increase in NO production. Moreover, it has been shown that kinin promotes angiogenesis via production of NO (27). In addition, several reports (11, 25) showed that NO increases endothelial cell proliferation and migration. Therefore, the elevated levels of cardiac NO after kallikrein gene transfer appear to be responsible for the observed growth of capillaries.

Fig. 7. Effect of kallikrein gene delivery on cardiac capillary density. A, left: factor VIII immunostaining of LV sections; magnification is ×400. Right, quantification of capillary density was determined by counting individual capillaries in 20 different fields (n = 4). B, left: rat endothelial cell antigen-1 (RECA-1) immunostaining of LV sections; magnification is ×400. Right, quantification of capillary density was determined by counting individual capillaries in 10 different fields (n = 2–4). Data are expressed as means ± SE.
In conclusion, kallikrein gene delivery in SHR resulted in attenuation of cardiac hypertrophy and fibrosis, along with a transient decline in blood pressure. The reduction in cardiac remodeling was accompanied by increases in NO and cGMP and decreases in TGF-β1, PAI-1, and phospho-JNK levels in the heart. The increase in capillary density after kallikrein gene delivery was most likely due to augmented NO production, resulting in neovascularization. Taken together, these findings indicate that the kallikrein-kinin system plays an important role in cardioprotection.

DISCLOSURES

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