Vascular transfer of adenovirus is augmented by nitric oxide in the rat heart

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Sasse, Alexander, Zhaoping Ding, Martina Wallich, Axel Gödecke, and Jürgen Schrader. Vascular transfer of adenovirus is augmented by nitric oxide in the rat heart. Am J Physiol Heart Circ Physiol 287:H1362–H1368, 2004. First published May 6, 2004; 10.1152/ajpheart.00193.2003.—Reversible opening of the endothelial barrier remains a major obstacle when hearts are transfected via the coronary system. Our aim was to establish an experimental system permitting the continuous analysis of vascular transfer of virus in the intact heart. Isolated saline-perfused rat hearts were inverted and covered with a latex cap to collect interstitial transudate (IT) on the pericardial surface. Adenovirus (10⁹ pfu/ml) was stably labeled with rhodamine fluorescent dye. Analysis of IT and coronary perfusate revealed that under baseline conditions, adenovirus in the IT reached 75% of its vascular concentration within 3 min. The nitric oxide donors S-nitroso-N-acetyl penicillamine (SNAP) and bradykinin (BK) were the most effective substances to increase total IT volume and adenoviral interstitial concentration. Perfusion with 9% serum markedly reduced IT volume flow and delayed the SNAP/BK effect. Our findings demonstrate that SNAP and BK effectively increased coronary transfer of adenovirus suggesting that the inverted isolated heart is a suitable model to optimize vascular transfer of virus under standardized conditions.

endothelial permeability; transvascular and transcoronary gene transfer; S-nitroso-N-acetyl penicillamine; interstitial transudate


LITTLE IS KNOWN OF THE FACTORS THAT INFLUENCE THE EXTENT AND KINETICS OF THE VIRAL TRANSPORT PROCESS. WHEREAS PERFUSION PRESSURE REMAINS ONE OF THE MOST IMPORTANT DRIVING FORCES FOR THE SHIFT OF ADENOVIRUS INTO THE INTERSTITIAL SPACE (17), SEVERAL SUBSTANCES, SUCH AS BRADYKININ (9, 33), SEROTONIN (9), RMP-7 (11), HISTAMINE (24), VEGF (8), NITRIC OXIDE (NO) (8), THE CGMP-SPECIFIC PHOSPHODIESTERASE INHIBITOR ZAPRINAST (33), INCREASED CALCIUM (9), β-THROMBIN (30), PROTAINE (22), AND MANNOIT (10) HAVE BEEN USED IN GENE TRANSFER PROTOCOLS WITH VARYING SUCCESS. THERE ARE PRESENTLY ALSO NO DATA AVAILABLE ON THE KINETICS OF THE PHARMACOLOGICALLY STIMULATED VIRUS UPTAKE (10, 11, 22).

THE PURPOSE OF THE PRESENT STUDY WAS TO ESTABLISH A MODEL PERMITTING THE CONTINUOUS EVALUATION OF THE UPTAKE OF FLUORESCENCE-LABELED ADENOVIRUS PARTICLES FROM THE VASCULAR INTO THE INTERSTITIAL SPACE BY USING THE ISOLATED INVERTED HEART. IN THIS SYSTEM, THE INTERSTITIAL TRANSUDATE (IT) WAS CONTINUOUSLY COLLECTED, AND THIS ENABLED US TO INVESTIGATE THE ROLE, OPTIMAL CONCENTRATION, AND KINETICS OF VARIOUS VASODILATORS ON FLUID FLOW AND THEIR EFFECT ON VIRUS PASSAGE.

MATERIALS AND METHODS

Materials. Latex MR Revultex was a gift of H. D. Cotterell (Hamburg, Germany). Rotilabo tube (model 9557.1) was from Roth (Karlsruhe, Germany). Donor horse serum, heat inactivated at 55°C, S-9135 was from Biochrome Seromed (Berlin, Germany). HES 200/0.5 was from Fresenius Kabi (Hamburg, Germany). Histamine, bradykinin, serotonin, and penicillin/streptomycin (cat. no. P 0781) were from Sigma (St. Louis, MO). Adenosine was from Boehringer-Mannheim (Mannheim, Germany), synaptosomal-associated protein (SNAP) was from Biomol (Hamburg, Germany), and RMP-7 (Cereport) was a gift from Preclinical Research and Development (Alkermes; Cambridge, MA). Glycine was from Degussa. Sephadex G-50 fine column was from Amersham-Pharmacia-Biotech. Centrifugal filter devices (model YM-10, 10,000 mol/wt) were from Centricron. Human embryonic kidney (HEK)-293 cells were from DSMZ (Braunschweig, Germany); HEK-293 N3S suspension cells were from Microbix Biosystems (Ontario, Canada). Jokliks medium (cat. no. 22300–107) was from GIBCO-BRL. Rhodamine (C-1171) and nanoparticles fluorospheres (0.044 µm; orange; 540/560 nm) were from Molecular Probes (Eugene, Oregon).

Isolated perfused heart: reverse heart model. All animal experiments were conducted in conformity with the “Guiding Principles for Research Involving Animals and Human Beings” (3a). Male Wistar rats (280–300 g, heart weight: ~1 g) were anesthetized with ether, and after opening of the thorax, the hearts were quickly removed, and the aorta was cannulated. Perfusion was initiated in a nonrecirculating manner using Krebs-Henseleit buffer equilibrated with 95% O₂-5% CO₂ at 37°C. Hearts were perfused at a constant pressure of 65 mmHg. Proximal to the aortic cannula coronary flow (ultrasonic flowmeter; Transonic) and pressure (Statham transducer model P23 XL) were recorded. To permit measurement of left ventricular pressure development, a latex balloon mounted on a catheter was inserted...
in the left ventricle via the left atrium. After an equilibration period of 20 min, the Langendorff-perfused heart was changed to the reverse heart model.

In the reverse heart model, the heart was fixed to a rigid catheter via the left ventricular balloon and inverted according to published protocols (7, 32). The ventricular surface was covered with a latex cap (thickness: ∼0.2 mm), which was previously cast as appropriate for the average shape of the heart. Thereafter the apex of the latex cap was connected to a silicon-tube, which ended 4 cm below the base of the heart, allowing for minimal suction. The IT, continuously produced and excreted on the epicardial surface into the pericardial space, was collected separately by collecting the outflow, weighing the collected fluid and excreted on the epicardial surface into the pericardial space, was sampled separately by collecting the outflow from the inverted heart via pulmonary artery and right atrium. Heart rate, developed left ventricular pressure, coronary flow, and coronary pressure remained unchanged after inversion of the heart and covering of the ventricles with the latex cap (Fig. 1).

Unless indicated otherwise, hearts were perfused with Krebs-Henseleit buffer containing (in mM) 116 NaCl, 4.6 KCl, 1.1 MgSO4, 1.18 KH2PO4, 24.9 NaHCO3, 8.32 glucose, 2.0 pyruvate, and 2.52 CaCl2 (salts from Merck). In specified experiments, hearts were perfused with 2.5–6.5% poly(O2-hydroxyethyl) starch (HES) or serum (heat-inactivated horse serum, added at a rate of 3 and 5% of the continuous measured coronary flow). Total protein concentration of the effluent perfusate in serum-perfused hearts was determined by using the Lowry method; perfused buffer with 3 and 5% serum contained 4.8 ± 2.4 (n = 12) and 8.8 ± 2.8 mg/ml BSA total protein (n = 8), respectively. Compared with the normal reference range in rats of representative gender and age (56.0 ± 3.0 g protein/l) the detected protein concentrations in our model are comparable to a concentration of 8.5% (in 3% serum perfusion) and 15.7% (in 5% serum perfusion) found in the in vivo situation (35).

**Virus production and labeling.** Adenovirus stocks were prepared by infection of human embryonic kidney (HEK)-293 cells with ΔE1/E3-recombinant Ad5.GFP (Qbiogene; Carlsbad, CA) at a multiplicity of infection of 25 pfu/cell. Cells and medium were harvested 48 h after infection, centrifuged at 2,000 rpm for 5 min, and resuspended in sterile PBS. The virus was released by four cycles of freezing and thawing. Viral preparations were purified by CsCl density centrifugation and virus band was extracted and dialyzed at 4°C in 4 liters of solution [3.16 liters H2O, 400 ml PBS, 40 ml 10 mM MgCl2, 400 ml 87% glycerol (Sigma), 40 ml of 50 µM CaCl2] for 4 h. This was repeated twice. Virus was then stored at 4°C for immediate processing or at −80°C for later preparation.

HEK-293 cells for virus assays and HEK-293 N3S suspension cells for virus production were grown in Joklik medium supplemented with 10% horse serum and 1% penicillin/streptomycin. Viral titer was estimated by measuring the absorbance of the viral DNA at 260 nm (A260) after incubation in lysis buffer (1:20 in 0.1% SDS, 10 mM Tris-HCl pH 7.4, 1 mM EDTA; 56°C, 10 min). The titer was calculated from these data assuming a molecular weight of 2.5·10^7 g·mol for the adenoviral genomic DNA and a DNA concentration of 50 µg/ml at an A260 = 1. Virus titer was also determined by plaque assay with HEK-293 cells as described elsewhere (29).

Adenovirus was stably labeled with rhodamine fluorescence dye according to published protocols (23). In brief, rhodamine was dissolved in DMSO at a concentration of 10 mg/ml and stored at −20°C. Buffer (490 µl buffer; 0.2 M NaHCO3, pH 8.3) and adenovirus (500 µl; 10^8 – 10^10 pfu/ml) were mixed; 10 µl rhodamine solution was added and after mixing were incubated for 30 min at room temperature. Glycine (110 µl; 2% dissolved in PBS) are added. Free rhodamine and rhodamine attached to adenovirus were separated by using a Sephadex G-50 column. Fluorescence was detected with Fluoromax 3 (Jobin-Yvon Horiba; Edison, NY). The virus titer of this solution was determined in parallel to fluorescence to enable the calculation of a standard curve for the assessment of virus titer from fluorescence measurement.

To determine the amount of rhodamine attached to the adenovirus, the virus preparation was centrifuged over a filter (cutoff 10,000 mol/wt) at 3,000 g for 30 min. Fluorescence (93.3 ± 4.2%; n = 4) remained above the filter representing rhodamine attached to adenovirus. The rhodamine-labeled adenovirus retained its infectious properties, because infected HEK-293 cells displayed positive green fluorescent protein (GFP) staining after 48 h.

**Statistics.** Results were analyzed by one-way ANOVA and unpaired t-test using GraphPad Prism version 3.0 (GraphPad Software). Data are expressed as means ± SD. In all experiments with measurement of IT flow or fluorescence, baseline values were subtracted from results.

**RESULTS**

Mean IT fluid collected under baseline conditions via the pericardial cover within the first hour at a coronary perfusion pressure of 65 mmHg was 41.5 ± 2.3 µl/min (n = 6). Mean coronary flow equaled 14.9 ± 2.3 ml/min (n = 6). Therefore, the IT-flow represents 0.43% of the fluid perfused through the coronary system. IT-flow remained stable over the first 2 h of perfusion; thereafter, IT-flow continuously increased in a linear fashion (slope 1.03 ± 0.07, r² = 0.56). All further experiments were conducted within the first 120 min of heart perfusion.

To evaluate the effect of changes in oncotic pressure on IT flow, the perfusion medium was supplemented with heat-inactivated horse serum (9 and 16% of normal total protein in vivo) and HES (2.5, 5.0, 6.5%). From the results shown in Fig. 2, it can be seen that 9 and 16% serum decreased IT flow to
30 ± 10.1 and 13.8 ± 5.3%, respectively. Similarly, HES, a substance known to increase the intravascular oncotic pressure (1) also dose dependently decreased IT. At 2.5 and 5% HES, IT flow was ~50%, whereas 6.5% HES further reduced IT flow to 20%.

In a further experimental series, influence of substances known to alter capillary permeability were analyzed. From the data summarized in Fig. 3, it can be seen that in the absence of serum, serotonin (5 μM), potent bradykinin-2 receptor agonist RMP-7 (25 nM) (10), bradykinin (1 μM), and the NO-donor SNAP (1 μM) significantly increased IT. The concentration chosen for each substance maximally increased IT as was derived from separate dose-finding studies. The most potent substance was SNAP, which doubled IT flow. Histamine (5 μM) and adenosine (3 μM), despite substantially increasing coronary flow, proved to be ineffective. The combined application of bradykinin (1 μM) and serotonin (5 μM) increased IT by 67 ± 29% (n = 2), an effect similar to a single application of bradykinin or serotonin. In the presence of 9% serum (Fig. 3B), only the bradykinin- and SNAP-mediated increase of IT flow reached the level of significance. The vasodilator adenosine, which maximally increased coronary flow, did not change IT flow. In the histamine experiments, we noted that the hearts became severely edematous with an increase of heart weight from 1.05 ± 0.08 to 1.32 ± 0.1 g wet wt (n = 7, P < 0.001, t-test).

Substances tested not only differed in their ability to increase IT, they also differed in the time required to reach maximum IT. From the data summarized in Table 1, it is evident that SNAP is the most rapid-acting substance reaching its maximum effect after 23 s. In contrast, serotonin required >3 min to reach its maximal effect on IT.

To investigate the passage of the adenovirus from the vascular space to the IT, rhodamine-labeled adenovirus was perfused through the coronary system and the IT, and the coronary effluent perfusate was collected. Fig. 4 shows representative data from a single experiment; the adenovirus reached the IT with a time delay of 2 min, arrived at its peak concentration level after ~7 min, and nearly approximated the virus concentration in the effluent perfusate compared with the respective

![Fig. 2. Effect of the heat-inactivated serum and O-2-hydroxyethyl (HES) on the IT fluid collected when perfusing in the reverse heart model. n, Number of experiments. Percents are relative share of serum and HES, respectively.](image)

![Fig. 3. Changes of IT relative to baseline conditions (100%) in percent mean ± SD. Hearts are perfused with Krebs-Henseleit buffer in the reverse heart model and histamine (5 μM), serotonin (5 μM), RMP-7 (25 nm), bradykinin (1 μM), synaptosomal-associated protein (SNAP; 1 μM), and adenosine (3 μM). A: data of saline-perfused hearts. B: results from hearts perfused with 9% heat-inactivated serum additive. Displayed flow rates are measured after steady-state conditions were reached depending on kinetics of individual substances (see Table 1). *P < 0.05, **P < 0.01 compared with controls; n, number of experiments.](image)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mean Time-To-Peak IT Effect</th>
<th>No. Experiments</th>
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<tbody>
<tr>
<td>Histamine (5 μM)</td>
<td>65 ± 23</td>
<td>4</td>
</tr>
<tr>
<td>Serotonin (5 μM)</td>
<td>201 ± 28</td>
<td>6</td>
</tr>
<tr>
<td>RMP-7 (25 nM)</td>
<td>37 ± 18</td>
<td>12</td>
</tr>
<tr>
<td>Bradykinin (1 μM)</td>
<td>32 ± 15</td>
<td>12</td>
</tr>
<tr>
<td>SNAP (1 μM)</td>
<td>23 ± 14</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SD time-to-peak interstitial transudate flow (IT) in seconds. Minimal concentration of the permeabilizing substances required to achieve maximal IT-flow are in brackets. SNAP, synaptosomal-associated protein.
virus concentration in the vascular space. After cessation of virus perfusion, there was, as expected, a delayed washout from IT. In all of the experiments, concentration of the adenovirus in interstitial fluid reached 74.9 ± 35.3% (n = 4) compared with concentration found in effluent perfusate. Additionally, the total amount of virus reaching the pericardial space and perfused via the coronary system was calculated. Of the total virus perfused, 0.404 ± 0.027% (n = 4) reached the epicardial space; the effluent perfusate contained 99.6 ± 1.9% of the perfused virus. After a washout phase, no relevant amounts of virus are detected in the effluent perfusate. Therefore, <1% of perfused adenovirus actually penetrated the myocardial space under experimental conditions.

To prove that fluorescence detected in IT on the epicardial surface did in fact represent rhodamine-labeled adenovirus, the IT was centrifuged for 30 min at 3,000 g through Centricon filters (cutoff 10,000 mol wt; see Materials). Of detected fluorescence, 94.25 ± 3.4% (n = 6) was found to be still associated with the virus. In separate experiments, IT containing rhodamine-labeled virus was added to HEK-293 cells in culture, and GFP-positive staining was found after 48 h.

To investigate whether the transendothelial migration of the adenovirus was solely dependent on the size of the perfused virus particles (73.1 nm OD, Ref. 12) experiments with fluorescent nanoparticles (44 nm) were performed. As shown in Fig. 4B, the concentration of the nanoparticles detected in the IT was far from reaching the equilibrium with the effluent perfusate. The average nanoparticle concentration reached in the interstitial fluid in four experiments was 23.5 ± 2.8%.

In a final experimental series, we explored the influence of bradykinin and SNAP on adenoviral migration into the IT. As shown in Fig. 5, it can be seen that SNAP and bradykinin almost doubled adenovirus concentration in the IT compared with baseline conditions. This was associated with an increase of virus concentration in the IT above concentrations measured in the effluent perfusate; in the SNAP and bradykinin experiments, the adenovirus concentration in the IT rose by 47.1 ± 34.1 and 41.9 ± 5.4%, respectively, above concentrations detected in effluent perfusate (both P < 0.001) (Fig. 6). Surprisingly, this effect was almost completely blunted by the application of 9% serum. Note, however, that the maximum values for controls were not different after the addition of 9% serum. Although less effective, serotonin significantly increased adenovirus concentration in IT to 108% compared with effluent perfusate.

Similar to the time-to-peak measurement of IT (Table 1), there were also pronounced differences in the time required for the virus to reach maximum concentrations in the IT as shown in Table 2. SNAP reduced the time-to-peak value substantially; under control conditions the time to peak was almost doubled if perfused with 3% serum. Again, bradykinin and SNAP almost halved this time interval.

**DISCUSSION**

This study reports that the isolated reverse heart presents a suitable model that permits the continuous measurement of two important parameters: 1) determination of water flux from the vascular space to the interstitial space as influenced by various vasodilators and in parallel and 2) measurement of the kinetics of adenoviral particle migration across the vascular barrier and its concentration in the interstitial relative to vascular space. We found that substances increasing local NO, such as SNAP and bradykinin, significantly increased the interstitial virus concentration, which is relevant for future gene therapy protocols.
Previous publications (32) have validated the isolated reverse heart model showing that interstitial substrate concentrations could be reliably determined. Lactate and glucose concentrations were measured in this model under conditions of anoxia. Moreover, interstitial adenosine and inosine kinetics under various states of oxygenation were determined by using the reverse heart model (7). The present study extends the utility of this model to the analysis and characterization of adenoviral particle and water flux across the endothelial barrier. With the use of the oncotically active components serum and HES, in the reverse heart model, both were associated with a decrease of interstitial fluid volume, underscoring the ability to accurately assess endothelial permeability in the intact heart. However, the presented model due to limited temporal stability is not suited to study transgenic expression.

In the saline-perfused heart, the adenovirus in the interstitial fluid reaches a concentration equal to ~75% of the concentration in the perfusate medium. However, when hearts were stimulated with SNAP or bradykinin, the interstitial virus concentration significantly increased above the respective vascular concentration despite the fact that interstitial fluid flow also increased. The most straightforward interpretation of this surprising finding is that SNAP and bradykinin by some unknown mechanism caused an active virus uptake across the vasculature. A recent ultrastructural study suggested that adenovirus vectors can traverse the continuous endothelium of the heart via a transcellular vesicular transport pathway (14). Alternatively, it is conceivable that there was more active water reabsorption induced by SNAP and bradykinin that

![Fig. 5. Reverse heart model in the absence of serum. Rhodamine-labeled adenovirus is detected in the IT fluid or effluent perfusate. Time 0 represents start of adenovirus perfusion. Top boxes, duration. Data shown represent one typical experiment, respectively. Virus titer is calculated via standard curve from fluorescence data. A: perfusion with 1 μM bradykinin. B: perfusion with 1 μM of the NO-donor SNAP.](image)

![Fig. 6. Concentration of rhodamine-labeled adenovirus in the IT measured in the reverse heart model. Adenovirus concentration is expressed as mean concentration in the IT relative to the virus concentration in the perfusate mean ± SD. Perfusion with Krebs-Henseleit buffer with and without 9% heat-inactivated serum. Hearts are also perfused with SNAP (1 μM), bradykinin (BK, 1 μM), and serotonin (5 μM). ***P < 0.001, **P < 0.01, respectively, compared with controls.](image)

### Table 2. Perfusion of the reverse heart model with rhodamine-labeled adenovirus

<table>
<thead>
<tr>
<th></th>
<th>Serum, %</th>
<th>Time Interval To Peak Value, min</th>
<th>No. Experiments/Group</th>
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<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td>7:35 ± 1:24</td>
<td>4</td>
</tr>
<tr>
<td>Serum</td>
<td>9</td>
<td>14:03 ± 1:54</td>
<td>4</td>
</tr>
<tr>
<td>SNAP (1 μM)</td>
<td>9</td>
<td>3:30 ± 0:42</td>
<td>3</td>
</tr>
<tr>
<td>SNAP (1 μM)</td>
<td>9</td>
<td>6:53 ± 0:31</td>
<td>3</td>
</tr>
<tr>
<td>Bradykinin (1 μM)</td>
<td>9</td>
<td>7:43 ± 0:47</td>
<td>3</td>
</tr>
<tr>
<td>Bradykinin (1 μM)</td>
<td>9</td>
<td>8:12 ± 0:38</td>
<td>2</td>
</tr>
<tr>
<td>Serotonin (5 μM)</td>
<td>4</td>
<td>4:12 ± 1:15</td>
<td>2</td>
</tr>
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</table>

Values are mean ± SD time (h:min) required to reach the peak concentration of adenovirus in the IT. Hearts are perfused with Krebs-Henseleit buffer with and without heat-inactivated serum.
would, as a result, increase interstitial virus concentration. NO has been reported to increase the permeability of individual microvessels (36); however, the mechanism is not well understood (28). Vasodilatation as such does not seem to play a role, because adenosine, which maximally increased interstitial fluid flow, did not increase the interstitial virus concentration.

The nonfenestrated and continuous coronary endothelium is likely to be the major barrier for fluid transport and virus uptake into the myocardium. However, very little is known about the mechanisms of virus transport through endothelial cells. The pathway of the adenovirus does not appear to be through gaps between adjacent endothelial cells, which average ∼20 nm, although this space might be widened through endothelial contractions (3, 34). Preliminary evidence was recently provided showing that adenovirus (73 nm) most likely passes across the endothelial barrier through a vesicular transport (transcytosis) (14). Vesicles of endothelial cells have a mean diameter of 70 nm continually fusing and separating, leading to the formation of channels (28). Whether NO can stimulate transcytosis of virus particles through endothelial cells as suggested by our study remains to be investigated. Whereas the relatively large adenovirus particle is quite efficiently transported across the endothelial barrier, the permeability for considerably smaller nanoparticles (44 nm) is significantly lower (Fig. 4). Although this might, in part, be due to a different surface charge of the nanoparticles, our data point to a potentially specific adenoviral transport mechanism across the endothelium.

Transfection efficiency for intracoronally applied adenovirus is determined by the following factors: 1) the interstitial virus concentration, which is a direct function of endothelial permeability; 2) the duration of exposure of the cells with the virus; and 3) the density of the coxsackie and adenovirus receptor on cardiomyocytes (18, 27). The present experimental model is the first permitting in a beating heart the continuous measurement of the interstitial virus concentrations and the kinetics of these changes. Again the NO-donors SNAP and bradykinin were the most potent substances to rapidly increase the kinetics of virus transport. Whereas under serum-free control conditions the adenovirus required 7.4 min to reach its peak value in the interstitial fluid, this value was reached after 3.3 min when treated with SNAP. The addition of serum (9%) generally doubled the time-to-peak value interval, which is relevant for in vivo conditions in which serum is usually present. Our findings are consistent with the observation that plasma proteins are adsorbed to the endothelial cell glyocalyx and form part of the structure making up the molecular filter at the cell surface tighter (2). More importantly, serum reversed the NO-induced effect on interstitial virus concentration, thereby reducing the utility of NO for practical application in vivo. Due to the presence of hemoglobin as an important NO scavenger, the situation in vivo will be even less favorable for the use of NO. However, in a recent study (7a), we succeeded in transfecting 43% of the in vivo rat heart by flushing the coronary system with serum-free medium before the application of virus.

Histamine has been often used in the literature to augment the viral transfection efficiency in the heart (24). In our experiments, however, histamine did not significantly increase the IT volume. Moreover, we found that histamine delayed the time-to-peak interstitial fluid levels and caused tachyphylaxia and visible edema of the heart. Histamine, due to its side effects, therefore appears not to be the preferred agent to facilitate gene transfer into the rat heart.

In conclusion, the present study has used a novel approach to study transvascular gene transfer into the rat heart. We made the unexpected observation that vasodilators such as SNAP and bradykinin, which most likely work through the formation of NO, substantially augment the kinetics and extent of interstitial accumulation of adenovirus in the heart. Inclusion of NO donors into a future cardiac gene therapy protocol should therefore facilitate the transfection rate of the heart.

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