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Silencing of sodium/hydrogen exchanger in the heart by direct injection of naked siRNA

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Morgan PE, Correa MV, Ennis IL, Diez AA, Pérez NG, Cingolani HE. Silencing of sodium/hydrogen exchanger in the heart by direct injection of naked siRNA. *J Appl Physiol* 111: 566–572, 2011. First published May 19, 2011; doi:10.1152/japplphysiol.00200.2011.—Cardiac Na^+/H^+ exchanger (NHE1) hyperactivity is a central factor in cardiac remodeling following hypertension, myocardial infarction, ischemia-reperfusion injury, and heart failure. Treatment of these pathologies by inhibiting NHE1 is challenging because specific drugs that have been beneficial in experimental models were associated with undesired side effects in clinical practice. In the present work, small interference RNA (siRNA) produced in vitro to specifically silence NHE1 ($siRNA_{NHE1}$) was injected once in vivo into the apex of the left ventricular wall of mouse myocardium. After 48 h, left ventricular NHE1 protein expression was reduced in $siRNA_{NHE1}$-injected mice compared with scrambled siRNA by 33.2 ± 3.4% ($n = 5$; $P < 0.05$). Similarly, NHE1 mRNA levels were reduced by 20 ± 2.0% ($n = 4$). At 72 h, $siRNA_{NHE1}$ spreading was evident from the decrease in NHE1 expression in three portions of the myocardium (apex, medium, base). NHE1 function was assessed based on maximal velocity of intracellular pH (pH_i) recovery (dpH_i/dt) after an ammonium prepulse-induced acidic load. Maximal dpH_i/dt was reduced to 14% in $siRNA_{NHE1}$-isolated left ventricular papillary muscles compared with scrambled siRNA. In conclusion, only one injection of naked $siRNA_{NHE1}$ successfully reduced NHE1 expression and activity in the left ventricle. As has been previously suggested, extensive NHE1 expression reduction may indicate myocardial spread of siRNA molecules from the injection site through gap junctions, providing a valid technique not only for further research into NHE1 function, but also for consideration as a potential therapeutic strategy.

Na^+/H^+ exchanger; myocardium

CARDIAC Na^+/H^+ EXCHANGER (NHE1) is an integral protein of the myocyte plasma membrane. NHE1 removes H^+ from the cytosol in exchange for Na^+, thus playing a central role in the regulation of intracellular pH (pH_i) and Na^+ concentration (20). The NHE family has 10 members. Isoforms NHE1–NHE5 are located in the plasma membrane, whereas the others are located intracellularly. Isoforms 2–5 have tissue-restricted expression, in contrast to the isoforms found in intracellular membranes. NHE1 is known as a "housekeeping" isoform; it is ubiquitously distributed and contributes to several processes, such as cell volume regulation and cellular proliferation. NHE1 is the main isoform in heart tissue. Hyperactivity of NHE1 protein has been implicated in myocardial ischemia-reperfusion injury, as well as in cardiac hypertrophy and heart failure (3, 9, 24). Enhanced NHE1 activity has been proposed to increase intracellular Na^+ concentration, driving the NHE to raise the intracellular Ca^{2+} concentration, leading to cell injury and/or cardiac hypertrophy (4, 8, 24). Pharmacological inhibition of NHE1 is beneficial in experimental models of different cardiac pathologies (4, 7, 10, 17–19, 24). However, clinical trials with NHE1 inhibitors (27, 33, 42) failed to yield positive results or revealed undesired cerebrovascular side effects (42). Pharmacological inhibition of NHE1 could affect the exchanger in tissues other than the myocardium, or could even inhibit unrelated proteins, as it was shown for carbonic anhydrase II (36). It is possible that the undesired side effects associated with general NHE1 inhibition might be reduced, if its application were restricted to the heart. We hypothesized that direct intramyocardial injection of small interference RNA (siRNA) would be a suitable tool for this purpose. The objective of the present work was to specifically silence NHE1 expression in the myocardium without affecting other tissues by injecting naked siRNA into the mouse myocardial wall. We present evidence that a single injection of naked siRNA against NHE1 ($siRNA_{NHE1}$) was enough to successfully reduce NHE1 expression and function in the entire left ventricle, and that the action of the siRNA molecules was limited to the heart without spreading of the silencing effect into other tissues.

MATERIALS AND METHODS

Animals. Six- to eight-week-old male BALB/cAnN mice were anesthetized with euthanyl (pentobarbital sodium; 100 mg/kg intraperitoneally). All procedures followed during this investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and to the Argentine Republic Law no. 14346 concerning animal protection and were approved by the Animal Welfare Committee of La Plata School of Medicine.

Tissue culture. Human embryonic kidney (HEK)-293 cells were grown at 37°C with air with 5% CO_2 in Dulbecco's modified Eagle's medium, supplemented with 10% (vol/vol) bovine serum and penicillin streptomycin (Invitrogen). Human embryonic kidney (HEK)-293 cells grown to 30–40% confluence were transiently transfected (21), using the calcium phosphate method with 1.6 μg hemagglutinin-NHE1 cDNA alone, or cotransfected with 1.6-μg NHE1 cDNA and increasing amounts of $siRNA_{NHE1}$, or cotransfected with 2 μg of a nonsilencing siRNA designed with a scrambled NHE1 target sequence ($siRNA_{SCR}$).

Immunodetection. One day posttransfection, HEK-293 cells were washed in PBS buffer (140 mM NaCl, 3 mM KCl, 6.5 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.5), and cell lysates were prepared by addition of 150 μl SDS-PAGE sample buffer to a 60-mm Petri dish. Samples of brain, lung, liver, and heart were washed with PBS buffer, and ventricles were dissected and homogenized (3 × 10 s with a PRO250 homogenizer) in four volumes of IPB buffer (1% Igepal, 5

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mM EDTA, 0.15 M NaCl, 0.15% deoxycholate, 10 mM Tris, pH 7.5), supplemented with protease inhibitors (Mini Complete, Roche Molecular Biochemical). The homogenates were centrifuged at 13,000 rpm for 10 min, and the supernatant kept for protein analysis before being suspended in an equal volume of SDS-PAGE sample buffer. Cell samples or ventricle lysates (200 μg protein) were resolved by SDS-PAGE on 7.5% acrylamide gels. Proteins were transferred to polyvinylidene fluoride membranes and then incubated with mouse monoclonal anti-NHE1 (MAB3140, 1:2,000; Chemicon), mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (sc-47724, 1:2,000, Santa Cruz), or mouse anti-β-actin (sc-47778, 1:1,000; Santa Cruz). Immunoblots were then incubated with a 1:5,000 dilution of donkey anti-rabbit IgG (sc-2317, Santa Cruz) or mouse anti-goat IgG (sc-2354, Santa Cruz), conjugated to horseradish peroxidase. Blots were visualized with enhanced chemiluminescence reagent (ECL, Millipore) and a Chemidoc Station (Bio-Rad), and quantified using Image analysis software.

siRNA synthesis. siRNA was synthesized in vitro by hybridization of complementary single-strand (ss) RNAs generated by a T7 RNA polymerase (AmpliScribe T7, Epicentre Biotechnologies). An RNA sequence of 19 bp used to knock down NHE1 mRNA has been reported previously (1). Before evaluating this sequence in vivo, we confirmed that it was unique to isoform 1 of NHE and did not target any other proteins, by performing a mouse NHE sequence alignment and confirming it with the National Center for Biotechnology Information BLAST tool (2). As a nonsilencing control, a double-stranded (ds) RNA was designed with the scrambled NHE1 target sequence. The scramble sequence used for the control siRNA did not recognize any mRNA as a potential target. DNA primers (Invitrogen) were as follows: T7-NHE1 sense 5′-GGTAATACGACTCACTATAG-GATAGGT TTCCATGTGATC-3′ and its complement 5′-GATCA-CATGGAAACCTATCCT ATAGTGAGTCGTATTACC-3′; T7-NHE1 antisense 5′-GGTAATACGACTCAC TATAGGATCACATGGAAAC-CTATC-3′ and its complement 5′-GATAGGTT TCCATGTGATC-CTATAGTGAGTCGTATTACC-3′; T7-scrambled sense 5′-GG TA-ATACGACTCACTATAGGGCATGTCGTCTAGTATTA-3′ and its complement 5′-TAATACTAGACGACATGCCCTATAGTGAGTCG-TATTACC-3′; and T7-scrambled antisense 5′-GGTAATACGACT-CACTATAGTAATACTAGA CGACATGCC-3′ and its complement 5′-GGCATGTCGTCTAGTATTACTATA GTGAGTCGTATTACC-3′. Each dsDNA duplex was used to generate 19-bp ssRNA molecules, according to the manufacturer's protocol (AmpliScribe T7, Epicentre Biotechnologies). dsRNA duplexes against NHE1 and scramble were generated by hybridization of each pair of ssRNA in the annealing buffer (100 mM NaCl, 50 mM Tris, pH 8.0) 1 min at 96°C, followed by a slow cooling at room temperature. Annealing was confirmed by electrophoresis, and quantification of dsRNA was done by light absorption at 260 nm (SmartSpec 3000, Bio-Rad).

Injection of siRNA into the mouse heart. Mice were anesthetized with intraperitoneal injection of ketamine (20 mg/kg)-xylazine (5

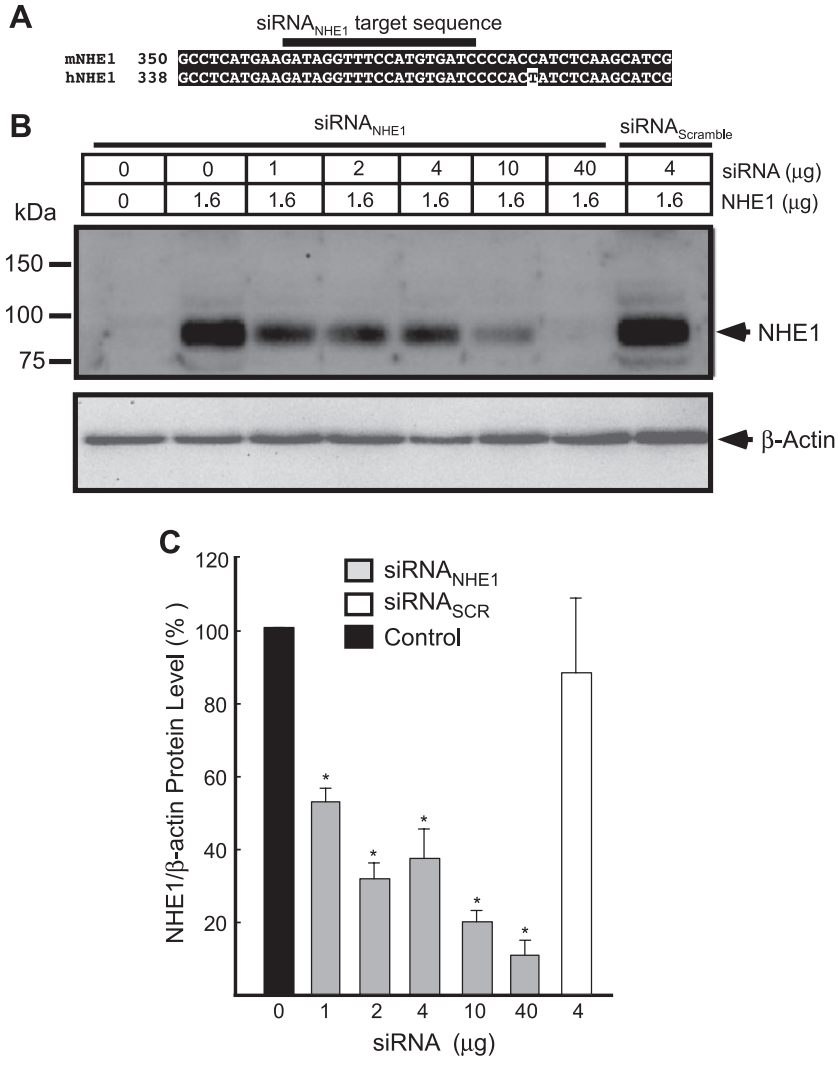

Fig. 1. In vitro-generated small interference RNA (siRNA) silence Na^+/H^+ exchanger (NHE1) (siRNA$_{NHE1}$) expression in human embryonic kidney (HEK)-293 cells. HEK-293 cells were transfected with 1.6 μg hemagglutinin-NHE1 cDNA alone or cotransfected with increasing concentrations of siRNA$_{NHE1}$ or a control scrambled siRNA (siRNA$_{SCR}$). HEK-293 cells were lysed, electrophoresed, and probed for NHE1 and β-actin, as loading controls. *A*: target sequence (horizontal bar) for siRNA$_{NHE1}$ shown on an mRNA sequence alignment of mouse (mNHE1) and human NHE1 (hNHE1). *B*: representative immunoblots of HEK-293 cell lysates corresponding to mock-transfected cells or cells transfected with NHE1 cDNA and siRNA$_{NHE1}$ or siRNA$_{SCR}$. *C*: bar graph shows average expression of protein corresponding to mock-transfected cells (solid bar, $n = 4$) or transfected with NHE1 cDNA and siRNA$_{NHE1}$ (shaded bars, $n = 5$) or siRNA$_{SCR}$ (open bar, $n = 5$), quantified by densitometry and normalized to the amount of β-actin. Values are means ± SE. *$P < 0.05$ vs. siRNA$_{SCR}$.

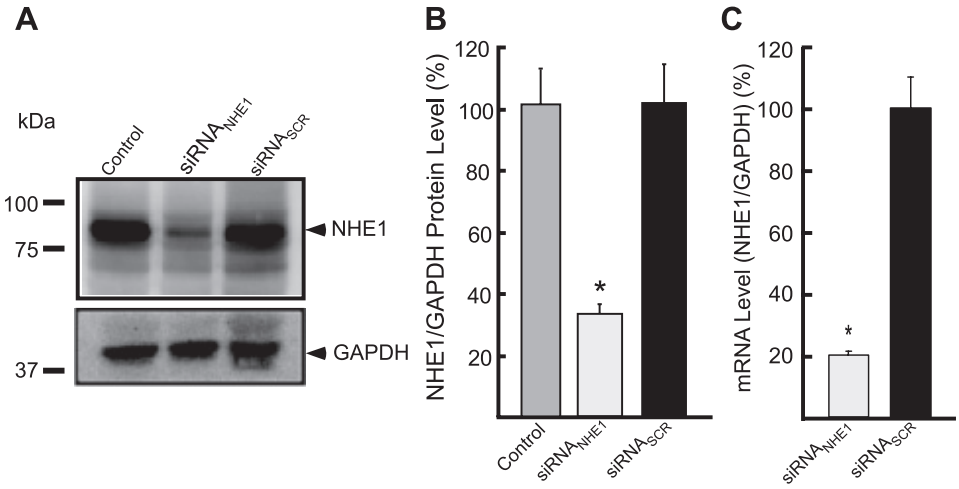

Fig. 2. Silencing of NHE1 in the mouse heart. Mice were injected once in the wall of the left ventricle with 20 μg of naked $siRNA_{NHE1}$ or $siRNA_{SCR}$ and killed 48 h later. *A*: immunoblots of heart lysates treated with $siRNA_{NHE1}$ ($n = 5$) or $siRNA_{SCR}$ ($n = 4$). *B*: bar graph shows average expression of protein, quantified by densitometry and normalized to the amount of GAPDH. Values are means ± SE. $*P < 0.05$. *C*: total RNA samples of cardiac left ventricle were used to study NHE1 mRNA expression by quantitative RT-PCR. Data for each sample were normalized with internal control using GAPDH mRNA expression. Values are means ± SE; $n = 4$ (*t*-test, $*P < 0.05$).

mg/kg) and intubated with an endotracheal tube for ventilation. During the surgery, animals were anesthetized with sevoflurane (~2%) used in a gas mixture with oxygen and delivered through ventilation by using a positive-pressure respirator (model 680, Harvard, South Natick, MA). For direct siRNA injection, mice underwent a left thoracotomy in the fourth or fifth intercostal space under general anesthesia. A single injection of 20 μg/10 μl saline solution of $siRNA_{NHE1}$ or $siRNA_{SCR}$ was injected into the left anterior ventricular wall using a 30 G needle (32). The needle was inserted into the ventricle wall in the direction from the base to the apex, penetrating ~1 mm deep. Subsequently, the chest was closed in layers, and the animals were allowed to recover.

Quantitative real-time PCR. Total RNA was extracted from frozen heart samples using the Qiagen RNeasy Mini kit. RNA (0.8 μg) was reverse-transcribed using the Omniscript RT kit (Qiagen). A dilution of the resulting cDNA was used to quantify the relative content of mRNA by real-time PCR (iCycler iQ real-time PCR detection system, Bio-Rad) using specific primers and SYBR Green as the fluorescent probe. The following primers were used: forward 5′-CCCTCACGTGCGCACACCC-3′ and reverse 5′-GACGTCTGATTGCAGGAAGG-3′. PCR was performed with *Taq* DNA polymerase (Invitrogen). Fluorescence data were acquired at the end of the extension step. A melt analysis was run for all products to determine the specificity of the amplification. The cycle threshold values for each gene were measured and calculated by computer software (iCycler IQ OSS, version 3.0a, Bio-Rad).

Measurement of pH_i, determination of NHE1 activity and intrinsic buffer capacity. pH_i was measured in isolated left ventricular papillary muscles following a previously described 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein-epifluorescence technique (29). The NHE1 activity was assessed based on the pH_i recovery from an ammonium prepulse-induced acute acid load (29). The experiments were performed in the nominal absence of bicarbonate (HEPES buffer) to assure that pH_i recovery after the acidic load was entirely because of NHE1 activation. The papillary muscles were acid loaded by transient (10-min) exposure to 30.0 mmol/l ammonium chloride, followed by washout with Na^+-containing HEPES buffer, and the recovery of pH_i was monitored. Intrinsic buffer capacity was determined as previously described (29).

Statistical analysis. Values are presented as means ± SE. Statistical significance between any two groups was determined using Student's *t*-test, and between more than two groups by one-way ANOVA followed by the Student-Newman-Keuls method. A final value of $P < 0.05$ was considered significant.

RESULTS

The $siRNA_{NHE1}$ that we used recognizes a 19-bp mRNA sequence specific to NHE1 (Fig. 1*A*) that spans 360–378 bp (1). To verify that this siRNA was effective, HEK-293 cells were transiently transfected with a constant amount of human hemagglutinin-tagged NHE1 cDNA (1.6 μg) alone or cotransfected with increasing concentrations of either $siRNA_{NHE1}$ or $siRNA_{SCR}$: 0, 1, 2, 4, 10, or 40 μg. After 24 h of transfection, HEK-293 cells were lysed and subjected to electrophoresis and

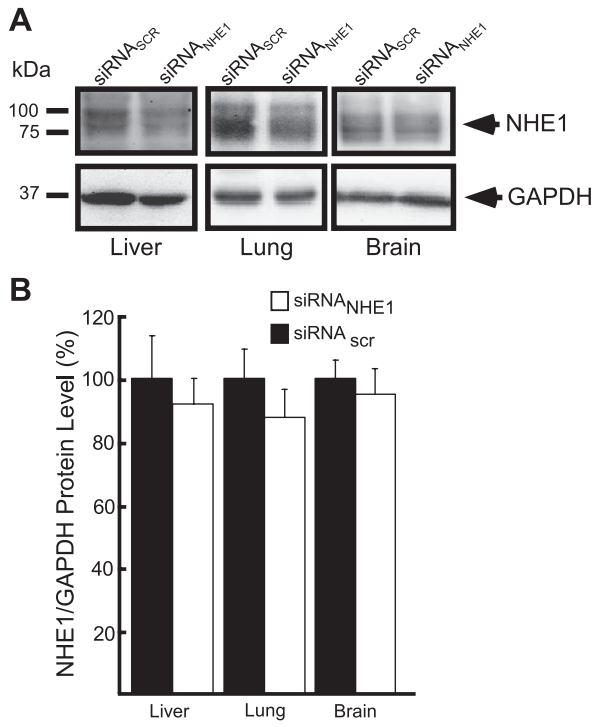

Fig. 3. NHE1 protein expression in mouse liver, lung, and brain. Mice were injected once in the wall of the left ventricle with 20 μg of naked $siRNA_{NHE1}$ or $siRNA_{SCR}$ and killed after 48 h. *A*: immunoblots of liver, lung, and brain lysates corresponding to mice treated with $siRNA_{NHE1}$ ($n = 5$) or $siRNA_{SCR}$ ($n = 4$). *B*: bar graph shows average expression of protein, quantified by densitometry and normalized to the amount of GAPDH. Values are means ± SE.

immunoblotting to detect NHE1 and β-actin. We found that, after 24 h of transfection with NHE1 cDNA, NHE1 expression increased dramatically (Fig. 1*B*). Expression of NHE1 normalized to β-actin was dose dependently reduced in cells transfected with $siRNA_{NHE1}$ compared with $siRNA_{SCR}$. (Fig. 1, *B* and *C*).

Once we confirmed the effectiveness of $siRNA_{NHE1}$ silencing in vitro, we tested its ability to knock down NHE1 in vivo in cardiac tissue. Adult mice were injected once, in the anterolateral wall of the left ventricle close to the apex, with 20 μg of siRNA (Fig. 2*A*). After 48 h, the mice were killed, and the heart ventricles removed, lysed, and analyzed by PAGE, followed by immunoblotting for NHE1 or GAPDH (Fig. 2*A*). NHE1 protein expression normalized to the amount of GAPDH was not affected by $siRNA_{SCR}$ injection compared with the control (102.1 ± 11.9 and 100 ± 11.2%, respectively; $n = 4$, $P < 0.05$). However, $siRNA_{NHE1}$ injection reduced the expression of NHE1 to ~30% compared with $siRNA_{SCR}$-injected mice (Fig. 2*B*). Cardiac NHE1 mRNA expression was also investigated in mice injected with $siRNA_{NHE1}$ or $siRNA_{SCR}$. Total RNA was extracted from the left ventricle, and NHE1 mRNA was identified and quantified with quantitative RT-PCR. NHE1 mRNA expression was reduced by ~80% in the entire cardiac left ventricle of mice injected with $siRNA_{NHE1}$ compared with animals treated with $siRNA_{SCR}$ (Fig. 2*C*).

To confirm that the injected $siRNA_{NHE1}$ molecules were confined to the heart, we assessed the expression of NHE1 in the liver, lung, and brain of the injected animals. These tissue lysates were analyzed by PAGE followed by immunoblotting for NHE1 or GAPDH (Fig. 3*A*). In these tissues, NHE1 expres>sion normalized to GAPDH was the same in $siRNA_{NHE1}$- and $siRNA_{SCR}$-injected mice (Fig. 3*B*), suggesting that the effects of $siRNA_{NHE1}$ were restricted to the heart.

The extensive reduction in NHE1 expression in the left ventricle could have resulted from the diffusion of siRNA molecules from the injection site in the apex along the myocardium. Therefore, we evaluated NHE1 expression in three portions of the left ventricle: the apex, the base, and the intermediate portion at 72 h after the injection. NHE1 expression was reduced in each portion of the ventricle injected with the $siRNA_{NHE1}$ compared with the $siRNA_{SCR}$-injected hearts (Fig. 4). Although the reduction in NHE1 expression in the base tended to be smaller than in the area close to the injection site, this difference was not statistically significant. The diffusion of the $siRNA_{NHE1}$ was also evidenced by a functional study of NHE1 in left ventricle papillary muscles 72 h after injection. NHE1 activity was determined by the maximal velocity of pH_i recovery after an ammonium-induced acidic load (Fig. 5*A*). Papillary muscles from $siRNA_{NHE1}$-injected mice showed a significant reduction in maximal velocity of pH_i recovery compared with $siRNA_{SCR}$ and noninjected controls (Fig. 5*B*), revealing that the NHE1 silencing strategy affected, as expected, not only protein expression, but also exchanger activity. Accordingly, papillary muscles from $siRNA_{NHE1}$-injected mice had a lower basal pH_i (Fig. 5*C*) and a magnified acidification after acidic load (Fig. 5*D*) compared with the $siRNA_{SCR}$-injected and noninjected control mice. These findings are in

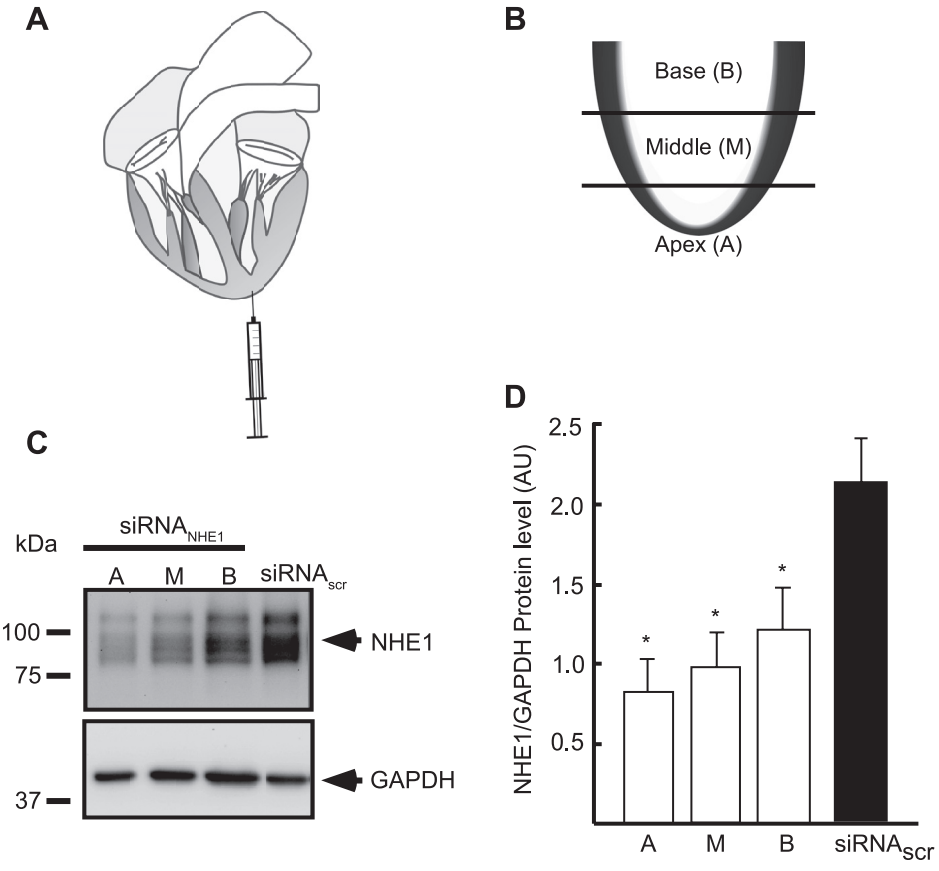

Fig. 4. NHE1 expression reduction distally from the injection site. *A*: mice were injected once in the apex of the left ventricle with naked $siRNA_{NHE1}$ or $siRNA_{SCR}$, as shown in the heart scheme, and killed after 72 h. *B*: left ventricle was divided into three parts (apex, medium, and base) to evaluate NHE1 expression. *C*: representative immunoblots of lysates of the different fractions of the left ventricle. *D*: bar graph shows average expression of protein, quantified by densitometry, and normalized to the amount of GAPDH. A significant reduction in NHE1 protein was detected in each fraction of the $siRNA_{NHE1}$ compared with the $siRNA_{SCR}$-injected hearts, confirming the spread of $siRNA_{NHE1}$ molecules. Although the reduction in NHE1 expression in the base seemed to be smaller than in the apex (close to the injection site), this difference did not reach statistical significance. Values are means ± SE; $n = 7$. *$P <$ 0.05.

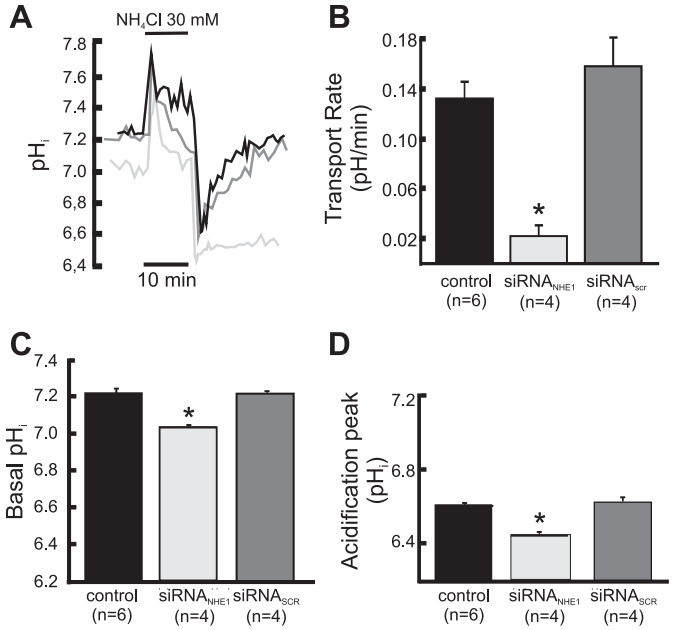

Fig. 5. Transport activity of NHE1 in papillary muscle of mouse heart. Mice were injected once in the apex of the left ventricle with 20 μg of naked $siRNA_{NHE1}$ or $siRNA_{SCR}$ and killed after 72 h. Papillary muscles were perfused with a bicarbonate-free (HEPES)-buffered medium. NH_4Cl (horizontal bar) was added and washed out to induce intracellular acidosis, as indicated in *A*. *A*: representative experiments recording intracellular pH (pH_i) corresponding to papillary muscles treated with $siRNA_{NHE1}$, control $siRNA_{SCR}$, and control without siRNA. NHE1 transport activity was calculated as the initial rate of alkalinization after NH_4Cl-induced acidosis. *B*: summary of the effects of the siRNA treatments on NHE1 exchange activity. *C*: summary of the resting pH_i, calculated as the steady-state pH recorded 1 min before addition of NH_4Cl. *D*: summary of the minimum pH_i obtained after the NH_4Cl induced acidosis. Values are means ± SE. $*P < 0.05$.

agreement with a decrease in the function of an alkalinizing mechanism, such as that involving NHE1 (26, 30).

DISCUSSION

In the present work, we provide evidence that a single injection of naked $siRNA_{NHE1}$ into the left ventricle of the heart spread from the inoculation site throughout the left ventricle, reducing NHE1 expression and function. This extensive effect is in agreement with the proposal that siRNA molecules diffuse in the myocardium from cell to cell through gap junctions (25, 35).

This report is the first using in vivo administration of siRNA to knock down NHE1 in the heart. However, the several attempts to genetically manipulate NHE1 have yielded disparate results. Among these, *NHE1* gene disruption (*Nhe1*$^{-/-}$ mice) was successfully used to prevented cardiac ischemia-reperfusion injury (38), but these mice exhibited growth retardation, seizures, and high postnatal mortality (6). Moreover, paradoxical results after myocardial ischemia-reperfusion were obtained with experiments performed in a transgenic model of cardiac-specific NHE1 overexpression (13). Permanent genetic modifications, such as null and overexpression mutations, usually generate an altered cellular environment that makes it difficult to interpret data. These issues and disparities encouraged us to consider the possibility of silencing cardiac NHE1 expression in adult animals using a siRNA technique.

The silencing of other proteins by in vivo naked siRNA has usually been done by systemic administration (11, 23, 37, 41). Of note, under these conditions, the effect is not restricted to the myocardium; siRNA molecules delivered in this way reach not only the heart but also other organs. In one of these studies, intended to evaluate the role of tumor necrosis factor-α converting enzyme in cardiac hypertrophy and fibrosis, a siRNA directed against tumor necrosis factor-α converting enzyme was administered to rats and mice by subcutaneous implantation of osmotic minipumps and also to mice via jugular vein injection (37). The authors found not only heart effects, but also a significant knockdown effect in resistance arteries. The jugular injection to mice was repeated every 5 days to obtain sustained inhibition of protein expression. We know of only two studies in which naked siRNA directed against proteins other than NHE1 were injected, similar to our report, directly into the myocardium of rats. One involved a single intracardiac injection of siRNA directed against Bcl2-associated athanogene (22). The other one used repeated injections (every 2 wk) of a siRNA directed against thyrotropin-releasing hormone (31).

Studies in cultured HeLa cells and human embryonic stem cells demonstrated that siRNA molecules can travel from one cell to another through gap junctions containing connexin 43 (35, 40), the predominant connexin isoform in adult hearts (14). Kizana et al. (25), working with neonatal rat ventricle myocytes, reported that both siRNA and microRNA molecules move through cells coupled by gap junctions composed of connexin 43. Although the mechanism by which siRNA is incorporated into myocytes is not clear, a receptor-mediated process has been suggested. The transmembrane protein SID1 mediates siRNA uptake in different organisms, such as nematodes and mammalian cells (16, 39). Mice express two paralogs, SIDT1 and SIDT2 (15). Tissue distribution analysis of the expressed sequence tag corresponding to mouse SIDT2 has revealed expression in the heart, indicating possibly receptor-mediated siRNA uptake in myocytes (28).

After myocardial injection, siRNA molecules might spread from the heart into the circulatory system and cause undesirable NHE1-silencing actions in tissues other than myocardium. We found no variations in NHE1 protein expression either in the liver of mice injected with NHE1-specific siRNA, or in the lung or brain. We inferred that, if any siRNA molecules were disseminated into blood, they would have negligible effects on other organs, probably because of the low concentration. Therefore, we conclude that the effects of siRNA were limited to the heart.

In the present work, we were interested in obtaining an in vivo NHE1 silencing effect restricted to the heart, suitable to clarify previous results in which NHE1 pharmacological inhibitors may have introduced undesired effects. Although we did not perform maneuvers to sustain siRNA action over a long period of time, an extension of our project is to analyze the possibility of administering siRNA in vivo with a different time frame; i.e., to induce regression of pressure overload-induced cardiac hypertrophy and/or failure. Accordingly, one option would be to repeat the injection of naked siRNA, as other groups have done (31, 37). However, the use of a viral vector to deliver the siRNA would also be a suitable alternative. In this regard, it is important to note that, although this approach would potentially provide long-term and stable gene

expression, the clinical use of viral vectors raises specific safety and ethical issues. Concerns include the possible generation of replication-competent viruses during vector production, mobilization of the vector by endogenous retroviruses in the genomes of patients, insertional mutagenesis leading to cancer, germline alteration resulting in transgenerational effects, and dissemination of new viruses from gene therapy patients (12, 34). These concerns reinforce the use of naked siRNA, although repeated injections would probably be necessary to prolong the effect.

Experiments by Baartscheer et al. (5) showed that chronic NHE1 inhibition causes regression of cardiac hypertrophy and failure. Therefore, we hypothesized about a potential translation of this technique to the clinical arena. If hyperactive myocardial NHE1 is deleterious in heart failure, a $siRNA_{NHE1}$ silencing effect would be a therapeutic strategy to consider either for patients in class IV of heart failure, or even as a bridge to heart transplantation.

In conclusion, the main novelty of our study was the finding that a single injection of naked $siRNA_{NHE1}$ into the heart extensively reduced NHE1 expression in the left ventricle, which was functionally correlated with a decrease in exchanger activity. These effects were specifically restricted to the heart without affecting other tissues. Based on our results, we think that this silencing technique may be a valuable therapeutic tool, not only in acute experimental models, but also for decreasing the hyperactivity in exchanger activity that occurs in certain myocardial pathologies, while avoiding the undesired effects of currently available pharmacological inhibitors. Further research would be necessary to determine the dose and frequency of siRNA administration to prolong the knockdown effect.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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