Targeted Disruption of Na\(^+\)/Ca\(^{2+}\) Exchanger Gene Leads to Cardiomyocyte Apoptosis and Defects in Heartbeat*

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Ca\(^{2+}\), which enters cardiac myocytes through voltage-dependent Ca\(^{2+}\) channels during excitation, is extruded from myocytes primarily by the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX1) during relaxation. The increase in intracellular Ca\(^{2+}\) concentration in myocytes by digitalis treatment and after ischemia/reperfusion is also thought to result from the reverse mode of the Na\(^+\)/Ca\(^{2+}\) exchange mechanism. However, the precise roles of the NCX1 are still unclear because of the lack of its specific inhibitors. We generated Ncx1-deficient mice by gene targeting to determine the in vivo function of the exchanger. Homozygous Ncx1-deficient mice died between embryonic days 9 and 10. Their hearts did not beat, and cardiac myocytes showed apoptosis. No forward mode or reverse mode of the Na\(^+\)/Ca\(^{2+}\) exchange activity was detected in null mutant hearts. The Na\(^+\)-dependent Ca\(^{2+}\) exchange activity as well as protein content of NCX1 were decreased by ~50% in the heart, kidney, aorta, and smooth muscle cells of the heterozygous mice, and tension development of the aortic ring in Na\(^+\)-free solution was markedly impaired in heterozygous mice. These findings suggest that NCX1 is required for heartbeats and survival of cardiac myocytes in embryos and plays critical roles in Na\(^+\)-dependent Ca\(^{2+}\) handling in the heart and aorta.

Ca\(^{2+}\) signaling is essential for the regulation of a wide variety of cellular functions and is a complex process involving multiple transporters, channels, and compartments. The Na\(^+\)/Ca\(^{2+}\) exchanger plays important roles in maintaining calcium homeostasis in many mammalian tissues (1). This exchanger catalyzes the electrogenic exchange of one intracellular Ca\(^{2+}\) ion for three extracellular Na\(^+\) ions in each reaction cycle (2, 3). Previous studies have defined three isoforms of the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX1, NCX2, and NCX3) that are coded by distinct genes in mammals (4–6). These isoforms are ~70% identical to one another in amino acid sequences. NCX1-specific transcripts are most abundant in the heart, although they are found in many other tissues (6, 7). In contrast, expressions of NCX2 and NCX3 genes are restricted to the brain and skeletal muscles, respectively (5, 6). Hydrophathy analysis shows that the NCX1 protein has 11 transmembrane regions with a large intracellular loop located between membrane-spanning segments 5 and 6 (4, 7).

In the heart, NCX1 is involved in excitation-contraction coupling as the dominant myocardial Ca\(^{2+}\) efflux system (8). NCX1 excludes Ca\(^{2+}\) that enters the cardiac myocytes through voltage-gated Ca\(^{2+}\) channels during contraction and returns the cell to its resting state. The increase in intracellular Ca\(^{2+}\) concentration in myocytes by digitalis treatment and after ischemia/reperfusion is also thought to result from the reverse mode of the Na\(^+\)/Ca\(^{2+}\) exchange mechanism (9). It was reported that an NCX1 inhibitor, KBR-7943, showed significant protection of cardiomyocytes against Ca\(^{2+}\) overload during anoxia and hypercontracture (10). The factors regulating NCX1 have been investigated in mammalian cells. Previous studies, using electrophysiological and other techniques, have shown that NCX1 is activated by [Ca\(^{2+}\)]i (3, 11) and external monovalent cations (12) and is inhibited by high cytoplasmic Na\(^+\) concentrations (11, 13), low cytoplasmic pH (14), and ATP depletion (15, 16). Recent studies, using molecular biological techniques, have revealed that a high affinity Ca\(^{2+}\) binding site in the central cytoplasmic loop of NCX1 is required for the regulation by Ca\(^{2+}\) (17, 18) and that protein kinase C-dependent regulation differs among the three NCX isoforms (19).

Although many previous studies strongly indicated the critical role of NCX1 in cardiac function, there are still many ambiguities because of lack of specific inhibitors. In addition, the role of NCX1 during development is not known. Therefore, we gen-


RESULTS AND DISCUSSION

Generation of Mutant Mice Lacking Ncx1—Exon 2 of Ncx1 is a first coding exon and encodes approximately two-thirds of the Ncx1 protein (26, 27). In targeting vector, this exon was re-

icated mutant mice that lack the Ncx1 gene.

EXPERIMENTAL PROCEDURES

Generation of Mutant Mice—We cloned the Ncx1 gene from a 129/Sv mouse genomic library. The targeting vector was constructed by insertion of the neo cassette into the 3-kilobase pair XbaI–XhoI fragment containing exon 2 of the Ncx1 gene. The diphtheria toxin-A fragment gene was ligated to the 3’ position of the targeting vector for negative selection (see Fig. 1A). The A3-1 embryonic stem (ES) cell line was transfected with the linearized targeting vector by electroporation. After G418 selection, homologous recombinants were identified by PCR and confirmed by Southern blot hybridization. PCR and Southern and Northern blot hybridizations were performed by standard procedures (20). Targeted ES cells were aggregated with eight cells from C57Bl/6J (B6) mice, and chimeric blastocysts were implanted into the uterus of pseudopregnant ICR mice. Chimeric male mice were then mated to female B6 mice to confirm the germline transmission.

In Situ Hybridization—In situ hybridization was performed as described previously (21). Briefly, day 9.5 embryos were fixed and sectioned at 15 μm. Antisense and sense RNA probes were prepared from a mouse NCX1 cDNA (XbaI–XhoI, 445 base pairs) with digoxigenin-UTP according to the manufacturer’s instructions (Roche Molecular Biochemicals). Thaw-mounted sections on polylysine-coated slides were prehybridized for 1 h at 50 °C in the hybridization buffer (2,54 mM CaCl2, 24.9 mM NaHCO3, 10.0 mM glucose, and 0.03 mM EDTA) containing 0.1–2 mM 45CaCl2 (1.5 mCi/ml). After 30 s of incubation, 45Ca2+ uptake activity was estimated by subtracting the 45Ca2+ uptake in normal BSS from that in Na+-free BSS.

Preparation of Thoracic Aortic Rings and Measurement of Tension—Thoracic aortic rings (~4 mm in length) were prepared from 12–15-week-old mice. Isometric tension was measured as described previously (25). Briefly, using two thin steel wires, the ring was tied to a force transducer and suspended in a water bath containing Krebs-Ringer bicarbonate buffer (118.5 mM NaCl, 4.74 mM KCl, 1.18 mM KH2PO4, 2.54 mM CaCl2, 24.9 mM NaHCO3, 10.0 mM glucose, and 0.03 mM EDTA) bubbled with 95% O2, 5% CO2 at 37°C. Aortic rings were stabilized at a resting tension of 1 g for 1 h before recording contractile responses. Aortic rings were soaked in Na+-free buffer (118.5 mM N-methyl-D-glucamine, 4.74 mM KCl, 1.18 mM MgSO4, 1.18 mM KH2PO4, 2.54 mM CaCl2, 25 mM choline bicarbonate, 10 mM glucose, and 0.01 mM EDTA) at 37°C and were pretreated with 10 μM ionomycin. Then the relaxation response induced by the addition of 10 mM EGTA was measured. Contractile responses were also measured by changing to Na+-free buffer after the treatment of 1 mM ouabain for 30 min.

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FIG. 1. Targeted disruption of the Ncx1 gene. A, structure of the wild-type allele, gene-targeting vector, and targeted allele. The probe for Southern blotting and PCR primers are shown. B, BamHI; K, KpnI; S, SpeI; Xb, Xbal; Xh, XhoI. B, Southern blot analysis. BamHI-digested DNA from offspring tails of heterozygous intercross was hybridized. The 8-kilobase pair (kb) fragment is the wild-type allele and the 6.6-kilobase pair fragment is the targeted allele. C, Northern blot analysis of day 9.5 embryos. +/+; wild type mouse; +/-, heterozygous mouse; −/−, homozygous mouse.

were fixed in 2.5% paraformaldehyde, 2% glutaraldehyde overnight, embedded in paraffin, and sectioned at 2 μm. Sections were then incubated with anti-PECAM antibody to identify endothelial cells (22).

[Ca2+]i Measurements—[Ca2+]i concentration was measured at room temperature as described previously (23) with some modifications. Briefly, whole hearts were excised from embryos at day 9.5 under the microscope and incubated in dye-loading solution containing 5 mM Fluo-3 for 30 min. Fluo-3-loaded hearts were then attached to a CELL TAK (Collaborative Biochemical Products)-coated glass plate in the flow-through chamber on the microscope. After washing with dye-free normal HEPES solution consisting of 140 mM NaCl, 2.0 mM KCl, 1.08 mM CaCl2, 1.0 mM MgCl2, 2.0 mM KOH, 5.5 mM glucose, 4 mM HEPES, pH 7.4, hearts were excited by a mercury arc lamp system at a 485-nm wavelength, and fluorescence at 530 nm was detected.

Measurement of [Na+]i-dependent 45Ca2+ Uptake—Measurement of [Na+]i-dependent 45Ca2+ uptake was performed as described previously (24). Briefly, for Na+-loading, confluent smooth muscle cells in 24-well dishes were incubated at 37°C for 30 min in balanced salt solution (BSS) (10 mM Tris/HEPES, pH 7.4, 146 mM NaCl, 4.0 mM KCl, 2.0 mM MgCl2, 0.1 mM CaCl2, 10 mM glucose, and 0.1% bovine serum albumin) containing 1 mM ouabain and 10 mM monensin. 45Ca2+ uptake was initiated by switching the medium to Na+-free BSS or to normal BSS, both of which contained 0.1–2 mM CaCl2 (1.5 μg/ml). After 30 s of incubation, 45Ca2+ uptake was terminated, and radioactivity was counted. [Na+]i-dependent 45Ca2+ uptake activity was estimated by subtracting the 45Ca2+ uptake in normal BSS from that in Na+-free BSS.

Preparation of Thoracic Aortic Rings and Measurement of Tension—Thoracic aortic rings (~4 mm in length) were prepared from 12–15-week-old mice. Isometric tension was measured as described previously (25). Briefly, using two thin steel wires, the ring was tied to a force transducer and suspended in a water bath containing Krebs-Ringer bicarbonate buffer (118.5 mM NaCl, 4.74 mM KCl, 1.18 mM KH2PO4, 2.54 mM CaCl2, 24.9 mM NaHCO3, 10.0 mM glucose, and 0.03 mM EDTA) bubbled with 95% O2, 5% CO2 at 37°C. Aortic rings were stabilized at a resting tension of 1 g for 1 h before recording contractile responses. Aortic rings were soaked in Na+-free buffer (118.5 mM N-methyl-D-glucamine, 4.74 mM KCl, 1.18 mM MgSO4, 1.18 mM KH2PO4, 2.54 mM CaCl2, 25 mM choline bicarbonate, 10 mM glucose, and 0.01 mM EDTA) at 37°C and were pretreated with 10 μM ionomycin. Then the relaxation response induced by the addition of 10 mM EGTA was measured. Contractile responses were also measured by changing to Na+-free buffer after the treatment of 1 mM ouabain for 30 min.


placed by the neomycin-resistant gene (neo) (Fig. 1A). A3-1 ES cells were transfectected with the targeting vector, and ES clones containing the homologously recombined gene were isolated. Chimeric mice were generated using these recombinant ES cells by the aggregation method. The heterozygous (NcxI<sup>+/−</sup>) mice were mated to generate null mutant mice. No homozygous (NcxI<sup>−/−</sup>) mutant offspring was identified among ~100 pups from heterozygous intercresses (Fig. 1B). Although NcxI<sup>−/−</sup> embryos could be recognized at day 9.5, NcxI<sup>−/−</sup> embryos were absorbed after day 10.5. The disruption of the NcxI gene in mutant embryos were confirmed by Northern blot analysis using RNA prepared from whole embryos (Fig. 1C) and reverse transcription-PCR (data not shown). There were no obvious blood vessels in the yolk sacs of NcxI<sup>−/−</sup> embryos (Fig. 2A). The body size of NcxI<sup>−/−</sup> embryos was smaller than that of their wild type (NcxI<sup>+/+</sup>) or NcxI<sup>+/−</sup> littermates (Fig. 2B). NcxI<sup>−/−</sup> embryos also exhibited an enlarged pericardics sac (arrow) (Fig. 2B). Otherwise, developments of limb buds, the cranial neural tube, and somites of NcxI<sup>−/−</sup> embryos was comparable with those of NcxI<sup>+/+</sup> embryos (28). Although NcxI<sup>+/−</sup> and NcxI<sup>−/−</sup> hearts showed spontaneous rhythmic contractions at day 9.5, ~70% of NcxI<sup>−/−</sup> embryos did not show any heartbeats, and the remaining NcxI<sup>−/−</sup> embryos showed very slow and arrhythmic contraction. To determine the localization of NCX1 transcripts in the embryos at day 9.5, we performed in situ hybridization analysis. This analysis using whole embryos revealed that NcxI is expressed abundantly and restrictedly in the heart at day 9.5 (Fig. 2C). These findings strongly suggest that NCX1 is essential for heartbeats in embryos.

Histological Analysis in Mice Lacking Ncx1—To determine the cause of embryonic lethality of NcxI<sup>−/−</sup> mutants, day 9.5 embryos were examined. Histological analysis revealed that the ventricular wall of NcxI<sup>−/−</sup> was very thin and that there were fewer cardiac myocytes in the ventricle of NcxI<sup>−/−</sup> mice than that of NcxI<sup>+/−</sup> littermates (Fig. 3, A–D). Reverse transcription-PCR analysis revealed that heart-specific genes such as Gata5, Nkx2.5, Gata4, atrial natriuretic peptide, and myosin light chain 2v genes were expressed in NcxI<sup>−/−</sup> mice as abundantly as in NcxI<sup>+/+</sup> and NcxI<sup>−/−</sup> littermates (data not shown), suggesting that there is no defect in cardiomyocyte differentiation. Some myocardial cells in NcxI<sup>−/−</sup> embryos showed morphology characteristic of apoptosis such as nuclear condensation and cell shrinkage (Fig. 3D) (29). In the neural tube and optic vesicle, many epithelial cells showed prominent nuclear condensation and cell shrinkage (Fig. 4, A–D). In addition, many epithelial cells were detached from the neural tube and neural crest. To evaluate the degree of apoptosis, we performed TUNEL analysis. Many TUNEL-positive cells were observed in the heart and neural tube of NcxI<sup>−/−</sup> embryos, whereas no positive cells were detected in the corresponding area of NcxI<sup>+/−</sup> embryos (Fig. 3, E and F and Fig. 4, E–H). Because NcxI expression was restricted to the heart and not detected in epithelial cells at day 9.5, apoptosis of neuroepithelial cells in NcxI-lacking mice may be secondary to ischemia caused by the lack of blood circulation although slight expression of NcxI was detected in epithelial cells, which was not detected by in situ hybridization analysis, cannot be ruled out.

As shown in Fig. 2A, obvious vessels were not observed in yolk sacs of the null mutant. We examined the development of vessels by staining with anti-PECAM antibody, which reacts with endothelial cells. Although honeycomb-like vasculature was present in both NcxI<sup>+/+</sup> and NcxI<sup>−/−</sup> yolk sac, branching vitelline vessels were not observed, and a plexus of capillaries was enlarged in the NcxI<sup>−/−</sup> yolk sac (Fig. 5A). Because NcxI is not expressed in yolk sac vessels at that embryonic stage, the impaired development of yolk sac vessels is also thought to be secondary to loss of heartbeats. We next examined hematopoiesis. In yolk sac circulation, blood islands were present in NcxI<sup>−/−</sup> as well as NcxI<sup>+/−</sup> yolk sac, suggesting that hematopoiesis is normal in NcxI<sup>−/−</sup> embryos. Capillaries were also formed but dilated in NcxI<sup>−/−</sup> embryos (Fig. 5B). This finding was consistent with the observations of anti-PECAM staining. In placental circulation, the primitive erythrocytes were detected in the placental labyrinth and chorionic plate of the NcxI<sup>−/−</sup> embryos but not of NcxI<sup>−/−</sup> embryos (Fig. 5C). These findings suggest that erythrocytes, which were normally formed in blood islands, did not circulate due to the deficiency of heartbeats. It has been reported that mechanical forces such as vascular endothelial growth factor and transforming growth factor-β are essential for vascular development in the
yolk sac (30). In general vasculogenesis of the yolk sac, the primary capillary plexus generated by sprouting develops into a mature vascular system through the remodeling (30). These findings suggest that the remodeling of the primary capillary plexus is impaired in Ncx1<sup>2</sup>/<sup>2</sup> embryos possibly because of the defects of blood circulation.

**Ca<sup>2+</sup>** Signaling in Cardiac Myocytes from Embryos Lacking Ncx1—To determine the mechanism of cardiomyocyte apoptosis and loss of heartbeats, we examined the Ca<sup>2+</sup> signaling in the organ-cultured heart of day 9.5 embryos using Ca<sup>2+</sup> indicator Fluo-3. Spontaneous intracellular Ca<sup>2+</sup> transient was observed in Ncx1<sup>+/+</sup> hearts in normal HEPES solution and was completely inhibited in 20 μM nifedipine-contained solution, as shown in Fig. 6, suggesting that the Ca<sup>2+</sup> cycling system in hearts of day 9.5 embryos requires Ca<sup>2+</sup> influx through L-type Ca<sup>2+</sup> channels, as observed in adult hearts. Although sporadic Ca<sup>2+</sup> transients at a very low frequency were observed (data not shown), Ncx1<sup>−−</sup> hearts did not show fast Ca<sup>2+</sup> transients, suggesting that NCX1 is required for Ca<sup>2+</sup> transients. To examine the role of NCX1 in Na<sup>+</sup>/Ca<sup>2+</sup> exchange activity, hearts
were perfused with Na\(^+\)-free solution (0 mM Na\(^+\) solution). Removal of Na\(^+\) markedly increased [Ca\(^{2+}\)], in Ncx\(^{-/-}\) hearts, and subsequent application of 140 mM Na\(^+\) decreased [Ca\(^{2+}\)], (Fig. 6A). However, Ncx\(^{-/-}\) hearts did not show any response to either removal or reapplication of Na\(^+\) (Fig. 6B). These findings clearly indicate that the reverse mode of Na\(^+\)/Ca\(^{2+}\) exchange activity was absent in the Ncx\(^{-/-}\) heart at day 9.5.

To evaluate the forward mode of Na\(^+\)/Ca\(^{2+}\) exchange activity in Ncx\(^{-/-}\) hearts, we examined the decline of caffeine-induced Ca\(^{2+}\) transients. After equilibrating the sarcoplasmic reticulum with Ca\(^{2+}\), 20 mM caffeine was applied to the hearts. Caffeine of millimolar concentration has been reported to release Ca\(^{2+}\) from the sarcoplasmic reticulum through ryanodine receptors and to inhibit Ca\(^{2+}\) uptake by sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (31). Indeed, 20 mM caffeine induced a marked increase in [Ca\(^{2+}\)]\(_{\text{i}}\) in both Ncx\(^{+/+}\) and Ncx\(^{-/-}\) hearts (Fig. 6, A and B), suggesting that sarcoplasmic reticulum contains Ca\(^{2+}\) and that ryanodine receptors are functional in both Ncx\(^{+/+}\) and Ncx\(^{-/-}\) hearts at day 9.5. The decay phase of the caffeine-induced Ca\(^{2+}\) transient appears to be slower in the Ncx\(^{-/-}\) heart (Fig. 6B). To clarify if this was due to disruption of the forward mode of the Na\(^+\)-dependent Ca\(^{2+}\) exchange activity, we applied caffeine to the hearts in 0 mM Na\(^+\) solution (Fig. 6, C and D, red line). The decay phase was dramatically prolonged in 0 mM Na\(^+\) solution (Fig. 6C, red line) in a Ncx\(^{+/+}\) heart compared with that in 140 mM Na\(^+\) solution (Fig. 6C, black line), which was consistent with previous findings (23, 32, 33). This finding suggests that NCX1 plays an important role in the decay of caffeine-induced Ca\(^{2+}\) transients. In contrast, the decay phase of caffeine-induced Ca\(^{2+}\) transient was not affected by the presence of Na\(^+\) in Ncx\(^{-/-}\) hearts (Fig. 6D, red line and black line), indicating that the forward mode Na\(^+\)/Ca\(^{2+}\) exchange activity is completely lacking in Ncx\(^{-/-}\) hearts.

The decay of caffeine-induced Ca\(^{2+}\) transients has been reported to be mediated by sarcolemmal Ca\(^{2+}\)-ATPase and mitochondria as well as NCX1 (23, 32). We investigated the functional contribution of sarcolemmal Ca\(^{2+}\)-ATPase to the decay phase of caffeine-induced [Ca\(^{2+}\)], transients using Ncx\(^{-/-}\) hearts. Sarcolemmal Ca\(^{2+}\)-ATPase was inhibited by raising the extracellular Ca\(^{2+}\) levels. As shown in Fig. 6D, the decay phase of caffeine-induced [Ca\(^{2+}\)], transient elicited by a solution of 10 mM Ca\(^{2+}\), 0 mM Na\(^+\), 20 mM caffeine, and 20 mM nifedipine concentration (blue line) was markedly prolonged in compari-

![Anti-PECAM staining and histological analysis of yolk sacs and placenta.](image)
son with that by either a solution of 0 mM Ca\(^{2+}\), 140 mM Na\(^{+}\), 20 mM caffeine, and 20 \(\mu\)M nifedipine concentration (black line) or a solution of 0 mM Ca\(^{2+}\), 0 mM Na\(^{+}\), 20 mM caffeine, and 20 \(\mu\)M nifedipine concentration (red line). These findings clearly indicate that sarcolemmal Ca\(^{2+}\)-ATPase is critically involved in the decay phase of caffeine-induced [Ca\(^{2+}\)]\(_{i}\) transients.

Abnormalities in Adult Ncx1\(^{+/+}\) Mice—We next examined expression levels of NCX1 protein and the Na\(^{+}\)/Ca\(^{2+}\) exchange activity in Ncx1\(^{+/+}\) adult mice. Protein levels of NCX1 in the heart, kidney, aorta, and smooth muscle cells of Ncx1\(^{+/+}\) mice were half that found in Ncx1\(^{++/}\) mice (Fig. 7A, inset, and data not shown). Intracellular Na\(^{+}\)-dependent Ca\(^{2+}\) uptake in cultured smooth muscle cells of Ncx1\(^{+/+}\) mice was also down-regulated to half that of Ncx1\(^{++/}\) mice (Fig. 7A). The maximum velocities of Ca\(^{2+}\) uptake in smooth muscle cells in both Ncx1\(^{+/+}\) and Ncx1\(^{++/}\) mice were 1.35 and 0.71 nmol/30 s/mg of protein, respectively (Fig. 7B). Thus, the Na\(^{+}\)/Ca\(^{2+}\) exchange activity was well correlated to NCX1 protein levels in both Ncx1\(^{+/+}\) and Ncx1\(^{++/}\) mice. To determine whether this correlation was also observed at the tissue level, we examined Ca\(^{2+}\) uptake-dependent relaxation of aortic rings (Fig. 8, A and B). Aortic rings were precontracted by treatment with ionomycin and subsequently with EGTA. In the presence of extracellular Na\(^{+}\), there were no significant differences in the rate and degree of relaxation between Ncx1\(^{+/+}\) and Ncx1\(^{++/}\) mice. This suggests that non-NCX1 Ca\(^{2+}\) efflux systems may be up-regulated to compensate for the reduced amount of NCX1 protein in Ncx1\(^{++/}\) mice. In the absence of extracellular Na\(^{+}\), the rate and degree of relaxation were markedly decreased in Ncx1\(^{++/}\) mice but not in Ncx1\(^{+/+}\) mice. These findings suggest that the
smooth muscle relaxation of Ncx1\(^{-/-}\) aorta is extracellular Na\(^+\)-independent unlike Ncx1\(^{+/+}\) mice. We also examined the effect of Na\(^+\) removal on tension development in aortic rings of Ncx1\(^{+/+}\) and Ncx1\(^{-/-}\) mice (Fig. 8, C and D). When aortic rings were treated with 0.1 mM ouabain for 30 min and then placed in Na\(^+\)-free solution, the developed tension was significantly reduced in adult Ncx1\(^{-/-}\) mice (36% of high K\(^+\)-induced tension) as compared with Ncx1\(^{+/+}\) mice (94% of high K\(^+\)-induced tension). These findings suggest that altered expression levels of NCX1 have a profound effect on vascular tension development. Therefore, the NCX1 modulator can be a useful tool to control vascular contractility, and Ncx1\(^{-/-}\) mice would be very useful to elucidate physiological and pathological functions of NCX1.

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