The Na\(^+\)/Ca\(^{2+}\) Exchanger 1 (NCX1) Variant 3 as the Major Extrusion System in Renal Distal Tubular Transcellular Ca\(^{2+}\)-Transport

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Key Words
Sodium calcium exchange  ·  NCX1  ·  Kidney  ·  Calcium transport

Abstract

Background/Aims: Fine-tuning of renal calcium (Ca\(^{2+}\)) reabsorption takes place in the late distal convoluted and connecting tubules (DCT2/CNT) of the kidney via transcellular Ca\(^{2+}\) transport. Here, Ca\(^{2+}\) enters the cell at the apical side via the epithelial Ca\(^{2+}\) channel transient receptor potential vanilloid 5 and is subsequently extruded at the basolateral side by the concerted actions of the plasma membrane Ca\(^{2+}\) ATPases and the Na\(^+\)/Ca\(^{2+}\) exchanger 1 (NCX1). NCX1 is responsible for ~70% of basolateral Ca\(^{2+}\) extrusion. The aim of this study was to determine the predominant NCX1 variant in the kidney and its role in Ca\(^{2+}\) transport. Methods: DCT2/CNT specific tubules were used to show the abundance of NCX1 specific isoforms. Renal NCX1 variants were cloned from mouse kidney tissue. Human Embryonic Kidney 293(T) cells were transiently transfected with NCX1.3, and Fura-2 measurements and \(^{45}\)Ca\(^{2+}\) uptake assays were performed to determine several characteristics of NCX1.3 in the reverse mode. Results: NCX1.3 was demonstrated to be the predominant NCX1 variant in the DCT2/CNT, next to NCX1.2 and NCX1.7. NCX1.3 could be inhibited by SN-6, an NCX-specific inhibitor, whereas stimulation of the cAMP/PKA or PKC-mediated pathway did not affect Ca\(^{2+}\) influx as measured in the reverse mode. Lowering intracellular Ca\(^{2+}\) concentrations resulted in a decreased Ca\(^{2+}\) uptake. Conclusion: NCX1.3 is the predominant NCX variant in the DCT2/CNT tubules. Its function is dependent on intracellular Ca\(^{2+}\) concentrations.

Introduction

The Na\(^+\)/Ca\(^{2+}\) exchanger I (NCX1) is widely expressed in the body and generally controls intracellular calcium (Ca\(^{2+}\)) levels by extruding Ca\(^{2+}\) from the cell. However, in the kidney, NCX1 is also involved in the highly regulated process of transcellular Ca\(^{2+}\) reabsorption, regulating final excretion of Ca\(^{2+}\) via the urine and thus overall Ca\(^{2+}\) balance [1, 2]. In the late distal convoluted and connecting tubules (DCT2/CNT) of the nephron, the epithelial Ca\(^{2+}\) channel transient receptor potential vanilloid 5 (TRPV5) is responsible for entry of Ca\(^{2+}\) from the tubular lumen into the cells [3]. Thereafter, Ca\(^{2+}\) is shuttled toward the basolateral side by the Ca\(^{2+}\)-binding protein,
Table 1. Oligonucleotide sequences applied in real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Application</th>
<th>Forward primer (5'-3')</th>
<th>Reverse primer (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slc8a1</td>
<td>Cloning</td>
<td>ATGCCTCGATTAAGTCTCCCA</td>
<td>TTAGAAGCCCTTATGTGGCA</td>
</tr>
<tr>
<td>Slc8a1</td>
<td>PCR analysis splice site</td>
<td>AGAGGATTGGCATCATGGA</td>
<td>CAGTGGGCTTGTACCATC</td>
</tr>
<tr>
<td>Slc8a1</td>
<td>Real-time PCR</td>
<td>CTCCTTTGTGGCTGAAGAC</td>
<td>CAGTGGGCTTGTACCATC</td>
</tr>
<tr>
<td>Gapdh</td>
<td>Real-time PCR</td>
<td>TAACTCAATGGGTGGAGG</td>
<td>GGTTCACACCACACAAAC</td>
</tr>
<tr>
<td>TRPV5</td>
<td>Real-time PCR</td>
<td>CTGGAGCTTGTGGTCTC</td>
<td>TACCTCTAGGCTCACAC</td>
</tr>
<tr>
<td>Parv</td>
<td>Real-time PCR</td>
<td>CGCTAGGACATCAAGAGG</td>
<td>AGCTTTAGCCACAGAGTG</td>
</tr>
<tr>
<td>Slc12a1</td>
<td>Real-time PCR</td>
<td>GCTTCTGATCTTTGTCAGGC</td>
<td>CATCATGAAATCAGCGTCTCC</td>
</tr>
</tbody>
</table>

*Slc8a1 = Murine NCX1; Gapdh = murine glyceraldehyde-3-phosphate dehydrogenase gene; TRPV5 = murine epithelial Ca2+ channel TRPV5; Parv = murine parvalbumin; Slc12a1 = murine NKCC2.*

Calbindin-28K [4, 5], where it is extruded toward the blood by the ATP-dependent plasma membrane Ca2+-ATPases (PMCA 1/4) and the Na+-Ca2+ exchanger (NCX1) [6]. NCX1 is considered to be the predominant extrusion mechanism, accounting for ~70% of the extruded Ca2+ [7–9], thereby playing a key role in the process of renal transcellular Ca2+ reabsorption.

Three NCX isoforms exist, NCX1, NCX2 and NCX3, which are structurally highly similar [8, 10]. NCX consists of 10 transmembrane segments and a large regulatory cytosolic loop. The cytosolic loop contains 2 Ca2+-binding domains (CBD1 and CBD2), of which the CBD2 region is subject to alternative splicing [10, 11]. Alternative splicing results in at least 17 different NCX1 proteins, with a tissue specific distribution. The different splice variants are produced from a combination of 6 small exons (A, B, C, D, E, and F), with exons A and B being mutually exclusive [11, 12]. Exon A is expressed primarily in excitable tissues such as the heart and the brain, while exon B is present in non-excitable tissues including the kidney [13]. In the kidney, 3 different NCX1 variants have been identified, NCX1.2 (exons BCD), NCX1.3 (exons BD) and NCX1.7 (exons BDF) [12, 14, 15]. Since the alternative splicing region is located within the CBD2 domain, NCX1 variants have different responses to regulatory Ca2+, due to differences in response kinetics, dynamic range and affinity of allosteric sensors [13, 16].

NCX1 was first cloned from cardiac tissue (NCX1.1, exons ACDEF), where its crucial role in myocyte contraction has been extensively studied [17, 18]. However, studies on the function and regulation of the kidney-specific variants are more limited.

The aim of the present study was to investigate which NCX1 variants are expressed in Ca2+-transporting epithelia in mouse kidney, more specifically DCT2/CNT, and to functionally characterize the predominant variant NCX1.3.

**Materials and Methods**

**Cloning of NCX Variants from Mouse Kidney**

To obtain NCX1 cDNA, total mouse kidney mRNA was extracted using TRIzol® (Invitrogen, Breda, The Netherlands) and processed using RQ1 DNase and M-MLV Reverse Transcriptase (Invitrogen) using RNasin® (Promega, Madison, WI, USA) as a RNase inhibitor, according to the manufacturer's protocols. Next, a PCR was performed on the kidney cDNA to obtain the full length NCX1 coding sequence, by using primers annealing to the ends of the open reading frame (table 1, based on NCBI Reference Sequence NM_011406.2). Sequences were added for restriction digestion sites using *MluI* restriction enzyme recognition sites, respectively: forward: `cgcgtATGCTTGTAGTATTAGTCTCCCA`, reverse: `tgctgcCTAGAGACCTTATGTGGGCA`. PCR was performed using Phusion® High-fidelity DNA polymerase (Invitrogen, Breda, The Netherlands). PCR products were isolated from agarose gel, *MluI* and *AscI* restriction enzyme recognition sites were digested and ligated into the pCINeo-IRES-GFP vector. Subsequently, pCINeo-mNCX1.2-IRES-GFP, pCINeo-mNCX1.3-IRES-GFP and pCINeo-mNCX1.7-IRES-GFP plasmid DNA were recovered from transformed *E. coli* clones and identified by sequence analysis. All obtained constructs were verified by sequencing of the NCX1 open reading frame and applied restriction sites.

**PCR/Gel Electrophoresis**

PCR was performed using Taq polymerase (1 μl) in 10 mM Tris-HCl, 50 mM KCl, 0.01% (v/v) gelatin, 2.5 mM MgCl2, 1 mM dNTPs and 0.2 μM of each primer (table 1). DCT2/CNT cDNA was isolated by fluorescent sorting (table 1). PCR products were run on a 2% (w/v) agarose gel.

**Real-Time PCR**

cDNA of DCT2/CNT was prepared as described above. Real-time PCR was performed using iQSYBR Green Supermix (Bio-Rad, Veenendaal, The Netherlands). Expression levels in DCT2/CNT
fluorescent tubules were compared to expression in total cortex material, Gapdh was used as a housekeeping gene. Primer sequences are presented in table 1.

**Cell Culture and Transfection**

Human embryonic kidney (HEK)293(T) cells were grown in Dulbecco’s Modified Eagle’s Medium (Bio Whittaker Europe, Vervier, Belgium) containing 10% (v/v) fetal calf serum (PAA, Linz, Austria), 2 mM l-glutamine, 10 μl/ml non-essential amino acids at 37°C and 5% (v/v) CO2. Cells were transiently transfected using Lipofectamine® 2000 (Invitrogen, 2 μl/μg DNA). Cells were transfected either with pCINeo-IRES-GFP (Mock) or pCINeo-NCX1.3-IRES-GFP.

**Intracellular Ca2+ Measurements Using Fura-2**

After 24 h of transfection, HEK293 cells were re-seeded onto fibronectin-coated coverslips (Ø 25 mm). Twenty-four hours later, cells were loaded with 3 μM Fura-2 AM (Molecular Probes, Eugene, OR, USA) and 0.01% (v/v) Pluronic F-129 (Molecular Probes, Eugene, OR) in KREBS buffer (in mM: 5.5 KCl, 147 NaCl, 1.2 MgCl2, 1.5 CaCl2, 10 D-glucose, 10 HEPES-NaOH, pH 7.4) at 37°C for 20 min. Then cells were washed with KREBS, allowed to equilibrate for 10 min and placed on a 37°C stage of an inverted microscope (Axiovert 200 M, Zeiss, Jena, Germany), continuously being perfused with 37°C KREBS buffer. Sixty seconds after start of the measurements, perfusion buffer was changed to KREBS containing 1 μM thapsigargin (Sigma, St. Louis, MO, USA). At 220 s buffer was changed to KREBS with NaCl replaced by 147 mM N-methyl-D-glucamine (NMDG) in presence of thapsigargin. Details of monitoring [Ca2+]i using Fura-2 and quantitative image analysis have been described previously [20]. In short, the Fura-2 probe is excited at 340 and 380 nm, with a sampling interval of 3 s. After background correction, the fluorescence emission ratio of 340 and 380 nm excitation was calculated.

**45Ca2+ Uptake Assays**

HEK293T cells were re-seeded into poly-L-lysine (Sigma) coated 24-well plates. Na+-dependent uptake of 45Ca2+ was measured generally using the assay as described by Iwamoto et al. [21]. Cells were pre-incubated for 30 min with physiological buffer (in mM: 4 KCl, 147 NaCl, 2 MgCl2, 1.0 CaCl2, 10 d-glucose, 10 HEPES, NaOH, pH 7.4) at 37°C for 20 min. Then cells were washed with KREBS, allowed to equilibrate for 10 min and placed on a 37°C stage of an inverted microscope. Calcium-containing buffer (in mM: 1.5 CaCl2, 10 D-glucose, 10 HEPES-NaOH, pH 7.4) at 37°C for 20 min. Then cells were washed with KREBS, allowed to equilibrate for 10 min and placed on a 37°C stage of an inverted microscope (Axiovert 200 M, Zeiss, Jena, Germany), continuously being perfused with 37°C KREBS buffer. Sixty seconds after start of the measurements, perfusion buffer was changed to KREBS containing 1 μM thapsigargin (Sigma, St. Louis, MO, USA). At 220 s buffer was changed to KREBS with NaCl replaced by 147 mM N-methyl-D-glucamine (NMDG) in presence of thapsigargin. Details of monitoring [Ca2+]i using Fura-2 and quantitative image analysis have been described previously [20]. In short, the Fura-2 probe is excited at 340 and 380 nm, with a sampling interval of 3 s. After background correction, the fluorescence emission ratio of 340 and 380 nm excitation was calculated.

**Immunoblotting**

NCX1 protein expression was analyzed using transiently transfected HEK293T cell lysates (in 150 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.5 using HCl, 1% (v/v) Triton X-100 and protease inhibitors), immunoblotted overnight at 4°C, using 1:500 diluted mouse anti-NCX1 antibody (Abcam, Cambridge, UK, ab6495) and with mouse anti–β-actin 1:10,000 (Sigma) as a loading control. Secondary anti-mouse antibody conjugated with HRP was used and visualized using femto ECL (Thermo scientific, Waltham, MA, USA).

**Results**

Cloning of NCX1.2, NCX1.3 and NCX1.7 from Mouse Kidney

In the kidney, 3 different splice variants were reported to be expressed: NCX1.2 (exons BCD), NCX1.7 (exons BDF) and NCX1.3 (exons BD) [14, 15, 22]. An overview of the renal splice variants is presented in figure 1a. All 3 reported renal variants were obtained after cloning NCX1 from mouse kidney, namely NCX1.2, NCX1.3 and NCX1.7. The different variants were identified by complete sequencing (data not shown). Sequences were identical to GenBank accession numbers XM_006523942, XM_006523944, XM_006523940, respectively. By comparing PCR products using primers flanking the alternative splice region, NCX1.3 was the predominant NCX1 variant in the kidney DCT2/CNT segments (fig. 1b), while other variants were detected as well. The identity of the NCX1.3 band was further confirmed by an SpI restriction digest, specifically digesting exon B (data not shown). To confirm the identity of our DCT2/CNT material, the relative amount of NCX1, TRPV5, Na+-K+-2Cl–-cotransporter (NKCC2) and parvalbumin as compared to total kidney cortex was determined using real-time PCR. Both NCX1 and TRPV5 were highly enriched in the DCT2/CNT material, whereas NKCC2 and parvalbumin, expressed in the thick ascending limb and DCT1 segments, respectively, were not (fig. 1c).

**NCX1.3 Cloned from Mouse Kidney Is Functional in HEK293 Cells**

To study the function of NCX1.3 cloned from mouse kidney, HEK293 cells were transiently transfected with the pCINeo-mNCX1.3-IRES-GFP construct. Depending on the electrochemical gradient, NCX can either extrude Ca2+ from the cell (forward mode) or take up extracellular...
Ca\textsuperscript{2+} (reverse mode). The intracellular Ca\textsuperscript{2+} sensor Fura-2 was used to demonstrate reverse mode NCX1.3 activity. First, Ca\textsuperscript{2+} was released from the intracellular stores by perfusing the cells with KREBS buffer containing the sarco/endoplasmic reticulum Ca\textsuperscript{2+}-ATPase inhibitor thapsigargin (1 μM). As a result, untransfected cells showed a clear rise in intracellular Ca\textsuperscript{2+} concentration, whereas NCX1.3-expressing cells did not demonstrate this response (fig. 2a, b). In order to study NCX1 activity, at 220 s, the reverse mode function was induced by switch-
Characterization of Renal NCX1.3

Regulation of NCX1.3 by PKA/PKC Phosphorylation and Intracellular Ca²⁺

Since the process of transcellular Ca²⁺ transport in the kidney is highly regulated by many hormones and factors [23, 24], regulation of NCX1.3 activity was further investigated. NCX1.3 activity was studied using radioactive ⁴⁵Ca²⁺ uptake during reverse mode function induced by switching to Na⁺-depleted medium for 30 s. Firstly, general characteristics of the murine NCX1.3 variant were studied. The NCX1 inhibitor for the reverse mode function SN-6 (50 μM) [25, 26] inhibited NCX1.3 function by 32 ± 8% when cells were pre-treated for 30 min. Pre-treatment with TPA (100 nM) or forskolin (10 μM), to induce activation of PKC or cAMP/PKA-mediated pathways, respectively, did not affect NCX1.3 activity (fig. 3a).

Application of either buffer significantly decreased NCX1.3 activity with 54 ± 8% or 25 ± 9%, respectively (fig. 3c). NCX1.3 protein levels were not affected by treatment with SN-6, TPA or forskolin. The immunoblot with beta-actin was used as loading control (b). NCX1.3 transfected HEK293T cells were pre-treated for 30 min with BAPTA-AM and EGTA-AM (25 μM) (c). Representative immunoblot for NCX1 expression, of cells used parallel to ⁴⁵Ca²⁺ uptake, treated with BAPTA-AM or EGTA-AM. The beta-actin immunoblot was used as loading control (d). * p < 0.05 as compared to Mock, # p < 0.05 as compared to DMSO/EtOH control (n = 9).

**Fig. 3.** Regulation of NCX1.3 as measured by influx of ⁴⁵Ca²⁺. HEK293T cells were transfected with Mock construct or NCX1.3 (DMSO/EtOH controls). Uptake of ⁴⁵Ca²⁺ was measured in cells pre-treated for 30 min with 50 μM SN-6, 100 nM TPA (both in DMSO) or 10 μM forskolin (in EtOH) in physiological buffer containing NaCl and ouabain. Reverse-mode of NCX1.3 was induced upon switching to NMDG containing medium for 30 s (a). Representative immunoblot for NCX1 expression, of cells used parallel to ⁴⁵Ca²⁺ uptake, treated with SN-6, TPA or forskolin. The immunoblot with beta-actin was used as loading control (b). NCX1.3 transfected HEK293T cells were pre-treated for 30 min with BAPTA-AM and EGTA-AM (25 μM) (c). Representative immunoblot for NCX1 expression, of cells used parallel to ⁴⁵Ca²⁺ uptake, treated with BAPTA-AM or EGTA-AM. The beta-actin immunoblot was used as loading control (d). * p < 0.05 as compared to Mock, # p < 0.05 as compared to DMSO/EtOH control (n = 9).
ments of the different compounds, as shown by immuno-
blotting of lysates from HEK293T cells cultured in par-
allel to the cells used for $^{45}\text{Ca}^{2+}$ uptake studies (fig. 3b, d).

**Discussion**

In this study, NCX1.2, NCX1.3 and NCX1.7 were cloned from mouse kidney cDNA. NCX1.3 was con-
firmed as the predominant NCX1 variant in murine kid-
ney and more specifically in DCT2/CNT nephron seg-
ments. Interestingly, stimulation of the PKC or cAMP/ 
PKA-mediated pathway did not result in altered $\text{Ca}^{2+}$ in-
flux via NCX1.3. However, intracellular $\text{Ca}^{2+}$ levels did 
regulate NCX1.3 activity.

In the kidney, $\text{Ca}^{2+}$ extrusion is performed by plasma 
membrane $\text{Ca}^{2+}$-ATPases and NCX1. In a previous study, 
the role of NCX1 function in this process was demon-
strated in DCT2/CNT specific tubules ex vivo [31]. The 
extact identity of the splice variants playing a role has been 
unclear, as splice variants described in different species 
were not consistent. In rat kidney, NCX1.3 (exons BD) 
and NCX1.7 (exons BDF) were found, whereas in rabbit 
variants, NCX1.2 (exons BCD) and NCX1.3 were detect-
ed [11, 12, 15]. In an immortalized mouse DCT cell 
line, expression of NCX1.6 (exons ACD) together with 
NCX1.2 and NCX1.3 was demonstrated [14, 32]. Inter-
estingly, Gotoh et al. [33] recently reported NCX2 protein 
expression in the kidney. The NCX2 heterozygote knock-
out mice presented with natriuresis and hypercalciuria, 
suggesting a potential important role for NCX2 in the 
kidney and in $\text{Ca}^{2+}$ homeostasis.

In this study, we focused on NCX1 and NCX1.2 (BCD), 
NCX1.3 (BD) and NCX1.7 (BDF) were cloned from 
mouse kidney tissue. NCX1.3 was identified as the pre-
dominant variant in the kidney as well as in DCT2/CNT, 
which was in accordance with previous literature [15, 22].

Murine NCX1.3 was cloned previously by Li et al. [34] 
from mouse osteoclasts but functional studies were never 
performed using this construct. NCX1.2 and NCX1.3 
were described to be functional in a mouse DCT cell line; 
however, NCX1.7 was not expressed [14]. In another 
study, NCX1.3 and NCX1.7 were compared, demonstrat-
ing a difference in buffer capacity and oxidative stress re-
response [35, 36]. However, this was later partly explained 
by the used NCX construct, which contained a difference 
of one residue at position 218, which is not present in the 
splice region [37]. For NCX1.2, not much is known about 
its role and regulation. The role of the mutually exclusive 
exons A and B has been investigated earlier, functionally 
comparing NCX1.3 (exons BD) to the brain specific vari-
ant NCX1.4 (exons AD) [22, 38]. NCX1.3 was shown to 
be considerably more susceptible to intracellular $\text{Na}^{+}$-de-
pendent inactivation compared to NCX1.4, and high in-
tracellular $\text{Ca}^{2+}$ concentrations could alleviate $\text{Na}^{+}$-de-
pendent inactivation in NCX1.4 only [22, 38]. Possibly, 
this characteristic could be explained by the hypothesis 
that the CBD2 region of splice variants containing exon 
B, are thought not to bind $\text{Ca}^{2+}$, as this region remains 
completely unstructured even when $\text{Ca}^{2+}$ levels are high 
[39]. In our study, NCX1.3 variant was functionally char-
acterized, since it was the most abundantly expressed 
variant in DCT2/CNT tubules, expressing most value for 
the role of NCX1 in transcellular $\text{Ca}^{2+}$ transport.

First, Fura-2 measurements in NCX1.3 overexpressing 
HEK293 cells demonstrated reverse mode NCX1.3 activ-
ity. Next, NCX1.3 was further characterized by applying 
the NCX inhibitor SN-6. A concentration of 50 μM was 
able to decrease $\text{Ca}^{2+}$ influx with 30%. Iwamoto et al. [26] 
showed a decrease for NCX1.1 (exons ACDEF) of almost 
90% with 30μM SN-6. This difference in amount of inhi-
bition could be due to the different splice variants; how-
ever, it is thought that SN-6 interacts at the so-called ex-
changer inhibitory peptide (XIP) region, which is present 
in both splice variants [26]. Potentially the difference in 
response on SN-6 is caused by the used cell type or differ-
ence in $\text{Ca}^{2+}$ concentration. Interestingly, the present 
results demonstrated no effect of PKC signaling on NCX1.3 
activity when activated using TPA, which is in contrast to 
Hwang et al. [37]. However, phosphorylation of NCX re-
 mains controversial and seems largely dependent on spe-
cific circumstances [21, 40]. Presumably, NCX1 is part of 
a macromolecular complex, containing kinases and phos-
phatases, which might differ among cell lines, tissues and 
different circumstances. Also, phosphorylation processes 
might indirectly affect NCX1 activity [40, 41]. Activation 
of NCX1 by PKA was only reported for NCX1.1 and not 
for NCX1.3, consistent with the present results [13].

Finally, we further substantiated that intracellular 
$\text{Ca}^{2+}$ is of key importance for NCX1.3 activity by applying 
the intracellular $\text{Ca}^{2+}$ buffers BAPTA-AM and EGTA-
AM. This was in accordance with previous literature, 
which showed a similar effect for NCX1.1 [30, 42]. In-
creased $\text{Ca}^{2+}$ concentrations are known to stimulate 
NCX1 activity, via binding to the $\text{Ca}^{2+}$-binding domains 
[27, 28, 43]. Another hypothesis is regulation by the $\text{Ca}^{2+}$ 
sensing protein calmodulin (CaM). By decreasing intra-
cellular $\text{Ca}^{2+}$ concentrations, CaM would not be able to 
potentially stimulate NCX1. However, interaction of 
CaM with NCX1 has never been observed so far [44, 45].
though Li et al. [44] identified a predicted CaM binding site in the XIP region of NCX1. However, this region was shown to bind CaM as a peptide only, and interestingly XIP peptides were shown to inhibit NCX. NCX regulation via CaM would be interesting, since all other important players in Ca\(^{2+}\) reabsorption in the DCT2/CNT, namely TRPV5 and PMCA, are also regulated by CaM [46, 47].

In conclusion, the present study identifies NCX1.3 as the main NCX1 expressed in DCT2/CNT tubules facilitating transcellular Ca\(^{2+}\) reabsorption and demonstrates that intracellular Ca\(^{2+}\) is important for NCX1.3 activity.

References


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Disclosure Statement

The authors declare no conflict of interest.

Characterization of Renal NCX1.3

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