Forefront of Na⁺/Ca²⁺ Exchanger Studies: Physiology and Molecular Biology of Monovalent Cation Sensitivities in Na⁺/Ca²⁺ Exchangers

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Abstract. Sensitivities of the reverse-mode Na⁺/Ca²⁺ exchange activity measured as the Na⁺-dependent Ca²⁺ uptake to extracellular monovalent cations K⁺, Li⁺, and Na⁺ were compared between the K⁺-dependent (NCKX2) and the K⁺-independent Na⁺/Ca²⁺ exchanger (NCX1) overexpressed in a fibroblast cell. Interestingly, the exchange activity of NCKX2 was not influenced by Li⁺ while it was increased by K⁺. On the contrary, the activity of NCX1 was increased by Li⁺. Thus, the cation sensitivities to K⁺ and Li⁺ markedly differed between NCKX2 and NCX1. In addition, Na⁺ exerted a significantly smaller inhibitory effect on the activity in NCKX2 than in NCX1. The Na⁺/Ca²⁺ exchange activities of NCKX2 and NCX1 are considered to be regulated differentially via the respective binding site domains that have distinct sensitivities to the external monovalent cations.

Keywords: Na⁺/Ca²⁺ exchanger (NCX), K⁺-dependent NCX (NCKX)2, NCX1

Introduction

The Na⁺/Ca²⁺ exchangers of ion transport proteins are classified into two families. One family is the K⁺-independent Na⁺/Ca²⁺ exchangers (NCX) expressed in cardiac myocytes and brain neurons, which transport 3 (1) or 4 (2, 3) Na⁺ ions in exchange for 1 Ca²⁺. The second family is the K⁺-dependent Na⁺/Ca²⁺-K⁺ exchangers (NCKX) expressed in the retinal photoreceptors and brain neurons, which transport 4 Na⁺ ions in exchange for every 1 Ca²⁺ and 1 K⁺ (4 – 7). The most contrastive feature between NCX and NCKX is such a different sensitivity to the monovalent cation K⁺. The Na⁺/Ca²⁺ exchangers play a major role in expelling cytosolic Ca²⁺, utilizing inward Na⁺-gradient on the plasma membrane in the case of the NCX family while utilizing both inward Na⁺-gradient and outward K⁺-gradient in the case of the NCKX family (8).

External monovalent cations were shown to activate the Na⁺/Ca²⁺ exchange activity of the NCX family in cardiac myocytes (9), squid axons (10 – 12), and NCX overexpressing fibroblast cells (13, 14). However, it has not yet been understood well how the Na⁺/Ca²⁺ exchange activities of the NCKX family are regulated by external cations. On the analogy of the different sensitivity to a monovalent cation K⁺ between NCX and NCKX, different sensitivities to the other monovalent cations are also predictable. Thus we have extended to NCKX2 the functional analysis on how the Na⁺/Ca²⁺ exchange activities are modified by the monovalent cations other than K⁺, such as Na⁺ and Li⁺.

In the present study, we have demonstrated that the sensitivities to all external monovalent cations examined vary significantly between NCKX2 and NCX1. Li⁺ increased the exchange activity of NCX1 but did not alter that of NCKX2. Intriguingly, NCKX2 thus lacked sensitivity to the external Li⁺. Also, Na⁺ inhibited the Na⁺/Ca²⁺ exchange activity less potently in NCKX2 than in NCX1.

Na⁺/Ca²⁺ exchange activities of NCKX2 and NCX1

Na⁺/Ca²⁺ exchange activities were measured by the ⁴⁰Ca²⁺ uptake method from the trasfected CCL39 cells expressing either NCKX2 or NCX1, which were pre-
loaded with Na\(^+\), utilizing its ionophore of monensin (Fig. 1). In NCKX2, the addition of mM level extracellular K\(^+\) was required for a prominent time-dependent increase of the Ca\(^{2+}\) uptake. The exchange activities were also recorded with the whole-cell patch clamp method as extracellular Ca\(^{2+}/K\(^+\)) -induced outward currents from the NCKX2 and NCX1 (Fig. 2). Both outward currents of NCKX2 and NCX1 similarly exhibited a time-dependent decay of so-called inactivation. The effect of a novel blocker of the NCX family, an isothiourea derivative KB-R7943, was then compared between NCKX2 and NCX1. The outward currents of NCKX2 were scarcely modified while those of NCX1 were suppressed by 10 \(\mu\)M KB-R7943. In addition, the external K\(^+\)-dependent [Ca\(^{2+}\)]\(_i\) rises in the NCKX2 transfectant cells loaded with Fura-2 via the reverse-mode exchange activity were confirmed (data not shown). Thus, we could demonstrate a strict requirement for the external potassium ion restrictedly in NCKX2 but not in NCX1 with respect to any reverse-mode Na\(^+\)/Ca\(^{2+}\) exchange activity of the \(^{45}\)Ca\(^{2+}\) uptake, the electrogenic whole-cell outward currents and the intracellular Ca\(^{2+}\) rise.

**Sensitivities of NCKX2 and NCX1 to monovalent cations**

The sensitivities of NCX1 to extracellular monovalent cations other than K\(^+\) were already examined by our group (14); Li\(^+\) of the nonsubstrate ion activates the Na\(^+\)/Ca\(^{2+}\) exchange activity and Na\(^+\) of the substrate ion inhibits it. We have extended to NCKX2 the examination on the sensitivities to Li\(^+\) and Na\(^+\) and have compared these sensitivities between NCKX2 and NCX1. Li\(^+\) did not alter the exchange activity of NCX2 but activated that of NCX1 (Fig. 3). Na\(^+\) significantly inhibited the exchange activity in NCKX2 (IC\(_{50}\) = 58 ± 5 mM, n = 4) less potently than in NCX1 (IC\(_{50}\) = 39 ± 2 mM, n = 4). Thus, the sensitivities to all external monovalent cations of K\(^+\), Li\(^+\), and Na\(^+\) differed between NCKX2 and NCX1.

**Membrane topology of \(\alpha\)-repeat structures responsible for the cation binding sites**

The \(\alpha\)-1 and \(\alpha\)-2 repeats are conserved structural domains that are considered to be functionally important regions for cation transporting and binding in the cardiac Na\(^+\)/Ca\(^{2+}\) exchangers (14 – 16). Judging from the susceptibility analysis by thiol-specific and membrane-impermeable MTSET (2-trimethylammonioethylmethane thiosulfonate) and membrane-permeable NEM (N-ethyl-...
maleimide) on the cysteine-substituted amino acid residues of Gly-207, Ile-209, Ile-563, and Asp-571, here we propose a topological model of the $\alpha$-1 and $\alpha$-2 repeats in the nine transmembrane segment model of the NCKX2 molecule (Fig. 4). All the results from the MTSET experiments suggested that the reentrant structures of the $\alpha$-1 and $\alpha$-2 repeats are situated extracellularly and intracellularly, respectively. Thus, these topological features of the putative cation binding sites in NCKX2 resembled those of NCX1.

We have already proposed as shown in Fig. 5 that two amino acid residues in the $\alpha$-2 repeat are responsible for the Li$^+$ activation in NCX1 from their mutational analysis and considered that Li$^+$ is likely to bind as an activator to these sites distinct from the transport sites, and it is not transported by NCX (14). The two amino acid residues of V820 and Q826 responsible for Li$^+$ binding sites in NCX1 were not conserved in the corresponding residues of NCKX2. The replacement of the residue V820 in NCX1 by I in NCKX2 and the residue Q826 by G in NCX1 may result in lowering the Li$^+$ sensitivity in NCKX2. Intriguingly, the same residues V820 and Q826 have been proposed to be responsible for the KB-R7943 inhibition in NCX1, which is most likely to yield the simultaneous insensitivity to both Li$^+$ and KB-R7943 in NCKX2.

Conclusions and future directions

The present study on the cloned Na$^+/Ca^{2+}$ exchangers demonstrated differential sensitivities between K$^+$-dependent and -independent exchangers for external monovalent cations of not only K$^+$ but also Na$^+$ and Li$^+$. Cysteine susceptibility analysis showed that the $\alpha$-1 and $\alpha$-2 repeat structures of the putative binding sites for external monovalent and multivalent cations were located extracellularly and intracellularly, respectively. It may be an underlying mechanism of the insensitivity of NCKX2 to Li$^+$ and KB-R7943 that the two amino acid residues proposed as the Li$^+$ and KB-R7943 binding sites.

Fig. 4. Membrane topology of the $\alpha$-1 and $\alpha$-2 repeats responsible for the putative cation binding sites determined by substituted cysteine susceptibility analysis using MTSET. The $\alpha$-1 and $\alpha$-2 repeat are located as reentrants extracellularly and intracellularly, respectively.

**$\alpha$-1 repeat**

NCKX2 168 KGLISDDVMAGATEMAAGCSAPELFTSLLIGF---IAHSNVGLGTVGSAVFNILFVIGCMALFSRE  
NCX1 92 VRWNETTVSNILTIMAAGCSAPEHILSIVVCGHNFTAGDDGETIVGSAFNMFFIALLVYVVPD

**$\alpha$-2 repeat**

NCKX2 536 TIGISEEIMGLTIAAGTSIIPDLITLVIVAK---KGLGMAVVSSVGSNIFDITVGLPLFWLTYIT  
NCX1 793 TIGLKDSVTAVVFLGTSVIPRFSFASKVAN---QDVADASIGNVTSNAVNFGLGIGVAWSIAAI

Fig. 5. Comparison of the amino acid alignment of each $\alpha$-1 and $\alpha$-2 repeat between NCKX2 and NCX1. The same amino acid residues conserved between both exchangers are surrounded by squares. Triangles show the residues responsible for the Li$^+$ and KB-R7943 bindings that has been proposed in NCX1.
sites in NCX1 are not conserved in NCKX2. In order to locate the cation binding sites and ion transport pathway of the exchanger molecule, extensive mutational analyses on the membrane α-repeat domains of NCKX2 and NCX1 are required.

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References