Forum Minireview

Forefront of $\text{Na}^+/\text{Ca}^{2+}$ Exchanger Studies: Regulation Kinetics of $\text{Na}^+/\text{Ca}^{2+}$ Exchangers

Satoshi Matsuoka^{1,*}¹Department of Physiology and Biophysics, Kyoto University Graduate School of Medicine, Kyoto 606-8501, Japan

Received June 14, 2004; Accepted July 27, 2004

Abstract. Five isoforms of $\text{Na}^+/\text{Ca}^{2+}$ exchanger have been identified: NCX1, NCX2, NCX3, NCX-SQ1, and CALX. In all of the exchangers, the $\text{Na}^+/\text{Ca}^{2+}$ exchange current, which was recorded in inside-out membrane patches, was regulated by cytoplasmic Ca^{2+} . However, the mode of regulation is different among the exchangers. NCX1, NCX2, and NCX-SQ1 are positively regulated by cytoplasmic Ca^{2+} , but CALX is negatively regulated. NCX3 apparently has both positive and negative regulation mechanisms. In this review, I briefly summarize the Ca^{2+} -dependent regulation mechanisms of the exchangers.

Keywords: $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX), transporter, regulation, patch clamp

Introduction

The $\text{Na}^+/\text{Ca}^{2+}$ exchange is the main mechanism that extrudes intracellular Ca^{2+} in exchange for extracellular Na^+ . At present, five isoforms of $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) have been identified: NCX1 (1), NCX2 (2), NCX3 (3), NCX-SQ1 (4), and CALX (5). NCX1, NCX2, and NCX3 are mammalian isoforms. NCX-SQ1 and CALX were cloned from squid axon and *Drosophila*, respectively. The electrophysiological properties of these isoforms, which were expressed in *Xenopus* oocytes or cultured cells, have been extensively studied using the inside-out giant membrane patch technique (6). This technique allows studying the regulation kinetics of the exchangers by cytoplasmic Na^+ and Ca^{2+} (Na^+ -dependent inactivation and Ca^{2+} -dependent regulation). The Na^+ -dependent inactivation and the Ca^{2+} -dependent regulation are common features to all the $\text{Na}^+/\text{Ca}^{2+}$ exchange isoforms: NCX1 (7), NCX2 (2), NCX3 (8), NCX-SQ1 (4), and CALX (9). The Na^+ -dependent inactivation is characterized as the decay of outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current during the cytoplasmic application of Na^+ . The Ca^{2+} -dependent regulation is characterized as the modulation of outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current by cytoplasmic Ca^{2+} . The modes and extents of regulation by cytoplasmic Ca^{2+} differ among the isoforms. In this review, I briefly

summarize the Ca^{2+} -dependent regulation mechanisms of NCXs.

Regulation of NCXs by Ca^{2+}

NCX1 is expressed ubiquitously in many tissues and is a dominant isoform in the heart. The function of NCX1 has been most extensively studied among $\text{Na}^+/\text{Ca}^{2+}$ exchange isoforms. The regulation of $\text{Na}^+/\text{Ca}^{2+}$ exchange by cytoplasmic Ca^{2+} can be easily studied by recording the outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current in the inside-out patch, namely, exchanging extracellular Ca^{2+} for cytoplasmic Na^+ . The submicromolar concentration of cytoplasmic Ca^{2+} increases the peak amplitude of outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current, even though the electrochemical gradient of Ca^{2+} decreases. The half maximum concentration (K_h) for Ca^{2+} to the increase of peak outward current is approximately $0.2 \mu\text{M}$ (10, 11). This effect can be explained by relief of exchanger from an inactive state (I_1 inactivation). The regulatory Ca^{2+} also increases the steady state amplitude of the $\text{Na}^+/\text{Ca}^{2+}$ exchange current by attenuating the Na^+ -dependent inactivation (I_2 inactivation). The K_h for Ca^{2+} to the attenuation of I_2 inactivation is higher by one order than the one for I_1 inactivation (approximately $2 \mu\text{M}$; 10, 11). Additionally, the regulatory Ca^{2+} increases the apparent affinity for cytoplasmic Na^+ (10). Figure 1 shows an example of the effect of regulatory Ca^{2+} on the steady state outward $\text{Na}^+/\text{Ca}^{2+}$ exchange

*Corresponding author. FAX: +81-75-753-4349
E-mail: matsuoka@card.med.kyoto-u.ac.jp

Fig. 1. Outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current of NCX1 recorded in inside-out giant membrane patch. Modified from Hryshko et al. (9).

current (9). The removal of cytoplasmic $1 \mu\text{M}$ Ca^{2+} attenuated the outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current and subsequent re-application of Ca^{2+} augmented the current. Applying trypsin or chymotrypsin to the cytoplasmic surface of the patch membrane eliminated both the I_1 and I_2 inactivation and fully activated the $\text{Na}^+/\text{Ca}^{2+}$ exchange current. The lower panel of Fig. 1 is a scheme of the positive regulation by cytoplasmic Ca^{2+} . Without cytoplasmic Ca^{2+} , the majority of NCX1 is in an inactive state and cannot exchange Na^+ for Ca^{2+} . When regulatory Ca^{2+} binds, NCX1 moves to an active state and can exchange the ions.

Site directed mutagenesis revealed that the regulatory Ca^{2+} binds to regions in the large cytoplasmic loop (loop f) (10, 12) and that the XIP region is responsible for the Ca^{2+} regulation (13). The region for alternative splicing may be important to determine the extent of the Ca^{2+} regulation (14).

NCX2, which is mainly expressed in the brain and skeletal muscles, has an essentially similar Ca^{2+} regulation to NCX1 (2). The Ca^{2+} regulation of NCX-SQ1, a squid isoform, is also similar to NCX1 (4). In both isoforms, regulatory Ca^{2+} augments the outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current, even though the Ca^{2+} dependence of the isoforms is slightly different from that of NCX1.

CALX was cloned from *Drosophila melanogaster* (5). The CALX is also regulated by cytoplasmic Ca^{2+} , but the effect is opposite to those of the above isoforms. Namely, the outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current of CALX was attenuated by regulatory Ca^{2+} (9, 15). Figure 2 shows the $\text{Na}^+/\text{Ca}^{2+}$ exchange current recorded with the same experimental protocol as that used in

Fig. 2. Outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current of CALX recorded in inside-out giant membrane patch. Modified from Hryshko et al. (9).

Fig. 1. The removal of cytoplasmic Ca^{2+} augmented the outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current, and re-application of Ca^{2+} attenuated the current. This Ca^{2+} effect is probably not due to the competitive inhibition of Ca^{2+} to Na^+ because partial proteolysis by chymotrypsin eliminated the effect. The physiological relevance of this unique effect of regulatory Ca^{2+} is not clear. However, this is a striking difference to the other isoforms.

The electrophysiological properties of NCX3, which is mainly expressed in the brain and skeletal muscles, have not yet been extensively studied. Linck et al. (8) studied the functional properties of NCX3 in the inside-out patch excised from BHK cells expressing NCX3, and they reported that overall functions of NCX3 are similar to those of NCX1 except for the regulation by ATP. NCX3 expressed in BHK cells was not activated by cytoplasmic ATP.

It has been difficult to measure the NCX3 exchange current in the giant membrane patch excised from oocytes. We overcame this problem by increasing the amount of cRNA injected by about ten times, compared to our usual amount. Figure 3 shows a typical record of the outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current of NCX3 recorded with a similar experimental protocol as those used in Figs. 1 and 3. Upon the application of cytoplasmic $1 \mu\text{M}$ Ca^{2+} , the $\text{Na}^+/\text{Ca}^{2+}$ exchange current immediately increased, and subsequently decreased within about 5 s. The steady state current was only slightly larger than the current in the absence of Ca^{2+} . Partial proteolysis by trypsin eliminated both the activation and decay of the $\text{Na}^+/\text{Ca}^{2+}$ exchange current (data not shown). These data are quite different from

NCX3

Fig. 3. Outward $\text{Na}^+/\text{Ca}^{2+}$ exchange current of NCX3 recorded in inside-out giant membrane patch.

those of other isoforms. The initial increase by Ca^{2+} can be explained by the recovery of the exchanger from the I_2 inactive state. However, to explain the subsequent decay of $\text{Na}^+/\text{Ca}^{2+}$ exchange current, a novel Ca^{2+} -induced inactivation mechanism must be considered. Namely, in the experimental protocol of Fig. 3, the regulatory Ca^{2+} first activated the NCX3 by relieving the exchanger from the Ca^{2+} free-induced inactive state (I_2 inactivation), then the regulatory Ca^{2+} facilitated the exchanger to enter the Ca^{2+} -induced inactive state. Thus, NCX3 is a unique exchanger that possesses the properties of both Ca^{2+} -induced activation and inactivation.

Concluding remarks

Regulation of NCXs by Ca^{2+} was briefly discussed. NCX1, NCX2, and NCX-SQ1 are positively regulated by Ca^{2+} . CALX is negatively regulated. NCX3 apparently has both positive and negative regulations by Ca^{2+} . The mechanisms of NCX3 regulation by Ca^{2+} are complicated, and the physiological relevance of the dual Ca^{2+} regulation is as yet unknown. Further studies are necessary to clarify the exact mechanisms.

Acknowledgments

I greatly appreciate Professor Akinori Noma for his encouragement and valuable discussion, and I also thank Drs. Hai-Tian Fan, Satomi Kita, Munekazu Shigekawa, and Takahiro Iwamoto for their collaboration. This

work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, as well as a grant from the Vehicle Racing Commemorative Foundation (to S.M.).

References

- Nicoll DA, Longoni S, Philipson KD. Molecular cloning and functional expression of the cardiac sarcolemmal $\text{Na}^+-\text{Ca}^{2+}$ exchanger. *Science*. 1990;250:562–565.
- Li Z, Matsuoka S, Hryshko LV, Nicoll DA, Bersohn MM, Burke EP, et al. Cloning of the NCX2 isoform of the plasma membrane $\text{Na}^+-\text{Ca}^{2+}$ exchanger. *J Biol Chem*. 1994;269:17434–17439.
- Nicoll DA, Quednau BD, Qui Z, Xia YR, Lusis AJ, Philipson KD. Cloning of a third mammalian $\text{Na}^+-\text{Ca}^{2+}$ exchanger, NCX3. *J Biol Chem*. 1996;271:24914–24921.
- He Z, Tong Q, Quednau BD, Philipson KD, Hilgemann DW. Cloning, expression, and characterization of the squid $\text{Na}^+-\text{Ca}^{2+}$ exchanger (NCX-SQ1). *J Gen Physiol*. 1998;111:857–873.
- Schwarz EM, Benzer S. *Calx*, a Na-Ca exchanger gene of *Drosophila melanogaster*. *Proc Natl Acad Sci USA*. 1997;94:10249–10254.
- Hilgemann DW. The giant membrane patch. In: Sakmann B, Neher E. editors. *Single-channel recording*, 2nd ed. New York: Plenum Press; 1995. p. 307–327.
- Matsuoka S, Nicoll DA, Reilly RF, Hilgemann DW, Philipson KD. Initial localization of regulatory regions of the cardiac sarcolemmal $\text{Na}^+-\text{Ca}^{2+}$ exchanger. *Proc Natl Acad Sci USA*. 1993;90:3870–3874.
- Linck B, Qiu Z, He Z, Tong Q, Hilgemann DW, Philipson KD. Functional comparison of the three isoforms of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX1, NCX2, NCX3). *Am J Physiol*. 1998;274:C415–C423.
- Hryshko LV, Matsuoka S, Nicoll DA, Weiss JN, Schwarz EM, Benzer S, et al. Anomalous regulation of the *Drosophila* $\text{Na}^+-\text{Ca}^{2+}$ exchanger by Ca^{2+} . *J Gen Physiol*. 1996;108:67–74.
- Matsuoka S, Nicoll DA, Hryshko LV, Levitsky DO, Weiss JN, Philipson KD. Regulation of the cardiac $\text{Na}^+-\text{Ca}^{2+}$ exchanger by Ca^{2+} . Mutational analysis of the Ca^{2+} -binding domain. *J Gen Physiol*. 1995;105:403–420.
- Fujioka Y, Hiroe K, Matsuoka S. Regulation kinetics of $\text{Na}^+-\text{Ca}^{2+}$ exchange current in guinea-pig ventricular myocytes. *J Physiol (Lond)*. 2000;529:611–623.
- Levitsky DO, Nicoll DA, Philipson KD. Identification of the high affinity Ca^{2+} -binding domain of the cardiac $\text{Na}^+-\text{Ca}^{2+}$ exchanger. *J Biol Chem*. 1994;269:22847–22852.
- Matsuoka S, Nicoll DA, He Z, Philipson KD. Regulation of cardiac $\text{Na}^+-\text{Ca}^{2+}$ exchanger by the endogenous XIP region. *J Gen Physiol*. 1997;109:273–286.
- Dyck C, Omelchenko A, Elias CL, Quednau BD, Philipson KD, Hnatowich M, et al. Ionic regulatory properties of brain and kidney splice variants of the NCX1 $\text{Na}^+-\text{Ca}^{2+}$ exchanger. *J Gen Physiol*. 1999;114:701–711.
- Omelchenko A, Dyck C, Hnatowich M, Buchko J, Nicoll DA, Philipson KD, et al. Functional differences in ionic regulation between alternatively spliced isoforms of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger from *Drosophila melanogaster*. *J Gen Physiol*. 1998;111:691–702.