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Topics on the Na^+/Ca^{2+} Exchanger: Involvement of Na^+/Ca^{2+} Exchanger in the Vasodilator-Induced Vasorelaxation

Junji Nishimura[1,*]

[1]*Division of Molecular Cardiology, Research Institute of Angiocardiology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

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Abstract. Many kinds of vasodilators induce relaxation of the vascular smooth muscle cells (VSMCs) through the production of cyclic AMP (cAMP) or cyclic GMP (cGMP). The relaxant effects mediated by these second messengers are thought to be mainly due to the decrease in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), as well as the decrease in Ca^{2+} sensitivity of the contractile apparatus of VSMCs. To explain the cAMP- or cGMP-mediated decrease in $[Ca^{2+}]_i$, several mechanisms have been proposed, including the inhibition of Ca^{2+} influx due to a hyperpolarization, a stimulation of Ca^{2+} uptake into the intracellular store, and an increase in Ca^{2+} extrusion from VSMCs by stimulation of sarcolemmal Ca^{2+}-pump. VSMCs have two major systems for Ca^{2+} extrusion, namely, sarcolemmal Ca^{2+}-pump and Na^+/Ca^{2+} exchanger (NCX). However, the involvement of NCX in the vasodilator-induced relaxation of VSMCs has not been well established. In this article, the possible involvement of NCX in the vasodilator-induced relaxation of VSMCs will be reviewed.

Keywords: cyclic AMP, cyclic GMP, intracellular Ca^{2+} concentration, Na^+/Ca^{2+} exchanger, vascular smooth muscle

Introduction

Na^+/Ca^{2+} exchanger (NCX) can move Ca^{2+} either into (reverse mode) or out of the cells (forward mode), depending on the electrochemical driving force on the exchanger. It is generally accepted that NCX (both forward mode and reverse mode) plays a crucial role in the excitation-contraction coupling in the cardiac myocyte. However, the role of NCX in the vascular smooth muscle cells (VSMCs) has not been extensively investigated, compared with the cardiac muscle. Previous studies have defined three isoforms of the NCX (NCX1, NCX2, and NCX3) that are coded by distinct genes in mammals (1 – 3). NCX1-specific transcripts are most abundant in the heart, although they are found in many other tissues (3, 4). NCX activity and NCX1 gene expression have been reported in blood vessels (5 – 8) and functional studies indicated that this exchanger plays an important role in the regulation of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in VSMCs (9 – 13). Slaughter et al. (14) reported that the NCX has 3 – 6-fold greater transporting capacity than that of the sarcolemmal Ca^{2+} pump. It is thus not surprising that the activity of the exchanger is regulated in many different ways and extents.

Recently, genetically engineered mouse models for human diseases have been produced and a number of new findings have been accumulating. Concerning the NCX, there are some limitations in using the NCX-knockout mouse, since it has been reported from several laboratories that the NCX-knockout mouse is embryonic lethal (8, 15 – 17). This fetal death induced by the deficiency in NCX expression could easily be supposed to be due to the loss of NCX function in the cardiac muscle because NCX is believed to be more important in cardiac muscle than in VSMCs as mentioned above. However, it has recently been reported that the cardiac-specific knockout of NCX does not lead to embryonic

*Corresponding author. nisimura@molcar.med.kyushu-u.ac.jp
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fetal death (18). Similarly, Cho et al. (19) reported that the transgenic re-expression of NCX1 in the NCX null mutant mouse cardiac myocytes failed to rescue the lethal defects. These authors observed the lack of an organized vasculature in the yolk sacs and a vascular placental labyrinth layer in the NCX null mouse. These results might indicate that NCX functioning in the VSMCs is more important than that functioning in cardiac myocytes, at least, in terms of fetal survival. It has recently been reported that NCX-over-expressing transgenic mice that specifically express NCX1.3 (an NCX1 isoform produced by an alternative splicing of the NCX1 gene) in smooth muscle are hypersensitive to salt intake in terms of inducing hypertension, indicating that salt-sensitive hypertension is triggered by Ca^{2+} entry through NCX1 in vascular smooth muscle (20). It is thus clear that NCX is operating in a reverse mode during the pathogenesis of the salt-sensitive hypertension. However, the role of the forward mode NCX under the normal condition has neither been extensively investigated nor been explored in the genetically engineered mouse models.

Involvement of NCX in the cAMP-mediated decrease in $[Ca^{2+}]_i$

Cyclic AMP (cAMP) induces the relaxation of VSMCs through various mechanisms. However, in this section, we focus on the mechanism underlying the cAMP-mediated decrease in $[Ca^{2+}]_i$, since the brief outline of these mechanisms are described in the abstract section. The cAMP-mediated decrease in $[Ca^{2+}]_i$ is thought to be induced by the inhibition of Ca^{2+} influx due to a hyperpolarization by the stimulation of Ca^{2+}-activated K^+ channels (21), a stimulation of Ca^{2+} uptake into the intracellular stores (22), and an increase in Ca^{2+} extrusion from cells through the sarcolemmal Ca^{2+}-pump (23). As mentioned above, since NCX is playing an important role not only in cardiac muscle but also in VSMCs, it would be natural to consider that the forward mode NCX might be involved in the cAMP-mediated decrease in $[Ca^{2+}]_i$.

In mammalian cells, it has been reported that NCX1 is activated by $[Ca^{2+}]_i$ (24) and external monovalent cations (25) and is inhibited by high cytoplasmic Na^+ concentrations (24, 25), low cytoplasmic pH (26), and adenosine triphosphate (ATP) depletion (27, 28). In addition, the consensus phosphorylation sites have been identified, suggesting that the NCX may be a target for cAMP-dependent protein kinase (PKA) and/or protein kinase C (PKC) (12). However, a variety of conflicting physiological results have been obtained following PKA activation. Mene et al. (29) reported that both basal and vasoconstrictor-stimulated NCX activity in human mesangial cells were acutely inhibited by the cAMP-mediated pathways, including forskolin, dibutyryl cAMP, and receptor stimulation coupled with adenylate cyclase. However, it is also reported that the activity of the neural isoform of the NCX is preferentially increased by PKA activation (30). We have previously reported that isoproterenol increases the activity of the NCX based on the following observations (31): 1) Various types of K^+ channel blockers, even if used in combination, could not completely reverse the isoproterenol-induced decreases in $[Ca^{2+}]_i$ and tension induced by U46619, a thromboxane A_2 analogue, in normal PSS (137.3 mM Na^+). 2) Isoproterenol-induced decrease in $[Ca^{2+}]_i$ became only transient when U46619 was applied in the low Na^+ (25.2 mM) PSS. 3) Although isoproterenol induced a sustained decrease in $[Ca^{2+}]_i$ when the concentration of K^+ was reduced to 30 mM, isoproterenol induced only a transient decrease in $[Ca^{2+}]_i$ when the Na^+ concentration was reduced to 25.2 mM. 4) When Ca^{2+} was substituted with Ba^{2+}, which cannot be extruded by the Ca^{2+} pumps but can be extruded through NCX (32 – 35), isoproterenol decreased $[Ba^{2+}]_i$ in the presence of high Na^+ (137.3 mM), while isoproterenol did not decrease $[Ba^{2+}]_i$ in the presence of low Na^+ (25.2 mM). 5) An NCX inhibitor, 2,4-DCB (2',4'-dichlorobenzamil), inhibited the isoproterenol-induced relaxation. 6) Ouabain, an inhibitor of Na^+/K^+ ATPase, had only a partial effect on the isoproterenol-induced decrease in $[Ca^{2+}]_i$. These results indicated that isoproterenol decreases $[Ca^{2+}]_i$ and tension via activation of NCX, which is functionally expressed in porcine coronary arterial smooth muscle.

Concerning the site of action at which isoproterenol induces the Ca^{2+} extrusion through NCX, it is possible that isoproterenol activates Na^+-K^+ ATPase to induce activation of NCX by increasing the electrochemical driving force. The sarcolemmal Na^+-K^+ ATPase has been implicated in the mechanism of β-adrenoceptor agonist-induced relaxation of airway and vascular smooth muscle (36, 37). Stimulation of the enzymatic activity of Na^+-K^+ ATPase by cAMP may lead to generation of the Na gradient necessary to exclude Ca^{2+} via the NCX or hyperpolarization of the membrane, which in turn reduces Ca^{2+} influx through membrane potential-dependent Ca^{2+} channels (38). However, the present results could not be explained by this mechanism alone because ouabain, a specific inhibitor of Na^+-K^+ ATPase, failed to completely inhibit the isoproterenol-induced decrease in $[Ca^{2+}]_i$. Figure 1 illustrates the mechanism by which the cAMP-mediated pathway activates NCX and reduces $[Ca^{2+}]_i$. In addition, if this hypothesis is correct, we considered that the decrease in

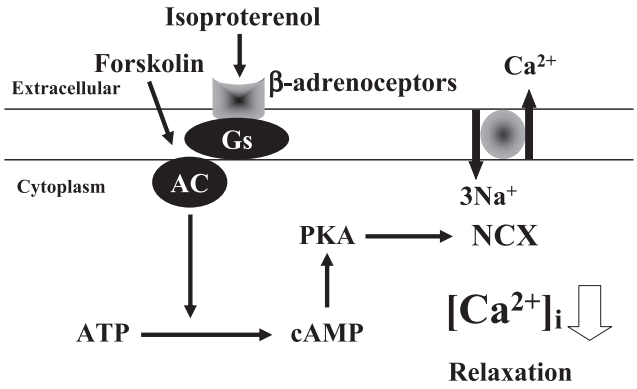

Fig. 1. The activation of NCX by the cAMP-mediated pathway. AC, adenylate cyclase; Gs, GTP binding protein *α*s; PKA, cAMP-dependent protein kinase.

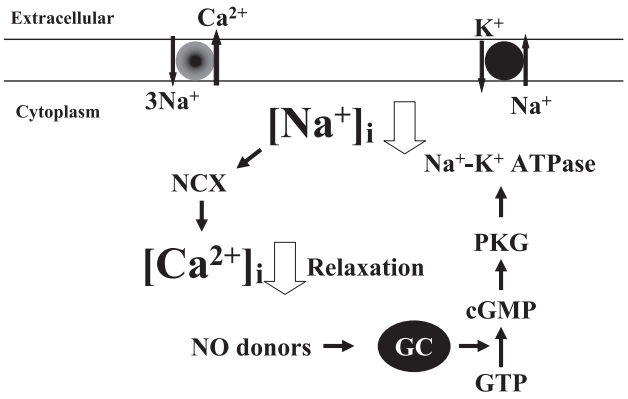

Fig. 2. The activation of NCX by the cGMP-mediated pathway. GC, guanylate cyclase; NO donors, nitric oxide donors; PKG, cGMP-dependent protein kinase.

$[Ca^{2+}]_i$ induced by the cAMP-mediated pathway should be enhanced in the NCX-over-expressing transgenic mice that specifically express NCX1.3 in smooth muscle. We are now investigating this in order to further confirm this hypothesis and already obtained positive data that shows that forskolin (an activator of adenylate cyclase)-induced decrease in $[Ca^{2+}]_i$ is enhanced in the NCX-over-expressing transgenic mice (manuscript in preparation).

Involvement of NCX in the cGMP-mediated $[Ca^{2+}]_i$ decrease

Nitric oxide (NO) donors such as nitroglycerin and isosorbide dinitrate (ISDN) have been commonly used in the treatment of the coronary artery disease. However, the mechanism underlying the NO donor-induced relaxation of the VSMCs is not fully understood. NO donors induce a relaxation of VSMCs mainly through the activation of the soluble guanylate cyclase and subsequent increases in cyclic GMP (cGMP) levels (39 – 41), although cGMP-independent mechanisms have also been reported (42, 43). The relaxation mediated by cGMP also involves a decrease in $[Ca^{2+}]_i$ (44) due to the activation of the sarcoendoplasmic reticulum Ca^{2+}-ATPase (45, 46), the plasma membrane Ca^{2+}-ATPase (39), the Na^+-K^+-ATPase (47 – 50), and various potassium channels (51 – 54). Potassium channel activation hyperpolarizes the cell membrane and inhibits the activity of L-type Ca^{2+} channels (55). In addition, a direct reduction of the sensitivity of the contractile apparatus to Ca^{2+} also mediates cGMP-induced relaxation (56).

Since both cAMP- and cGMP-induced vasorelaxations are accompanied by the reductions of $[Ca^{2+}]_i$ and Ca^{2+} sensitivity, it can be speculated that cGMP-induced decrease in $[Ca^{2+}]_i$ might also require the activation of NCX. Although the manuscript is in preparation, we obtained the similar results as in the case of the cAMP-mediated pathways. The ISDN-induced decreases in $[Ca^{2+}]_i$ and tension were significantly inhibited in low Na^+ PSS or by 2,4-DCB, an inhibitor of NCX. Another inhibitor of NCX, KB-R7943, also significantly inhibited ISDN-induced relaxation. The ISDN decreased $[Ba^{2+}]_i$ in normal concentrations of Na^+, while ISDN did not decrease $[Ba^{2+}]_i$ in low Na^+ PSS. These results are almost the same as those obtained by using isoproterenol as a stimulant (31). However, the major difference between the cAMP-mediated pathway and the cGMP-mediated was the effect of ouabain, a selective inhibitor of Na^+-K^+ ATPase. A large part of the ISDN-induced decreases in $[Ca^{2+}]_i$ and tension was inhibited by ouabain, while isoproterenol-induced decreases in $[Ca^{2+}]_i$ and tension could be only be partially inhibited by ouabain. These results indicated that the NCX plays a role in cGMP-mediated decreases in $[Ca^{2+}]_i$ and tension. However, the primary site of action for the cGMP-mediated pathway was considered to be activation of Na^+-K^+ ATPase. In support of this notion, it has been reported that Na^+-K^+ ATPase in the plasma membrane is activated by cGMP through cGMP-dependent protein kinase (PKG) (47 – 51, 57, 58). From these results, we considered that cGMP-mediated activation of PKG, due to increased cGMP production by the soluble guanylate cyclase, activated Na^+-K^+ ATPase, which decreased $[Na^+]_i$, thus increasing the Na^+ gradient across the plasma membrane. The increased Na^+ gradient would enhance the Ca^{2+} extrusion via the NCX to decrease $[Ca^{2+}]_i$, as shown in Fig. 2. Taken together, it is proposed that NCX is involved in the vasodilator-induced decrease in $[Ca^{2+}]_i$ and tension in both cAMP- (direct activation of NCX) and cGMP- (indirect activation of

NCX) mediated pathways. However, it should be noted that the activation of NCX by the cAMP- or cGMP-mediated pathways may not be a major mechanism for the cAMP- or cGMP-mediated vasorelaxation. Other mechanisms, especially the decrease in Ca^{2+} sensitivity, are also playing an important role in the vasodilator-induced vasorelaxation.

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