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Physiological Mini-Reviews

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Na$^+$/H$^+$ EXCHANGER AND MYOCARDIAL GROWTH.

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1. INTRODUCTION

The stretching of a papillary muscle induces a sudden and immediate rise in force **(Figure 1A: from points a to b)**, due to an augmentation in myofilament Ca^{2+} responsiveness. During the next 10 to 15 minutes a progressive increase in force develops known as the "slow force response" (SFR), that is due to a progressive increase in Ca^{2+} transient amplitude **(Figure 1B)**. The source for this increase in Ca^{2+} transient remained obscure until we proposed a link between Ca^{2+} influx mediated by Na$^+$/ Ca^{2+} (NCX) exchange in reverse mode and the activation of the Na$^+$/H$^+$ exchanger (NHE-1) caused by stretch;[1-4] being the increase in the Ca^{2+} transient secondary to the increase in intracellular Na$^+$ concentration ([Na$^+$]$_i$) **(Figure 1D "control")**. It is known that the increase in [Na$^+$]$_i$ can induce an increase in intracellular Ca^{2+} levels ([Ca^{2+}]$_i$) through the NCX either as a result of a decrease in Ca^{2+} efflux (decreased forward mode) or an increase in Ca^{2+} entry (increased reverse mode). The fact that after myocardial stretch there is no increase in diastolic [Ca^{2+}]$_i$[1,5] as would be expected for a decrease in Ca^{2+} efflux, suggests that the reverse mode of NCX is the mechanism involved in the increase in Ca^{2+} transient.[1]The SFR, the increase in [Na$^+$]$_i$ and the increase in Ca^{2+} transient can be abolished by blocking the Angiotensin II (Ang II) AT$_1$ receptors with losartan **(Figure 1C and 1D)**; the endothelin (ET) ET$_A$ receptors by BQ123 and by inhibition of the NHE-1.[6]
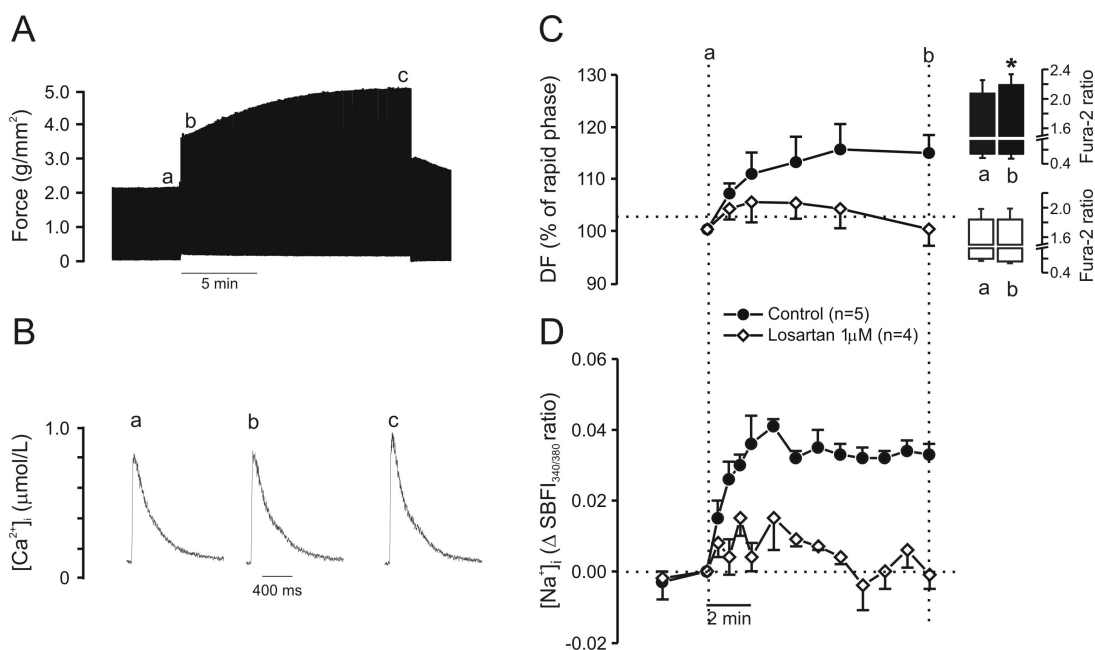

Figure 1. *Reproduced from News Physiol Sci 16:88-91, 2001 (used with permission of the American Physiological Society).*

2. NHE-1 AND SFR AFTER MYOCARDIAL STRETCH

A schematic representation of our proposed chain of events that follows myocardial stretch is presented in **Figure 2**. Endogenous Ang II is released from the myocytes activating AT_1 receptors in an autocrine fashion and inducing the release/formation of ET, which will simultaneously activate the NHE-1 and the anion exchanger (AE). The stimulation of the AE prevents the expected increase in pH_i by NHE-1 activation, but does not prevent the increase in $[Na^+]_i$. The increase in $[Na^+]_i$ drives the NCX in the reverse mode determining the increase in Ca^{2+} transient amplitude. A direct $[Na^+]_i$-independent stimulation of the NCX could also contribute to the increase in force when the NCX is already driven in reverse mode. Although we propose the stretch-induced mechanism as autocrine, we cannot rule out the possibility of endothelial cells or fibroblasts, contributing in a paracrine fashion.
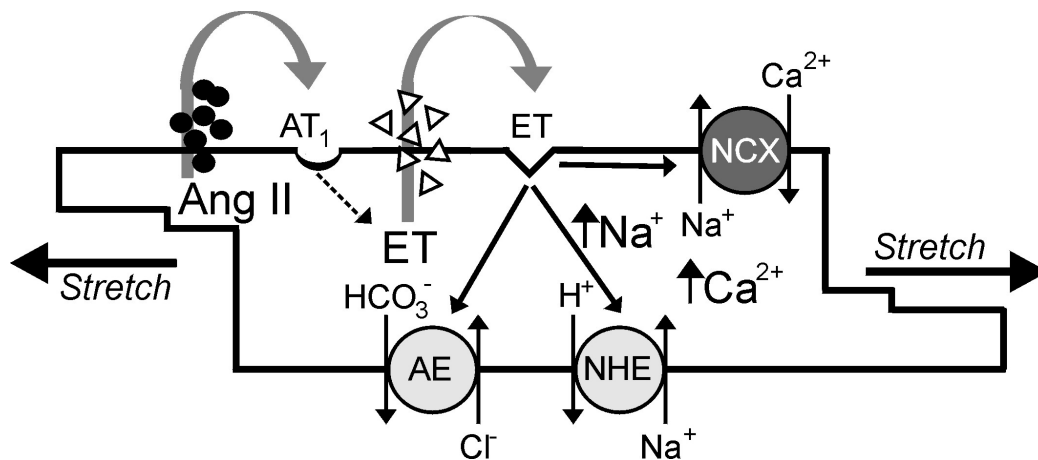

Figure 2. *Schematic representation of the proposed cascade of events following myocardial stretch. Modified from News Physiol Sci 16:88-91, 2001.*

3. NHE-1 AND MYOCARDIAL GROWTH

The NHE-1 is an integral membrane protein ubiquitously expressed in mammalian cells that electroneutrally exchange one intracellular H^+ for one extracellular Na^+ regulating intracellular pH (pH_i). This transport is sensitive to inhibition by amiloride or newer inhibitors[7-9] and also to competitive-inhibition by lithium.[10] **Figure 3** depicts a topological model of the NHE-1.[11] This exchanger is an 815 amino acid protein with a predicted molecular mass of 85kDa that can be separated into an N-terminal membrane associated domain and a long C-terminal tail, with the N- and C- terminal domains being cytoplasmic. The membrane domain consists of ~500 amino acids with 12 trans-membrane regions (TM) and 3 associated loops: intracellular loop 2 (IL2) between TM3 and TM4, IL4 between TM8 and TM9, and EL5 between TM9 and TM10[11] and it is associated with the ion transport across the plasma membrane.[7]

The NHE-1 cytoplasmic tail can be split into a number of sub-domains that are individually involved in various systems employed to regulate the exchanger activity **(Fig. 3)**. The first sub-domain includes two putative PIP2 binding sites between amino acids 513-564 involved in ATP regulation of NHE-1.[12] Although the exchanger does not directly use ATP, ATP depletion significantly decrease transport activity.[12] It also contains the

binding site for the ezrin, radixin, and moesin (ERM) family of proteins. Through a direct interaction with the ERM proteins and an indirect interaction with actin filaments via the ERM, NHE-1 is involved in maintaining the structural integrity of the cell as well as in cell migration.[13,14] Amino acids 515-530 contain the binding site for the calcineurin B homologous protein (CHP1),[15] an essential cofactor for NHE-1.[15]

The second sub-domain (amino acids 636-684) contains a high affinity (amino acids 636-656) and a low affinity (amino acids 657-684) calmodulin (CaM) binding sites.[16] The high affinity binding site functions as an "autoinhibitory domain" that binds Ca^{2+}-bound CaM and allows activation of the exchanger. Removal of the CaM high-affinity binding site results in a constitutively active NHE-1.[17]

The third sub-domain (amino acids ~700-815) contains a number of serine and threonine residues that are phosphorylated by protein kinases in response to hormone and growth factor stimulation as well as sustained acidosis.[18-20] The mitogen-activated protein kinases (MAPK) pathway is a major factor in myocardial NHE-1 regulation with ERK1/2 directly phosphorylating the C-terminal tail of the exchanger[21] as well as activating p90rsk, another NHE-1 kinase.[22] PKC and PKD are also able to influence NHE-1 activity in response to growth factor and hormone stimulation but they are not believed to directly phosphorylate the exchanger.[23,24]
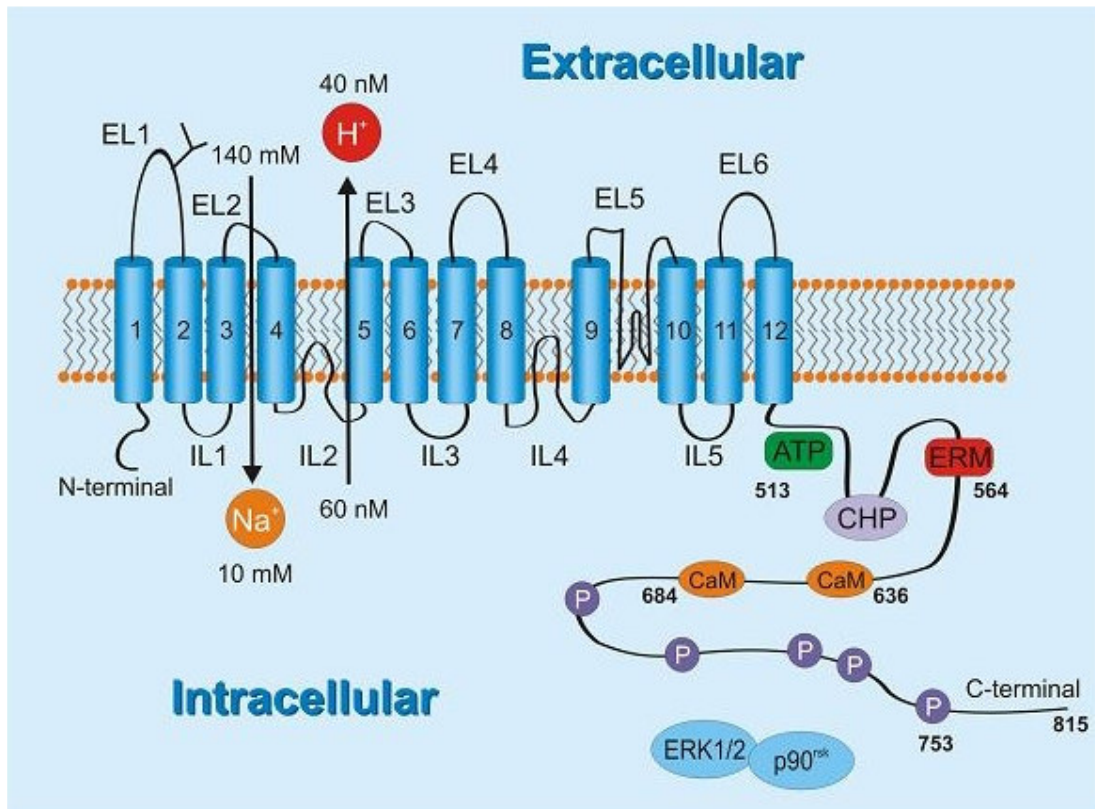

Figure 3. *Schematic Topology model of Na+/H+ exchanger isoform 1 (Wakabayashi) and regulatory elements. EL1-EL6: Extracellular loop 1-6; IL1-IL5: Intracellular loop 1-5; PIP2: Phosphatidylinositol bisphosphate; CHP: Calcineurin homologous protein; ERM: Ezrin/Radixin/Moesin proteins; CaM: Calmodulin.*

The possible link between the SFR, activation of NHE-1 and myocardial hypertrophy is supported by the fact that the specific blockade of the NHE-1 was shown to effectively regress cardiac hypertrophy in several experimental models.[25-29] The increase in $[Ca^{2+}]_i$ is a well recognized hypertrophic signal, acting through calcineurin, CaMKII or other kinase cascades.

We would like to emphasize that we do not propose the scheme depicted in **Figure 2** as the only mechanism leading to myocardial hypertrophy. Different intracellular pathways other than that involving the NHE-1 can lead to increases in $[Ca^{2+}]_i$ and hypertrophy.

4. NHE-1 INHIBITION AND REGRESSION OF CARDIAC HYPERTROPHY

The enhanced activity of the NHE-1 in the hypertensive hypertrophied myocardium of the SHR, was reported by us in 1995 and it was hypothesized that its inhibition may regress and/or prevent the development of hypertrophy.[30] The possible link between NHE-1 activity and hypertrophy was discussed in a recent review.[31] The hypertensive hypertrophy is a pressure overload model of hypertrophy characterized by concentric hypertrophy, fibrosis and expression of fetal genes. After myocardial infarction, myocardial hypertrophy develops as a consequence of volume overload of the surviving myocytes. Kusumoto et al. in 2001 proved that the specific NHE-1 inhibition with cariporide decreased hypertrophy and remodeling post-myocardial infarction.[29] Camilión de Hurtado et al reported in 2002 that the myocardial hypertophy of the SHR regressed with cariporide treatment.[25] The relationship between normalization of NHE-1 activity and regression of hypertrophy was reported after chronic treatment with losartan, enalapril or nifedipine,[32] and also by chronic cariporide treatment.[28] The latter study also showed the interesting fact that depending on the pharmacological intervention used, the decrease in hypertrophy was independent of the decrease in arterial pressure. Another feature of the hypertensive cardiac hypertrophy is enhancement of interstitial fibrosis, which was regressed by chronic blockade of NHE-1[33] although this effect took longer time to occur than the regression of myocyte hypertrophy, possibly as a reflection of the low turn-over rate of collagen metabolism or due to an effect related to apoptosis.[3,34] Also, the myocyte hypertrophy and the fibrosis of the myocardial hypertrophy induced by β–adrenergic stimulation was prevented by NHE-1 inhibition.[26,27]

The link between NHE-1 activity and cardiac hypertrophy has been established also for several neurohormonal models other than hypertension. In 2003, we reported that isoproterenol-induced cardiac hypertrophy was prevented by the co-administration of a NHE-1 inhibitor.[27] Hypertrophied hyperthyroid hearts have increased NHE-1 activity[35] and since NHE activity can be modulated by parathyroid hormone it is tempting to speculate about the possible involvement of the antiporter in this type of cardiac hypertrophy. Aldosterone induces hypertrophy and MAPK cascade activation accompanied by up-regulation of NHE-1 in neonatal rat cardiomyocytes.[28] Interestingly, in these experiments, treatment with a NHE-1 inhibitor blunted the up-regulation of the antiporter and cardiomyocyte hypertrophy although MAPK activity remained elevated.[28]

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XXII CONGRESO LATINOAMERICANO Y 1ER IBERO-AMERICANO DE CIENCIAS FISIOLÓGICAS

Organizado por la Sociedad Argentina de Fisiología, por decisión de la Asociación Latinoamericana de Ciencias Fisiológicas (ALACF) y con el auspicio de la Sociedad Española de Ciencias Fisiológicas
Buenos Aires, 4 al 7 de noviembre de 2006

El año próximo tendrá lugar en Buenos Aires el XXII Congreso de la Asociación Latinoamericana de Ciencias Fisiológicas (ALACF). Esta reunión congregará a científicos originarios de América Latina trabajando en sus países de origen, en Estados Unidos, en Europa y alrededor del mundo. Fisiólogos no latinoamericanos de primer nivel son también regularmente invitados. Esta vez la Sociedad Española de Ciencias Fisiológicas se asocia al evento, dándole especial interés y relevancia.

El objetivo central del Congreso es dar, a los fisiólogos trabajando y viviendo en Latinoamérica, la posibilidad de entrar en contacto con referentes en su campo de trabajo. Esto será especialmente cierto esta vez para aquellos radicados en el Cono Sur del continente (Bolivia, Brasil, Chile, Paraguay, Uruguay y Argentina).

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La estructura de base del Programa será la siguiente:

Horario	Sábado	Domingo	Lunes	Martes
08.30-10.30		Simposios	Simposios	Simposios
10.30-11.00		Pausa Café	Pausa Café	Pausa Café
11.00-12.00	Inscripción	Conferencias	Conferencias	Conferencias
12.00-13.00	Almuerzo	Almuerzo	Almuerzo	Almuerzo
13.00-15.30	Inscripción	Pres. Carteles	Pres. Carteles	Pres. Carteles
15.30-17.30	Simposios	Simposios	Simposios	Simposios
17.30-18.00		Pausa Café	Pausa Café	Pausa Café
18.00-19.00	Conferencias	Conferencias	Conferencias	Conferencias
19.30-21.00	Encuentro (get together)	Asamblea ALACF	Asamblea SAFIS	
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