Biochemical and Biophysical Research Communications 460 (2015) 50–52

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Review

Standing of giants shoulders the story of the mitochondrial Na^+Ca^{2+} exchanger

Israel Sekler

Ben Gurion University of the Negev, Israel

ARTICLE INFO

Article history:
Received 23 February 2015
Available online 18 May 2015

Keywords:
NCLX
MCU
Mitochondrial Ca^{2+} signaling
Na^+ signaling

ABSTRACT

It is now the 40th anniversary of the Journal of Molecular and Cellular Cardiology paper by Ernesto Carafoli and colleagues. This seminal study described for the first time mitochondrial Ca^{2+} extrusion and its coupling to Na^+. This short review will describe the profound impact that this work had on mitochondrial signaling and the cross talk between the mitochondria, the ER, and the plasma membrane. It will further tell how the functional identification and in particular its unique cation selectivity to both Li^+ and Na^+ eventually contributed to the identification of the mitochondrial Na^+/Ca^{2+} exchanger gene NCLX many years later. The last part will describe how molecular tools derived from NCLX identification are used to study the novel physiological aspects of Ca^{2+} signaling.

1. Introduction

In the chase today for publishing in high impact journals, the importance of groundbreaking studies is not immediately recognized, particularly when published in main stream journals. The Journal of Molecular and Cellular Cardiology paper of Ernesto Carafoli et al. that now celebrates 40 is a fine example of such fundamental and groundbreaking work that changed our view on mitochondrial Ca^{2+} signaling and is in the focus of our mini review.

2. Functional and molecular identification of the mitochondrial Na^+/Ca^{2+} exchanger

I will begin our story in the early 2000, when we functionally identified a Na^+/Zn^{2+} exchanger that could extrude Zn^{2+} out of epithelia and neurons by using the transmembrane Na^+ gradient [1]. My PhD student at the time, R. Palty reasoned that this unknown transporter may be related to the Na^+/Ca^{2+} exchanger superfamily because of its monovalent/divalent cation activity and decided to clone it by screening for a novel Na^+/Ca^{2+} exchanger member. He discovered a potentially new member of the Na^+/Ca^{2+} exchanger superfamily that was then called the unpretentious name FLJ22233. To our big disappointment at the time it did not mediate Na^+/Zn^{2+}, but instead Na^+/Ca^{2+} exchange [2]. The same gene was cloned and thought to be a member of the K^+-dependent Na^+/Ca^{2+} exchanger subtype of the Na^+/Ca^{2+} exchanger superfamily that was previously termed NCKX6 [3,4]. Subsequent phylogenetic analysis found, however, that it is the sole member of the CAX subgroup of the Na^+/Ca^{2+} exchanger superfamily. Indeed our studies indicated that it carried a K^+-independent Na^+/Ca^{2+} exchange [2].

Most of the Na^+/Ca^{2+} exchange members can distinguish between these monovalent cations and are inert to Li^+. Thus, replacing Na^+ by Li^+ is expected to cause reversal of the exchange, triggering Ca^{+2} influx instead of efflux. To our big surprise, the exchange mediated by FLJ22233 was not reversed, but Li^+ effectively supported extrusion of Ca^{2+} at a rate that was only slightly smaller than that supported by Na^+ [2]. We were very puzzled by this unusual finding and uncertain about its physiological significance. We initially thought that this unique ability to transport Li^+ by this exchanger, that we have now termed NCLX (standing for $Na^+/Li^+/Ca^{2+}$ exchanger), may be relevant to the bipolar syndrome. The apparent affinity of NCLX for Li^+ was however relatively low ~ 8 mM [2], a value that is much bigger than the maximal 0.5 mM reached during clinical courses [5]. We soon however came across the 1974 landmark study of Ernesto Carafoli et al.(6). This study was published during the time when our understanding of the functional mechanisms of transporters was minimal, and our knowledge about their molecular identity was null. Ernesto Carafoli showed in the 1974 paper that there is a rapid efflux of Ca^{2+} from cardiac mitochondria, which is strictly Na^+ dependent, and mitochondrial Ca^{2+} efflux was halted when Na^+ was omitted [6]. Intriguingly, the Na^+ affinity was very similar to that of NCLX. But what really rang a bell was the finding that Li^+ could replace Na^+ in promoting mitochondrial Ca^{2+} efflux, while other monovalent cations such as K^+ failed to do so. Remarkably, the rate at which Li^+ supported Ca^{2+} efflux was slightly lower compared to Na^+. The similarity between the kinetic properties of the mitochondrial exchanger and NCLX

http://dx.doi.org/10.1016/j.bbrc.2015.02.170
0006-291X/

indicated that NCLX and the mitochondrial exchanger are related to the same transporter. The evidence for that will be described toward the end of this short review. What is, however; also remarkable is that, despite the fact that Ernesto Carafoli's paper was published 40 years ago, the sophistication of the experimental approach that combined in vivo administration of isotopic Ca^{2+} followed by analysis of isolated mitochondria, is a highly demanding system even in today's standard. This landmark study by Ernesto Carafoli and colleagues was followed by many subsequent papers that studied the regulatory mode of this exchanger. Hence, the mitochondrial exchanger was found not only to transport cations but also to be allosterically regulated by pH and cations. For example, several studies indicated that its activity can be potentiated by K^+ [7]. There was also indication for strong regulatory allosteric interactions of H^+/Ca^{2+} [8]. Studies conducted on isolated mitochondria found that Ca^{2+} interacted with a domain at the mitochondrial inter-membrane space, triggering a strong allosteric inhibition of the exchanger [9]. Another intriguing observation was the stimulatory effect of cAMP agonists on the Na^+-dependent mitochondrial Ca^{2+} efflux in brown fat as well as other tissues [10,11].

The hypothesis that mitochondria control global Ca^{2+} signaling was however abandoned primarily because of the apparently low affinity for Ca^{2+} uptake observed in isolated mitochondria, raising doubts regarding the physiological relevance of this process [12]. In addition, the discovery of the ER as the major Ca^{2+} signaling hub shifted the attention toward this compartment.

The revival of the mitochondrial Ca^{2+} signaling role came by the ground breaking work of R. Rizzuto and T. Pozzan, who, by using mitochondrial Ca^{2+} reporters in situ, were the first to show that mitochondrial Ca^{2+} shuttling is a frequent and physiologically relevant event. Subsequent studies further showed an extensive cross talk between the mitochondria, the ER and the plasma membrane in controlling Ca^{2+} [13]).

Ironically, while these ground breaking developments promoted our studies in intact cells and tissues, they complicated our studies on the role of the exchanger. they could not offer a precise control on the cytosolic and mitochondrial exchange by its substrates Na^+ and Ca^{2+}. To overcome this barrier, cell-permeable inhibitors of the exchanger were added to the arsenal. The first were benzodiiazepine type Ca^{2+} channel blockers that, while effective in blocking the exchanger, were rather non-selective interacting with many other Ca^{2+} channels [14,15]. The next generation drug was CGP-37157, which has a 10-fold higher affinity and apparently a better selectivity toward other Na^+/Ca^{2+} exchanger members [16]. Subsequent studies, however, indicated that CGP-37157 may interact with other Ca^{2+} conducting pathways. For example, studies conducted on pancreatic beta cells suggested that, by raising mitochondrial Ca^{2+}, blocking the mitochondrial exchanger can increase metabolic rate and ATP production, thereby promoting insulin secretion [17]. However, later studies have indicated that CGP-37157 blocks LTCC, a major player in insulin secretion [18]. Similarly, other studies have shown that CGP-37157 may also modulate the activity of other transporters, among them the Ca^{2+} pump and the ryanodine receptor [19]. Recent studies further suggest that the neuronal rescue effect attributed to prevention of reversal of the mitochondrial Na^+/Ca^{2+} exchanger is in fact related to LTCC block [20]. Thus, a selective molecular tool was required for studying the role of the exchanger. A promising step toward this goal was made by the group of K.D. Garlid, which purified a 60 and 110 KD mitochondrial polypeptide that upon reconstitution carried a benzodiazepine sensitive $Na^+/Li^+/Ca^{2+}$ exchange. This lead was however not followed by cloning of the mitochondrial exchanger, although it provided important biochemical support for a link to the NCLX when it was later cloned [21].

Now we can return to the molecular identification of the mitochondrial exchanger NCLX. As discussed above, the kinetic properties of the mitochondrial exchanger discovered by Ernesto Carafoli were remarkably similar to those of NCLX, prompting us to determine if they are the same molecular species. We initially performed immuno-histochemical and immunoblotting analysis and found that NCLX was primarily targeted to the mitochondrial inner membrane. NCLX also shared a similar molecular weight of 60 and 110 KD, as reported by Garlid, representing the monomeric and dimeric appearance of NCLX in SDS gels, respectively [22]. Silencing of NCLX expression, or the expression of NCLX mutated at its catalytic domain, blocked mitochondrial Ca^{2+} efflux. Finally, a set of experiments carried out on digitonin-permeabilized cells reproduced the 40-year old Ernesto Carafoli's findings, showing that: 1) Ca^{2+} efflux by NCLX is dependent on Na^+, and 2) Li^+ could replace Na^+ in promoting Ca^{2+} efflux by NCLX, but at a slightly lower rate [22]. Thus, the localization of NCLX to the plasma membrane induced by the heavy overexpression in HEK cells met the land-mark paper of Ernesto Carafoli et al. and culminated in NCLX identification.

Another important predication that Ernesto Carafoli made in the 1974 paper (6) was that the affinity of the mitochondrial Na^+/Ca^{2+} exchanger responds to changes in cytosolic Na^+ elicited by physiological stimuli, and the last part of this minireview is devoted to this aspect of NCLX activity. We decided to focus on pancreatic beta-cells, because of the central role of cross-talk between the mitochondria and the plasma membrane in metabolic and Ca^{2+} signaling linked to insulin secretion. Furthermore, the density of voltage-gated Na^+ channels on these cells is high, but their physiological role is still poorly understood. Using TTX, a toxin selectively blocking these channels, we found that application of high glucose was followed, not only by strong cytosolic Ca^{2+} signaling, but also by a robust Na^+ rise. This cytosolic Na^+ was rapidly propagating into the mitochondria. When expression of NCLX was knocked down, mitochondrial Na^+ influx was diminished, indicating that the exchanger functions not only as mitochondrial Ca^{2+} extruder, but is also the major Na^+ uptake pathway into the mitochondria. Such cross-talk of Na^+ signaling between the plasma membrane and the mitochondria serves multiple purposes. By controlling the activity of NCLX, it paces the duration of the mitochondrial Ca^{2+} transients. It further controls the duration and magnitude of cytosolic and mitochondrial Na^+ transients [23]. This latter aspect lags behind the current focus on Ca^{2+} signaling, primarily because of the inferior quality of Na^+ reporters but is of major importance and warrants many more studies.

Having a molecular handle for studying the role of the mitochondrial exchanger opens the way to address several key questions, among them 1) the mode of NCLX regulation. Other members of the NCX superfamily have an allosteric Ca^{2+}-binding site in their regulatory domain, which is critical for their regulation by cytosolic Ca^{2+}. NCLX does not share this domain, however previous studies indicated that it is strongly regulated by the Parkinson-related kinase, Pink-1 [24]. Analysis of the NCLX sequence does indeed reveal several potential phosphorylation sites, and their molecular analysis can now be combined with functional analysis of NCLX activity to determine NCLX regulation in health and disease. 2) The NCLX partner, MCU forms complexes with several proteins, such as MICU1 and EMRE, which are essential for its affinity for Ca^{2+} and activity [25,26]. Furthermore, considering the complementary role of NCLX in mitochondrial Ca^{2+} shuttling it is intriguing to determine if NCLX is a physical component of this mitochondrial Ca^{2+} signalosome. 3) Finally, the generation of conditional NCLX knockdown strains will advance our knowledge of the link between mitochondrial Na^+ and Ca^{2+} signaling and major physiological processes in health and disease.

Conflict of interest

None.

References

[1] E. Ohana, D. Segal, R. Palty, D. Ton-That, A. Moran, S.L. Sensi, J.H. Weiss, M. Hershfinkel, I. Sekler, A sodium zinc exchange mechanism is mediating extrusion of zinc in mammalian cells, J. Biol. Chem. 279 (2004) 4278–4284.

[2] R. Palty, E. Ohana, M. Hershfinkel, M. Volokita, V. Elgazar, O. Beharier, W.F. Silverman, M. Argaman, I. Sekler, Lithium-calcium exchange is mediated by a distinct potassium-independent sodium-calcium exchanger, J. Biol. Chem. 279 (2004) 25234–25240.

[3] X. Cai, J. Lytton, The cation/Ca^{2+} exchanger superfamily: phylogenetic analysis and structural implications, Mol. Biol. E 21 (2004) 1692–1703.

[4] X. Cai, J. Lytton, Molecular cloning of a sixth member of the K^+-dependent Na^+/Ca^{2+} exchanger gene family, NCKX6, J. Biol. Chem. 279 (2004) 5867–5876.

[5] P. Sienaert, I. Geeraerts, S. Wyckaert, How to initiate lithium therapy: a systematic review of dose estimation and level prediction methods, J. Affect Disord. 146 (2013) 15–33.

[6] E. Carafoli, R. Tiozzo, G. Lugli, F. Crovetti, C. Kratzing, The release of calcium from heart mitochondria by sodium, J. Mol. Cell. Cardiol. 6 (1974) 361–371.

[7] M. Crompton, I. Heid, E. Carafoli, The activation by potassium of the sodium–calcium carrier of cardiac mitochondria, FEBS Lett. 115 (1980) 257–259.

[8] K. Baysal, G.P. Brierley, S. Novgorodov, D.W. Jung, Regulation of the mitochondrial Na^+/Ca^{2+} antiport by matrix pH, Arch. Biochem. Biophys. 291 (1991) 383–389.

[9] L.H. Hayat, M. Crompton, Ca^{2+}-dependent inhibition by trifluoperazine of the Na^+-Ca^{2+} carrier in mitoplasts derived from heart mitochondria, FEBS Lett. 182 (1985) 281–286.

[10] T.P. Goldstone, M. Crompton, Evidence for beta-adrenergic activation of Na^+-dependent efflux of Ca^{2+} from isolated liver mitochondria, Biochem. J. 204 (1982) 369–371.

[11] M.H. Al-Shaikhaly, J. Nedergaard, B. Cannon, Sodium-induced calcium release from mitochondria in brown adipose tissue, Proc. Natl. Acad. Sci. U. S. A. 76 (1979) 2350–2353.

[12] E. Carafoli, The fateful encounter of mitochondria with calcium: how did it happen? Biochim. Biophys. Acta 1797 (2010) 595–606.

[13] R. Rizzuto, M. Brini, M. Murgia, T. Pozzan, Microdomains with high Ca^{2+} close to IP3sensitive channels that are sensed by neighboring mitochondria, Science 262 (1993) 744–747.

[14] B. Koidl, N. Miyawaki, H.A. Tritthart, A novel benzothiazine Ca^{2+} channel antagonist, semotiadil, inhibits cardiac L-type Ca^{2+} currents, Eur. J. Pharmacol. 322 (1997) 243247.

[15] K.W. Kinnally, D.B. Zorov, Y.N. Antonenko, S.H. Snyder, M.W. McEnery, H. Tedeschi, Mitochondrial benzodiazepine receptor linked to inner membrane ion channels by nanomolar actions of ligands, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 1374–1378.

[16] M.A. Matlib, S.W. Lee, A. Depover, A. Schwartz, A specific inhibitory action of certain benzothiazepines and benzodiazepines on the sodium-calcium exchange process of heart and brain mitochondria, Eur. J. Pharmacol. 89 (1983) 327–328.

[17] B. Lee, P.D. Miles, L. Vargas, P. Luan, S. Glasco, Y. Kushnareva, E.S. Kornbrust, K.A. Grako, C.B. Wollheim, P. Maechler, J.M. Olefsky, C.M. Anderson, Inhibition of mitochondrial Na^+-Ca^{2+} exchanger increases mitochondrial metabolism and potentiates glucose-stimulated insulin secretion in rat pancreatic islets, Diabetes 52 (2003) 965–973.

[18] D.S. Luciani, P. Ao, X. Hu, G.L. Warnock, J.D. Johnson, Voltage-gated Ca^{2+} influx and insulin secretion in human and mouse beta-cells are impaired by the mitochondrial Na^+/Ca^{2+} exchange inhibitor CGP-37157, Eur. J. Pharmacol. 576 (2007) 18–25.

[19] J.T. Neumann, P.L. Diaz-Sylvester, S. Fleischer, J.A. Copello, CGP-37157 inhibits the sarcoplasmic reticulum Ca^{2+} ATPase and activates ryanodine receptor channels in striated muscle, Mol. Pharmacol. 79 (2011) 141–147.

[20] A. Ruiz, E. Alberdi, C. Matute, CGP37157, an inhibitor of the mitochondrial Na^+/Ca^{2+} exchanger, protects neurons from excitotoxicity by blocking voltage-gated Ca^{2+} channels, Cell. Death Dis. 5 (2014) e1156.

[21] W. Li, Z. Shariat-Madar, M. Powers, X. Sun, R.D. Lane, K.D. Garlid, Reconstitution, identification, purification, and immunological characterization of the 110-kDa Na^+/Ca^{2+} antiporter from beef heart mitochondria, J. Biol. Chem. 267 (1992) 1798317989.

[22] R. Palty, W.F. Silverman, M. Hershfinkel, T. Caporale, S.L. Sensi, J. Parnis, C. Nolte, D. Fishman, V. Shoshan-Barmatz, S. Herrmann, D. Khananshvili, I. Sekler, NCLX is an essential component of mitochondrial Na^+/Ca^{2+} exchange, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 436–441.

[23] Nita II, M. Hershfinkel, C. Kantor, G.A. Rutter, E.C. Lewis, I. Sekler, Pancreatic beta-cell Na^+ channels control global Ca^{2+} signaling and oxidative metabolism by inducing Na^+ and Ca^{2+} responses that are propagated into mitochondria, FASEB J. 28 (2014) 3301–3312.

[24] S. Gandhi, A. Wood-Kaczmar, Z. Yao, H. Plun-Favreau, E. Deas, K. Klupsch, J. Downward, D.S. Latchman, S.J. Tabrizi, N.W. Wood, M.R. Duchen, A.Y. Abramov, PINK1associated Parkinson's disease is caused by neuronal vulnerability to calcium- induced cell death, Mol. Cell. 33 (2009) 627–638.

[25] Y. Sancak, A.L. Markhard, T. Kitami, E. Kovacs-Bogdan, K.J. Kamer, N.D. Udeshi, S.A. Carr, D. Chaudhuri, D.E. Clapham, A.A. Li, S.E. Calvo, O. Goldberger, V.K. Mootha, EMRE is an essential component of the mitochondrial calcium uniporter complex, Science 342 (2013) 1379–1382.

[26] G. Csordas, T. Golenar, E.L. Seifert, K.J. Kamer, Y. Sancak, F. Perocchi, C. Moffat, D. Weaver, S. de la Fuente Perez, R. Bogorad, V. Koteliansky, J. Adijanto, V.K. Mootha, G. Hajnoczky, MICU1 controls both the threshold and cooperative activation of the mitochondrial Ca^{2+} uniporter, Cell. Metab. 17 (2013) 976–987.