

Lucio Annunziato *Editor*

Sodium Calcium Exchange: A Growing Spectrum of Pathophysiological Implications

Proceedings of the 6th International
Conference on Sodium Calcium Exchange

Advances in Experimental Medicine and Biology

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Conference on Sodium Calcium
Exchange

 Springer

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This book is dedicated to Mordecai Blaunstein and Kenneth Philipson as a tribute to their outstanding scientific careers: most of the current knowledge on NCX is due to their passionate work over the last 45 years and this conference would not have been possible without their memorable contribution.

Preface

From October 1 to October 5, 2011 more than 100 scientists from 21 countries of all the continents conveyed in the nice, little village of Lacco Ameno in the Island of Ischia in the Gulf of Naples, Italy, to take part to the 6th International Conference on Sodium Calcium Exchange. This book reports the text of the lectures of the Conference and the state-of-art of the topic discussed and it intends to represent a long lasting memory of the magic atmosphere of the meeting where science and friendship perfectly matched in a lovely natural setting.

The 6th International Conference on Sodium Calcium Exchange is the last of a series that begun with the meeting held in Stowe 1987, England and, thereafter, every 4–5 years each time in a different location. Nowadays, 35 years after this first meeting, the International Conference on Sodium Calcium Exchange is an highly expected appointment for the NCX community where new data are presented and fruitful discussions take place. The last 30 years experienced big changes in the field of NCX that, at the very beginning was a partially characterized protein object of the interest of few highly focused scientists and nowadays is a protein characterized even in its three dimensional structure with an ever growing spectrum of pathophysiological implications. Two lectures, given at the 6th International Conference on Sodium Calcium Exchange and reported in the present volume, gave an enthusiastic overview of the tremendous progress in the knowledge on the exchanger with the words of two leaders in the field, Mordecai Blaunstein and Kenneth Philipson to whom we intend to dedicate this book as a tribute to their outstanding scientific careers.

Looking back to the volumes of the Proceedings of the previous conferences it can be easily realized that all the milestones in the field have been documented in these books which, therefore, represent an important part of the literature on the exchanger. We are confident that the same will also happen for the present book that uncovers the most striking new findings on NCX that emerged since the previous Conference on Sodium Calcium Exchange, such as the structural dissection of the molecular determinants of Ca^{2+} sensitivity of the exchanger, the epigenetic regulation of *ncx1* gene, the molecular identification of the mitochondrial Sodium Exchanger, and the discovery of NCX in unexpected anatomical locations such as the female reproductive tract.

As a final remark we would like to emphasize that many of the participants to the Conference were young scientists under 35 years: it looks like that

besides the outstanding past of the fathers of NCX we should also celebrate the birth of a new generation of NCX proselytes that we hope will keep high the tradition of the NCX Conferences also in the future.

We would like to express our gratitude for their precious help in the organization of the meeting to all the members of the International (Luis Beaugé, Lorella MT Canzoniero, Ernesto Carafoli, Gianfranco Di Renzo, David Eisner, André Herchuelz, Takahiro Iwamoto, Lung-Sen Kao, Daniel Khananshvili, Jonathan Lytton, Kenneth D Philipson, Hannah Rahamimoff, John Reeves, Paul Schnetkamp, Karin R Sipido, Dandan Sun, Bruno Trimarco, Jin Zhang) and Local (Lorella MT Canzoniero, Mauro Cataldi, Gianfranco Di Renzo, Pasquale Molinaro, Anna Pannaccione, Giuseppe Pignataro, Antonella Scorziello, Agnese Secondo, Maurizio Tagliatela) Organizing Committees. Special thanks also to Mauro Cataldi for helping in the revision of the chapters and in the organization of the book, to Springer for taking care of the publication of the book, and to Studio Grafico Ciotola, Naples, Italy, for preparing all the illustrations of the book.

Lucio Annunziato, M.D.
Chairman of the Conference

Contents

Part I Historical Perspective

- 1 Livin' with NCX and Lovin' It: A 45 Year Romance** 3
Mordecai P. Blaustein
- 2 20 Years from NCX Purification and Cloning: Milestones** 17
Debora A. Nicoll, Michela Ottolia, Joshua I. Goldhaber, and Kenneth D. Philipson

Part II Structural and Functional Aspects of NCX

- 3 Ca²⁺ Regulation in the Na⁺/Ca²⁺ Exchanger Features a Dual Electrostatic Switch Mechanism**..... 27
Mark Hilge
- 4 Molecular Determinants of Allosteric Regulation in NCX Proteins** 35
Moshe Giladi and Daniel Khananshvili
- 5 NCX1: Mechanism of Transport** 49
Michela Ottolia and Kenneth D. Philipson
- 6 Structural Studies of the Ca²⁺ Regulatory Domain of *Drosophila* Na⁺/Ca²⁺ Exchanger CALX** 55
Lei Zheng, Mousheng Wu, and Shuilong Tong
- 7 Interplay of Ca²⁺ and Mg²⁺ in Sodium-Calcium Exchanger and in Other Ca²⁺-Binding Proteins: Magnesium, Watchdog That Blocks Each Turn if Able** 65
Dmitri O. Levitsky and Masayuki Takahashi

Part III Structural and Functional Aspects of NCKX

- 8 Functional and Structural Properties of the NCKX2 Na⁺-Ca²⁺/K⁺ Exchanger: A Comparison with the NCX1 Na⁺/Ca²⁺ Exchanger** 81
Haider F. Altimimi, Robert T. Szerencsei, and Paul P.M. Schnetkamp

- 9 NCKX5, a Natural Regulator of Human Skin Colour Variation, Regulates the Expression of Key Pigment Genes MC1R and Alpha-MSH and Alters Cholesterol Homeostasis in Normal Human Melanocytes.....** 95
 Stephen Wilson, Rebecca S. Ginger, Tony Dadd, David Gunn, Fei-Ling Lim, Magdalena Sawicka, Melanie Sandel, Paul P.M. Schnetkamp, and Martin R. Green
- 10 Expression and Regulation of Sodium/Calcium Exchangers, NCX and NCKX, in Reproductive Tissues: Do They Play a Critical Role in Calcium Transport for Reproduction and Development?** 109
 Hyun Yang, Kyung-Chul Choi, Eui-Man Jung, Beum-Soo An, Sang-Hwan Hyun, and Eui-Bae Jeung

Part IV Genetic and Epigenetic Regulation

- 11 Transcriptional Pathways and Potential Therapeutic Targets in the Regulation of *Ncx1* Expression in Cardiac Hypertrophy and Failure** 125
 Donald R. Menick, Mona S. Li, Olga Chernysh, Ludivine Renaud, Denise Kimbrough, Harinath Kasiganesan, and Santhosh K. Mani
- 12 Transcriptional Regulation of *ncx1* Gene in the Brain.....** 137
 Valeria Valsecchi, Giuseppe Pignataro, Rossana Sirabella, Carmela Matrone, Francesca Boscia, Antonella Scorziello, Maria Josè Sisalli, Elga Esposito, Nicola Zambrano, Mauro Cataldi, Gianfranco Di Renzo, and Lucio Annunziato

Part V Regulatory Mechanisms of NCX

- 13 Metabolic Regulation of the Squid Nerve Na⁺/Ca²⁺ Exchanger: Recent Developments.....** 149
 Luis Beaugé, Reinaldo DiPolo, Mariana Bollo, Alexandra Cousido, Graciela Berberían, and Alberto Podjarny
- 14 Regulation of Sodium-Calcium Exchanger Activity by Creatine Kinase.....** 163
 Ya-Chi Yang and Lung-Sen Kao
- 15 Coordinated Regulation of Cardiac Na⁺/Ca²⁺ Exchanger and Na⁺-K⁺-ATPase by Phospholemman (FXD1)** 175
 Joseph Y. Cheung, Xue-Qian Zhang, Jianliang Song, Erhe Gao, Tung O. Chan, Joseph E. Rabinowitz, Walter J. Koch, Arthur M. Feldman, and JuFang Wang

**Part VI Subcellular Localization and Function of NCX
in Ca²⁺-Storing Organelles and Mitochondria**

- 16 Mitochondria Na⁺-Ca²⁺ Exchange in Cardiomyocytes
and Lymphocytes** 193
Bongju Kim, Ayako Takeuchi, Orié Koga, Masaki Hikida,
and Satoshi Matsuoka
- 17 New Insights in Mitochondrial Calcium Handling
by Sodium/Calcium Exchanger** 203
Antonella Scorziello, Claudia Savoia, Agnese Secondo,
Francesca Boscia, Maria José Sisalli, Alba Esposito,
Annalisa Carlucci, Pasquale Molinaro, Luca Lignitto,
Gianfranco Di Renzo, Antonio Feliciello,
and Lucio Annunziato

Part VII NCX in Neurodegenerative Diseases

- 18 Genetically Modified Mice as a Strategy to Unravel
the Role Played by the Na⁺/Ca²⁺ Exchanger in Brain Ischemia
and in Spatial Learning and Memory Deficits** 213
Pasquale Molinaro, Mauro Cataldi, Ornella Cuomo,
Davide Viggiano, Giuseppe Pignataro, Rossana Sirabella,
Agnese Secondo, Francesca Boscia, Anna Pannaccione,
Antonella Scorziello, Sophie Sokolow, André Herchuelz,
Gianfranco Di Renzo, and Lucio Annunziato
- 19 NCX as a Key Player in the Neuroprotection Exerted
by Ischemic Preconditioning and Postconditioning**..... 223
Giuseppe Pignataro, Ornella Cuomo, Antonio Vinciguerra,
Rossana Sirabella, Elga Esposito, Francesca Boscia,
Gianfranco Di Renzo, and Lucio Annunziato
- 20 The Role of the Mitochondrial NCX in the Mechanism
of Neurodegeneration in Parkinson's Disease** 241
Alison Wood-Kaczmar, Emma Deas, Nicholas W. Wood,
and Andrey Y. Abramov
- 21 The Contribution of the Sodium-Calcium Exchanger
(NCX) and Plasma Membrane Ca²⁺ ATPase (PMCA)
to Cerebellar Synapse Function** 251
Chris J. Roome and Ruth M. Empson

**Part VIII Emerging Role of NCX Activity in Immune
and Glial Cells**

- 22 Sodium-Calcium Exchanger Modulates the L-Glutamate
Ca_i²⁺ Signalling in Type-1 Cerebellar Astrocytes**..... 267
Héctor Rojas, Claudia Colina, Magaly Ramos,
Gustavo Benaim, Erica Jaffe, Carlo Caputo,
and Reinaldo DiPolo

23	Immunosuppressive Drugs, Immunophilins, and Functional Expression of NCX Isoforms	275
	Hannah Rahamimoff, Benayahu Elbaz, Michael Valitsky, Mahdi Khatib, Marina Eskin-Schwartz, and Daniela Elmaz	
24	Calcium Influx Through Reversed NCX Controls Migration of Microglia	289
	Mami Noda, Masataka Ifuku, Yuki Mori, and Alexei Verkhratsky	
25	Sodium Fluxes and Astroglial Function	295
	Alexei Verkhratsky, Mami Noda, Vladimir Parpura, and Sergei Kirischuk	
26	New Roles of NCX in Glial Cells: Activation of Microglia in Ischemia and Differentiation of Oligodendrocytes	307
	Francesca Boscia, Carla D'Avanzo, Anna Pannaccione, Agnese Secondo, Antonella Casamassa, Luigi Formisano, Natascia Guida, Antonella Scorziello, Gianfranco Di Renzo, and Lucio Annunziato	
27	Human Macrophages and Monocytes Express Functional Na⁺/Ca²⁺ Exchangers 1 and 3	317
	Rosaria I. Staiano, Francesco Paolo Granata, Agnese Secondo, Angelica Petraroli, Stefania Loffredo, Lucio Annunziato, Massimo Triggiani, and Gianni Marone	
Part IX NCX in the Heart and Vascular Smooth Muscle		
28	New Insights into the Contribution of Arterial NCX to the Regulation of Myogenic Tone and Blood Pressure	329
	Jin Zhang	
29	Toward an Understanding of the Complete NCX1 Lifetime in the Cardiac Sarcolemma	345
	Donald W. Hilgemann, Mei-Jung Lin, Michael Fine, Gary Frazier, and Hao-Ran Wang	
Part X NCX Role in Hypertension, Heart Failure, Ischemia-Reperfusion, Arrhythmias and Diabetes		
30	Cardiac Sodium-Calcium Exchange and Efficient Excitation-Contraction Coupling: Implications for Heart Disease	355
	Joshua I. Goldhaber and Kenneth D. Philipson	
31	Cross Talk Between Plasma Membrane Na⁺/Ca²⁺ Exchanger-1 and TRPC/Orai-Containing Channels: Key Players in Arterial Hypertension	365
	Maria V. Pulina, A. Zulian, Sergey G. Baryshnikov, Cristina I. Linde, Eiji Karashima, John M. Hamlyn, Patrizia Ferrari, Mordecai P. Blaustein, and Vera A. Golovina	

32	T-Tubule Remodelling and Ryanodine Receptor Organization Modulate Sodium-Calcium Exchange	375
	Karin R. Sipido, Károly Acsai, Gudrun Antoons, Virginie Bito, and Niall Macquaide	
33	Na⁺/Ca²⁺ Exchange and the Plasma Membrane Ca²⁺-ATPase in β-Cell Function and Diabetes	385
	André Herchuelz, Evrard Nguidjoe, Lin Jiang, and Nathalie Pachera	
Part XI NCX Partners in Ionic Homeostasis: ASIC, NMDA, NHE and TRPC		
34	The Na⁺/H⁺ Exchanger NHE5 Is Sorted to Discrete Intracellular Vesicles in the Central and Peripheral Nervous Systems	397
	Viktoria Lukashova, Tushare Jinadasa, Alina Ilie, David Verbich, Ellis Cooper, and John Orłowski	
35	The Role of Na⁺/H⁺ Exchanger Isoform 1 in Inflammatory Responses: Maintaining H⁺ Homeostasis of Immune Cells	411
	Yejie Shi, Dong Kim, Marie Caldwell, and Dandan Sun	
36	Acid-Sensing Ion Channels in Pathological Conditions	419
	Xiang-Ping Chu and Zhi-Gang Xiong	
37	Nonselective Cation Channels and Links to Hippocampal Ischemia, Aging, and Dementia	433
	John F. MacDonald, Jillian C. Belrose, Yu-Feng Xie, and Michael F. Jackson	
	Erratum	E1
	List of Participants	449
	Author Index	451
	Subject Index	455

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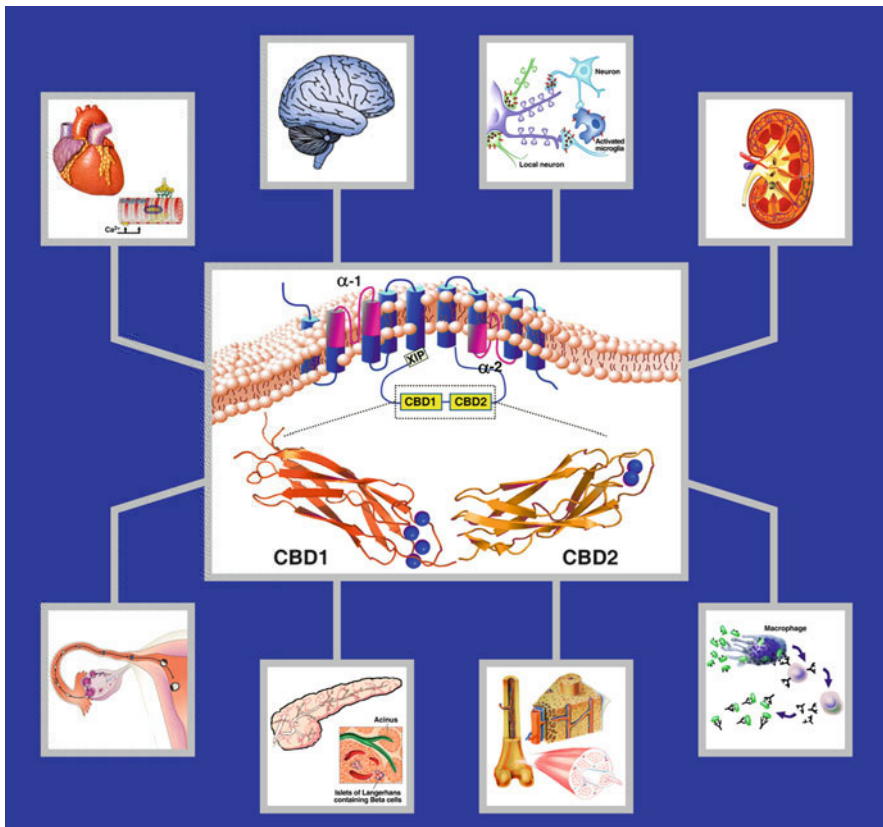
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Participants to the 6th Conference on Sodium Calcium Exchange, Lacco Ameno, Ischia, Naples, Italy, October 1-5, 2011



Part I

Historical Perspective

Livin' with NCX and Lovin' It: A 45 Year Romance

1

Mordecai P. Blaustein

Sit down before fact as a little child, be prepared to give up every conceived notion, follow humbly wherever, whatever abysses nature leads, or you will learn nothing.

Thomas H. Huxley,
letter to C. Kingsley, September 20, 1863

Abstract

This conference commemorates, almost to the day, the 45th anniversary of the discovery of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX). The discovery was serendipitous, as is so often the case with scientific breakthroughs. Indeed, that is what is so fascinating and romantic about scientific research. I will describe the discovery of NCX, but will begin by explaining how I got there, and will then discuss how the discovery influenced my career path.

Keywords

Cardiotonic steroids • Hypertension • PLasmERosomes • Synaptosomes • Vascular smooth muscle

1.1 For the Love of Physiology

I was introduced to cell physiology by Howard Schneiderman, a distinguished insect physiologist and developmental biologist, during my undergraduate days at Cornell University. I was interested in

In memory of Peter F. Baker, David E. Goldman, Alan L. Hodgkin, Howard A. Schneiderman, Daniel C. Tosteson, and Mani Matter.

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neurophysiology and the mind-brain problem but came under the spell of Daniel Tosteson when I was a medical student at Washington University in St. Louis. Dan convinced me to work on the Na^+/K^+ -ATPase (“sodium pump”), which had just been discovered (Skou 1957).

I spent a year and a half in Dan’s lab studying the red blood cell cardiotonic steroid-sensitive Na^+ pump. In 1963, after completing medical school and an internship at Boston City Hospital, I was offered a naval commission to work at the US Naval Medical Research Institute in Bethesda, MD (much better than a tour in Vietnam!).

Thus, I returned to neurophysiology, and under David Goldman (of the Goldman-Hodgkin-Katz

equation), I studied the effects of divalent cations and anesthetics on lobster nerve conduction (Blaustein and Goldman 1966; Blaustein 1968). I also was fortunate to spend a few weeks at Woods Hole with John Moore and Toshio Narahashi working on tetrodotoxin's action on squid axons (Moore et al. 1967).

I was planning to continue my career in cellular neurophysiology and arranged for a position in Alan Hodgkin's laboratory in Cambridge, England, with a special fellowship from the NIH. My family and I arrived in Cambridge in late August of 1966. After a family trip to Vienna for the International Congress of Biophysics, I left my wife Ellen and our two children (ages 3 and 5) in Cambridge and headed off to the Laboratory of the Marine Biological Association in Plymouth, England, for the fall squid season.

1.2 All Hands to the Pump

My expectation was to study squid axon electrophysiology, but Peter Baker, Alan's junior associate, a lecturer at Emmanuel College, Cambridge,

took a mini-sabbatical that fall, and he wanted all Plymouth squid researchers to work on the Na^+ pump. I was paired up with Rick Steinhardt, Richard Keynes' postdoctoral fellow, and we were tasked with studying the activation of the Na^+ pump by external cations. Richard came to Plymouth at the end of September; he showed us how to dissect squid axons (not knowing of my prior experience) and how to measure $^{22}\text{Na}^+$ efflux after injecting the giant axons (0.8–1.2-mm diameter) with a microsyringe that he and Alan designed (Hodgkin and Keynes 1956). Richard then went off to Homburg (Saar), Germany, to teach in a course on membrane biophysics organized by Hermann Passow and Robert Stampfli (more about this later).

Rick and I began our $^{22}\text{Na}^+$ efflux experiments on squid axons, and we rapidly identified a component that depended on external K^+ and was blocked by ouabain, i.e., the Na^+ pump component. When we removed external Na^+ and K^+ (Na_o and K_o , respectively), preparatory to adding back one monovalent cation at a time, we exposed a very large $^{22}\text{Na}^+$ efflux that did not depend upon K_o and was not blocked by ouabain (Fig. 1.1).

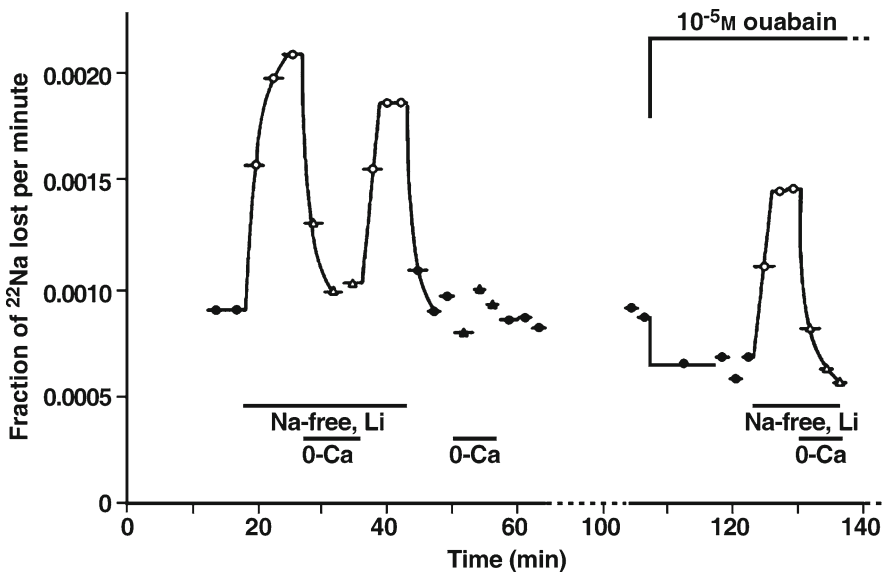


Fig. 1.1 Reduction of $[\text{Na}^+]_o$ activates a large Ca_o -dependent, ouabain-resistant $^{22}\text{Na}^+$ efflux in squid axons with high $[\text{Na}^+]_i$. Replacement of 460 mM NaCl in the artificial seawater (ASW) by LiCl greatly increased $^{22}\text{Na}^+$ efflux, measured as the fraction of $^{22}\text{Na}^+$ lost per minute. The increment was abolished by removal of the 11 mM CaCl_2 in the ASW (MgCl_2 was increased from

55 to 66 mM). This is evidence of " Na_o - Ca_o antagonism" and indirect evidence for $\text{Na}^+/\text{Ca}^{2+}$ exchange. Ouabain (10^{-5} M; added at 107 min) reduced the $^{22}\text{Na}^+$ efflux in control Na ASW (i.e., it inhibited the Na^+ pump), but it had no effect on the large Ca_o -dependent $^{22}\text{Na}^+$ efflux in Li ASW) (Reprinted from Baker et al. (1969), with permission)

This efflux persisted when the external NaCl and KCl were replaced by sucrose, LiCl, or choline Cl⁻. We consulted with Peter Baker, who agreed that internal Na⁺ could be exiting with an anion or exchanging for a cation. It was easiest to remove the other external cations, Ca²⁺ and Mg²⁺, so we first removed the Ca²⁺ – et voila! The large Na⁺ efflux was reversibly abolished, i.e., the Na⁺ efflux was external Ca²⁺-dependent (Fig. 1.1). Removal of external Mg²⁺ had negligible effect on the Na⁺ efflux, so we had our answer: Na/Ca exchange! This was just the first month of my fellowship!

Peter had read Ralph Niedergerke's articles on Na⁺-Ca²⁺ interactions in frog cardiac muscle, and he suggested that they might be of interest. There was no time to read, however, we were working 14–16-h days during October because I feared that a gale would interrupt our daily squid supply; the large squid did not survive in the relatively small holding tanks. In fact, the squid usually were injured in the nets and would die before the collection boat docked. Therefore, as soon as squid were caught, the fishermen removed the head and internal organs and placed the mantle (containing the giant axons) in a thermos of iced seawater. With the Na⁺ pumps thus turned off, the axons slowly gained Na⁺; the axons did not last overnight. As we shall see, this rise in the intracellular Na⁺ concentration, [Na⁺]_i, was fortuitous for the Ca²⁺ influx experiments.

In the meantime, we ordered some ⁴⁵Ca²⁺ to test the Na⁺/Ca²⁺ exchange idea directly. The ⁴⁵Ca²⁺ arrived the first week in November, but just before that, we had a gale. Finally, I had a chance to catch my breath. It was a miserable, stormy afternoon, and the laboratory building was deserted; I completed my data analysis for the last experiments and sauntered down the hall to the library. As soon as I started to read the description (Luttgau and Niedergerke 1958) of extracellular Na⁺-Ca²⁺ ([Na⁺]_o-[Ca²⁺]_o) antagonism and its influence on frog cardiac contraction (reduced [Na⁺]_o induces cardiac contraction), I got very excited. I immediately recognized that NCX must be widely distributed in both tissues and species, including vertebrate heart. Therefore,

since NCX apparently functions in the heart, it is the missing link to the puzzle that had stumped me ever since my first studies on the Na⁺ pump and, as an intern, my use of digitalis to treat patients with heart failure: How does Na⁺ pump inhibition by cardiotonic steroids increase the force of contraction of the heart? Because of both my clinical and research experiences, I frequently thought about this enigma.

Here was the answer: raising [Na⁺]_i promotes net Ca²⁺ gain by NCX and thereby enhances cardiac contraction. That “Eureka! moment” was even more thrilling than the discovery of NCX itself. I was, for a brief time, the only one in the world who understood how cardiotonic steroids enhance cardiac contraction! I was so exhilarated that I went off, alone, to the nearby Green Lantern restaurant, for a fine celebratory dinner with a bottle of claret. Then, slightly inebriated, I returned to the lab to reread Luttgau-Niedergerke, to be sure I was not delusional. It was a great day!

1.3 ⁴⁵Ca²⁺ Flux Studies: Verification of Na⁺/Ca²⁺ Exchange

The following Monday afternoon, Alan Hodgkin came down to Plymouth to see how I was getting on. After we dissected a few axons for the evening's ²²Na⁺ efflux experiments, he and I went to dinner with Trevor Shaw, another Plymouth squidder. We talked about the NCX, including my explanation of how Na⁺ pump inhibitors exert their cardiotonic effect. Alan asked a few questions but was, otherwise, impassive. I was crestfallen. How could he fail to be enthused by the story? Two days later, however, Alan asked me if I would mind if he remained in Plymouth to perform the ⁴⁵Ca²⁺ flux experiments with me. Would I mind? I was ecstatic! We had won him over.

Alan and I performed the first influx experiments the next Monday. Axons were incubated for 1 h in artificial seawater (ASW, the external fluid) containing either NaCl or LiCl as the predominant salt and labeled with ⁴⁵Ca²⁺. The axons were then washed in tracer-free solution, and the axoplasm