Increased Sodium-Lithium Countertransport Activity: A Cellular Dysfunction Common to Essential Hypertension and Diabetic Nephropathy

GIANPAOLO ZERBINI, DANIELA GABELLINI, DORA RUGGIERI, and ANNA MAESTRONI
Renal Pathophysiology Laboratory, Division of Medicine, San Raffaele Scientific Institute, Milan, Italy

Abstract. An increased activity of sodium-lithium countertransport (SLC) is a common finding in patients who have essential hypertension. The evidence that a similar dysfunction is shared also by patients with type 1 diabetes and nephropathy has suggested the hypothesis that a predisposition to essential hypertension may be the factor that, along with hyperglycemia, underlies the development of diabetic nephropathy. Despite the initial enthusiasm surrounding the potential use of SLC activity as a marker for the early detection and treatment of individuals who are predisposed to hypertension and diabetic nephropathy, its use has been so far restricted to epidemiologic studies, as specificity and sensitivity of the test are still too low to justify any clinical use. The recent finding, however, that the measurement of kinetic parameters of SLC can significantly increase the power to discriminate among individuals with and without hypertension or diabetic nephropathy could be of help toward a future clinical use of the measurement of this membrane transport. A second major point relates to the possibility that SLC per se might be directly involved in the pathogenesis of essential hypertension and diabetic nephropathy. This case has never been fully tested, as the gene responsible for this membrane transport has been, until recently, unknown. The recent identification of an alternative splicing of the first isoform of Na-H exchange that mediates SLC activity should allow for a rapid comprehension of the role of this transport in the pathophysiology of essential hypertension and diabetic nephropathy.

The presence in the erythrocyte membrane of a transport mediating the exchange of intracellular lithium for extracellular sodium was first suggested after the finding that, in lithium-treated patients who had affective disorders, lithium concentration was systematically lower in the erythrocytes than in the plasma (1,2). Since the first identification, sodium-lithium countertransport (SLC)—so called because sodium, at opposite with the sodium pump, is moved toward the intracellular compartment—was confirmed in the erythrocyte of humans and of a number of animal species (3,4).

SLC gained widespread reputation in 1980 after the demonstration by Canessa et al. (5) that elevated activity rates of this transport are a consistent concomitant of essential hypertension. It is interesting that although SLC activity rate can be affected by several environmental factors (6,7), family and genetic epidemiology studies have shown that the effect of polygenic inheritance and/or of recessive major gene is nonetheless a major determinant in the interindividual variability of SLC (8–10).

Since the original finding, an increased activity of SLC has always been considered an established marker of essential hypertension. For this reason, after the demonstration that hyperglycemia by itself is not sufficient to explain the development of diabetic nephropathy (11) and the discovery that arterial BP is higher in parents of patients with type 1 diabetes and proteinuria (12), it was consequential to evaluate SLC activity in patients with type 1 diabetes with and without nephropathy.

As a result, increased SLC activity in diabetic nephropathy was demonstrated by two independent studies (13,14), suggesting that an inherited predisposition to essential hypertension may be related to the susceptibility to nephropathy in patients with type 1 diabetes. Similar results were later confirmed by several (15–17) although not all studies (18,19). The reason for these discrepancies has never been completely clarified, even though methodological differences among different studies have been demonstrated (20,21). Finally, recent evidence indicates that an increased SLC activity predicts the development of both essential hypertension (22) and diabetic nephropathy (23), suggesting that this dysfunction cannot be considered simply a consequence of the development of these human diseases.

Potential Clinical Significance of SLC Activity Measurement

Altogether, the evidence that an increased SLC activity precedes the development of both essential hypertension and diabetic nephropathy and the reproducibility of the measurement over time even in patients with diabetes (Figure 1) is in line with the hypothesis that SLC activity might be of imme-
diate clinical use. Unfortunately, as clearly shown in virtually every study in which SLC has been measured (5,13,14,24) and also in Figure 2A, although SLC activity is significantly different among groups, a large overlap nonetheless exists between normotensive and hypertensive patients or between patient with diabetes with or without nephropathy, thus significantly reducing the clinical usefulness of SLC activity measurement. In the past few years, prompted by the finding of Rutherford et al. (25) that SLC activity as usually evaluated (standard assay at 150 mM external Na; see Figure 1A) does not totally saturate the transport for external Na, we investigated whether the measurement of kinetic parameters of SLC (V_{max} and K_{m} for external Na) could increase the sensitivity of the assay. Actually, this approach demonstrated that essential hypertension is characterized by an increase of both V_{max} and K_{m} for external Na of SLC and that the measurement of these parameters improves the sensitivity of the SLC in discriminating between the two groups of individuals (24). We more recently obtained similar results in patients who have diabetic nephropathy. As shown in Figure 2, diabetic nephropathy is also characterized by an increased V_{max} (Figure 2B), and K_{m} for external Na (Figure 2C) of SLC and differences among groups significantly increase when V_{max} or K_{m} for external Na are used instead of the standard assay at 150 mM external Na (Figure 2A). Altogether, these findings not only add one more clue to the hypothesis that essential hypertension and diabetic nephropathy share a common background but probably also allow a step ahead toward a future clinical application of SLC activity.

**SLC Activity Is Not Restricted to Erythrocyte**

Is SLC a membrane transport present only in erythrocyte? This question is of obvious importance: if SLC activity were indeed detectable only in erythroid cells, then its possible role in the pathogenesis of essential hypertension and diabetic nephropathy would be virtually irrelevant, as erythrocytes are most likely not involved in the pathogenesis of these diseases. Although SLC activity has been for many years thought to be measurable only in the mature erythrocyte, there nonetheless is evidence that this transport is present also in other kind of cells. In particular, an SLC-like activity has been demonstrated in the lymphocyte (26) and more recently in the human skin fibroblast (27). It is interesting that SLC activity in fibroblasts from patients with essential hypertension is increased when compared with fibroblasts obtained from normotensive subjects (28), extending the association between SLC overactivity and essential hypertension to nonerythroid cells and therefore, potentially, also to cells actively involved in the pathogenesis of human diseases.

**SLC in Other Human Diseases**

Increased SLC activity has been demonstrated in patients who have IgA nephropathy and rapid progression of the disease (29,30) and, more recently, also in patients with type 1 diabetes and proliferative retinopathy (31). These findings are in line with the hypothesis that a predisposition to essential

![Figure 1](image1.png)

**Figure 1.** Correlation between sodium-lithium countertransport (SLC) activity measured in 54 patients with type 1 diabetes with a mean duration of diabetes of 5.8 ± 0.5 yr (mean ± SEM) and SLC activity measured in the same patients 6 yr later. All of the patients were studied in the outpatient clinic of the San Raffaele Scientific Institute, Milan, Italy.

![Figure 2](image2.png)

**Figure 2.** (A) SLC activity measured with the standard assay (150 mM external Na) in 21 patients with type 1 diabetes and nephropathy and in 25 patients with type 1 diabetes and normoalbuminuria, matched for gender, age, and duration of diabetes. Medians are indicated by the bar. *P = 0.02. (B) V_{max} of SLC measured in the same patients as described above. **P = 0.004. (C) K_{m} for external Na of SLC measured in the same patients as described above. ***P = 0.0001. Methods used to measure SLC kinetic parameters are described in reference 24.
hypertension (unmasked by an increased SLC activity) may modulate the rate of progression of several renal diseases and, possibly, of diabetic complications.

Nature of SLC and Modulation of Its Activity

The nature of SLC and its role in human physiology have been investigated since its first identification in the human erythrocyte. As lithium is barely detectable in the human body, SLC was suggested to represent an in vitro mode of operation of some other sodium transport. Studies of Na flow across the membrane suggested that Na-H exchange—a membrane transport present in virtually every animal cell involved in regulation of intracellular pH, cell volume, and cell proliferation—exchanges also Li for Na, which has contributed to the hypothesis that SLC may reflect an operational mode of this system (33,34).

A major argument against the common nature of SLC and Na-H exchange, however, is represented by their different response to inhibitors. Sensitivity to amiloride is an almost invariable feature of Na-H exchange and of its isoforms (35), whereas SLC is totally insensitive to this drug (36). Moreover, the finding that the interindividual variability of SLC activity cannot be explained by polymorphisms of the gene encoding for the ubiquitous first isoform of Na-H exchange favors the alternative view that SLC may be mediated by a transmembrane carrier independent of amiloride-sensitive transports (37).

In the past few years, we have searched for the presence in the human erythrocyte of amiloride-insensitive Na-H exchange isoforms that could, at least potentially, mediate SLC. To this aim, we screened a cDNA library obtained from a pool of human bone marrows using two probes specific for the high homology region shared by the Na-H exchange isoforms 1 to 7 (38–40). After restriction and sequencing analyses of the several clones harboring partial transcripts of the Na-H exchange-isoform 1 gene, only Na-H exchange-isoform 1 transcripts were finally detected. Na-H exchange-isoform 1, because of its sensitivity to amiloride, already has been excluded as a possible mediator of the amiloride-insensitive SLC (41).

Aimed to the identification of the gene responsible for SLC activity, we recently succeeded in identifying in human erythrocytes and reticulocytes the presence of an alternative splicing of the above-described Na-H exchange-isoform 1, lacking the amiloride binding site. This amiloride-insensitive isoform of Na-H exchange, once transfected in Na-H exchange-deficient cells, restores SLC activity (42). This finding suggests that SLC is mediated by a deleted variant of the Na-H exchange-isoform 1 that differs from the full-length isoform because of amiloride insensitivity and in some way re-candidate Na-H exchange-isoform 1 as a gene potentially involved in the pathogenesis of essential hypertension.

Finally, as both essential hypertension and diabetic nephropathy are characterized by an increased SLC activity, great attention should also be paid to the genes that modulate SLC activity. It is interesting that two studies have focused particularly in this field. A very large study performed in baboons has suggested the baboon chromosome 5 (homologue of human chromosome 4) as a site potentially involved in the regulation of SLC activity (43), whereas a more recent genome-wide linkage study revealed the chromosome 15q as a candidate for the presence of a quantitative trait locus explaining SLC activity (44). Further studies now are necessary both in baboons and in humans to identify finally the single genes involved in the regulation of SLC activity.

Future Perspectives

After the first demonstration by Dr. Canessa >20 yr ago that SLC activity is increased in essential hypertension, a huge number of studies have followed, confirming the original finding, extending the identification of the same dysfunction also to diabetic nephropathy and to other diseases, and showing through an epidemiologic approach that the activity of SLC is under strict genetic control. The only piece of the puzzle still missing, i.e., the gene responsible for this membrane transport, has now been identified as Na-H exchange-isoform-1 through an amiloride-insensitive alternative splicing. As the genes potentially involved in the increased SLC activity seem to be close to the identification, it is possible to envisage for the near future the full comprehension of the role of SLC in the pathophysiology of essential hypertension and diabetic nephropathy.

Acknowledgments

The financial support of Telethon-Italy (grant no. E.816) is gratefully acknowledged.

References

20. Canessa M, Zerbini G, Laffel LMB: Sodium activation kinetics
22. Laurenzi M, Cirillo M, Panarelli W, Trevisan M, Stamler R,
23. van Norren K, Borggreven JM, Hovingh A, Willems HL, de Boo
25. Rutherford PA, Thomas TH, Wilkinson R: Increased erythrocyte

10. Hasstedt SJ, Wu LL, Ash KO, Kuida H, Williams RR: Hyper-
tension and sodium-lithium countertransport in Utah pedigrees:
Evidence for major locus inheritance. Am J Hum Genet 43:
14–22, 1988

11. Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR:
The changing natural history of nephropathy in type I diabetes.

12. Viberti GC, Keen H, Wiseman MJ: Raised arterial pressure in
parents of proteinuric insulin dependent diabetics. Br Med J 295:
515–517, 1987

13. Krolewski AS, Canessa M, Warram JH, Laffel LMB: Sodium-activ-
tion kinetics of red cells of patients with insulin-dependent diabetes

14. Mangili R, Breviario D, Mangili R, Gabellini D, Pozza G: Modes of operation of
an electroneutral Na/Li countertransport in human skin fibro-

Erythrocyte sodium-lithium countertransport rate is elevated in essential

16. Walker JD, Tariq T, Viberti GC: Sodium-lithium countertrans-
port activity in red cells of patients with insulin dependent diabetes

17. Lopes de Faria JB, Friedman R, Tariq T, Viberti GC: Prevalence of
increased sodium-lithium countertransport activity in type I dia-

18. Jensen JS, Mathiesen ER, Norgaard K, Hommel E, Borch-
Johnsen K, Funder J, Brahm J, Parving H-H, Deckert T: In-
creased blood pressure and erythrocyte sodium-lithium counter-
transport activity are not inherited in diabetic nephropathy.
Diabetologia 33: 619–624, 1990

19. Elving LD, Wetzels JFM, de Nobel E, Berden JHM: Erythrocyte
sodium-lithium countertransport is not different in type I (insu-
lin-dependent) diabetic patients with and without diabetic neph-

20. Canessa M, Zerbini G, Laffel LMB: Sodium activation kinetics
of red blood cell Na/Li countertransport in diabetes: Metho-

21. van Norren K, Borrgreven JM, Hovingh A, Willems HL, de Boo
T, Elving LD, Berden JH, De Pont JJ: Comparison of methods
for measurement of Na/Li countertransport across the erythro-

22. Laurenzi M, Cirillo M, Panarelli W, Trevisan M, Stamler R,
Dyer AR, Stamler J: Baseline sodium-lithium countertransport
and 6-year incidence of hypertension. The Gubbio population

23. Monciotti CG, Semplicini A, Morocutti A, Maioli M, Cipollina
MR, Barzon I, Palaro C, Brocco E, Trevisan M, Fioretto P,
Crepaldi G, Nosadini R: Elevated sodium-lithium countertransport
activity in erythrocytes is predictive of the development of

M, Semplicini A: Sodium-Lithium countertransport has low af-

25. Rutherford PA, Thomas TH, Wilkinson R: Increased erythrocyte
sodium-lithium countertransport activity in essential hyperten-
sion is due to an increased affinity for extracellular sodium. Clin

26. Grinstein S, Goetz JD, Rothstein A: 22Na fluxes in thymic
lymphocytes. I Na/Na and Na/H exchange through an amiloride-
insensitive pathway. J Gen Physiol 84: 565–584, 1984

27. Zerbini G, Mangili R, Gabellini D, Pozza G: Modes of operation of
an electroneutral Na/Li countertransport in human skin fibro-

Erythrocyte Na-Li countertransport rate is elevated in essential

Quarello F, Fusaroli M, Piccoli G: Increased sodium-lithium
countertransport activity in red cells of IgA nephropathy patients.

30. Kontessis PS, Friedman R, Tariq T, Moro F, Williams DG,
Hartley RB, Viberti GC: Sodium-lithium countertransport activity
as a determinant of deterioration of glomerular function in

31. Lopes de Faria JM, Silveira LA, Morgano M, Pavin EJ, Lopes de
Faria JB: Erythrocyte sodium-lithium countertransport and pro-
fibrative diabetic retinopathy. Invest Ophthalmol Vis Sci 41:
1482–1485, 2000

32. Aronson PS: Kinetic properties of the plasma membrane Na-H

33. Funder J, Wieth JO, Jensen HA, Ibsen KK: The sodium/lithium
exchange mechanism in essential hypertension: Is it a sodium/
proton exchanger? In: Topics in Pathophysiology of Hyperten-
sion, edited by Villareal H, Sambi MP, Boston, Martinus Nijhoff,
1984, pp 147–161

34. Aronson PS: Red-cell sodium-lithium countertransport and es-

35. Wakabayashi S, Shigekawa M, Pouysségur J: Molecular physi-
ology of vertebrate Na/H exchangers. Physiol Rev 77: 51–74,
1997

36. Pandey GN, Sarkadi B, Haas M, Gunn RB, Davis JM, Tosteson
DC: Lithium transport pathways in human red blood cells. J Gen

37. Lifton RP, Hunt SC, Williams RR, Pouysségur J, Lalouel J-M:
Exclusion of the Na-H antiporter as a candidate gene in human
essential hypertension. Hypertension 17: 8–14, 1991

38. Wakabayashi S, Shigekawa M, Pouysségur J: Molecular physi-
ology of vertebrate Na/H exchangers. Physiol Rev 77: 51–74,
1997

39. Counillon L, Pouysségur J: The expanding family of eucaryotic

40. Numata M, Orlowksi J: Molecular cloning and characterization
of a novel (Na, K)/H exchanger localized to the trans-golgi

41. Aronson PS: From flies to physiology—Accidental findings
along the trail of renal NaCl transport. J Am Soc Nephrol 5:
2001–2013, 1995

42. Zerbini G, Maestroni A, Breviario D, Mangili R, Casari G:
Alternative splicing of NHE-1 mediates Na-Li countertransport

43. Kammerer CM, Cox LA, Mahaney MC, Rogers J, Shade RE:
Sodium-lithium countertransport activity is linked to chromo-
some 5 in baboons. Hypertension 38: E35–E36, 2001

44. Weder AB, Delgado MC, Zhu X, Gleiberman L, Kan D, Chakra-
varti A: Erythrocyte sodium-lithium countertransport and blood
pressure: A genome-wide linkage study. Hypertension 41: 842–
846, 2003