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THE Li^+/Na^+ EXCHANGE IN HYPERTENSION

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1. ABSTRACT

The red cell membrane Li^+/Na^+ exchange is a heteroexchange that operates in either direction across the cell membrane. It binds either Li^+ or Na^+ on one side of the membrane and it exchanges the transported species for either Li^+ or Na^+ on the op posite side in a stoichiometric ratio of 1:1. In the population, Li^+/Na^+ exchange is unimodally distributed but skewed to the right. Such distribution results from superimposition of two normal distributions. Many laboratories have shown that red-cell Li^+/Na^+ exchange is increased in patients with essential hypertension, compared with normotensive controls. Among the various alterations of cell membrane cation transport reported in hypertension, the increase of red-cell Li^+/Na^+ exchange has been most widely investigated and confirmed. Moreover, increased Li^+/Na^+ exchange has been found in some clinical conditions related to hypertension, such as overweight and diabetes. Among diabetic patients, Li^+/Na^+ exchange is particularly high in patients with nephropathy, hypertension, and microalbuminuria, leading to the hypothesis that it can be considered a cellular marker of the risk of developing diabetic nephropathy. Furthermore, it is associated with severe and drug-resistant hypertension, insulin resistance, vascular and cardiac hypertrophy, hyperlipidemia, obesity, family history of hypertension, and of major cardiovascular accidents suggesting that high Li^+/Na^+ exchange could be a biochemical marker for increased cardiovascular risk. Regardless of its the pathophysiological significance, its measurement could be of clinical use as an intermediate phenotype of increased cardiovascular risk.

2. INTRODUCTION

Lithium, the lightest of the alkali metals, is widely present in nature but occurs normally in the body only at trace levels. Its salts have been widely used since the early fifties for the treatment of manic disorders to reduce the relapse and recurrence of manic-depressive episodes. Its therapeutic index is narrow (2 to 3 mEq/L), with therapeutic blood levels between 0.8 and 1.5 mEq/L and toxic levels above 2.5 mmol/L. Acute toxic effects include vomiting, diarrhea, ataxia, convulsions, and coma (1). Blood levels during chronic administration should therefore be periodically monitored. Clinical studies found that during Li^+ administration the concentration of Li^+ in red-cell water is usually only one-third that in plasma (2). Since at steady-state concentration the ratio between red cells and plasma for a passively distributed cation should be around 1.2, this observation suggested an uphill extrusion of Li^+.

In 1975, Haas *et al.* first reported the existence of a ouabain-insensitive Li^+/Na^+ exchange (countertransport or antiport, LNE) in human red cells (3). Their findings were independently confirmed by Duhm *et al.* (4), who also underlined the existence of large interindividual differences for Li^+ distribution across the human red cell membrane (5). The basic characteristics of this transport pathway were described by Pandey *et al.* (6), Sarkadi *et al.* (7), and Ehrlich *et al.* (8).

After a few reports in manic-depressive patients, interest in the clinical measurement of LNE in hypertension was raised by the finding by Canessa *et al.* of an increased red cell LNE in patients with essential hypertension but not in patients with secondary hypertension or normotensive controls (9). The suggestion was made that LNE maximal velocity (V_{max}) was heritable and that an increased LNE could be a genetic marker of susceptibility to hypertension (10).

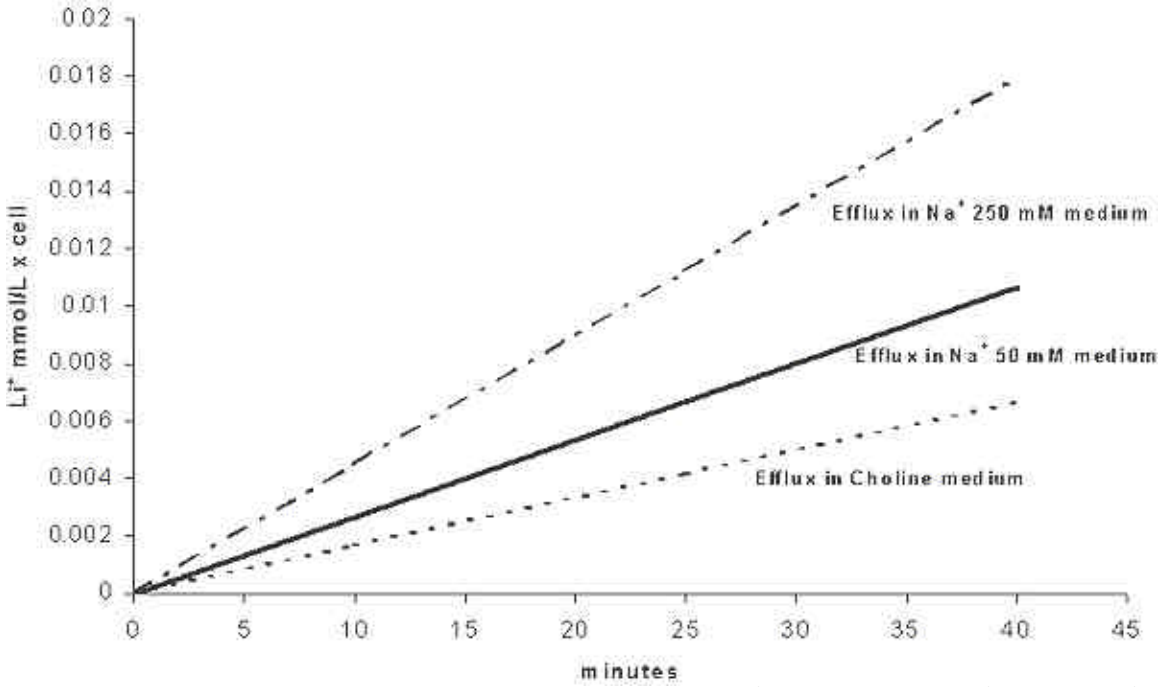

Figure 1. Lithium efflux in the presence and in the absence of extracellular Na^+. The velocity of Na^+-stimulated Li^+ efflux (NLE) is calculated from the difference between the slope of the line of the increasing Li^+ concentration in the presence and in the absence of Na^+(choline medium).

These observations prompted a large number of investigations (more than 200 between 1980 and 2000 according to a MEDLINE search) on LNE in hypertension and in other related clinical conditions (diabetes mellitus, renal insufficiency, hyperlipidemia), sometimes with conflicting results, due to methodological bias and differences in selection of patients.

3. KINETIC PROPERTIES AND CLINICAL ASSAY OF LNE

The red cell membrane LNE is a heteroexchange that operates in either direction across the cell membrane. It binds either Li^+ or Na^+ on one side of the membrane and exchanges the transported species for either Li^+ or Na^+ on the opposite side in a stoichiometric ratio of 1:1.

LNE is ATP independent (4). The energy for uphill translocation is derived from the disequilibrium of Na^+across the cell membrane, maintained by the Na^+Pump.

The rate of Li^+ transport from inside to outside is linear. The difference between the slope of the line of the increasing Li^+ in the medium at a given Na^+concentration and the slope of the efflux of Li^+ in a choline medium represents the transport rate (Figure 1).

The transport rate is saturable with respect to sodium concentration because only a finite number of exchangers exist in the membrane. The kinetics of this process is described by the Michaelis-Menten equation, where v is the transport rate measured as the quantity of Li^+ efflux at that Na^+ concentration in an hour:

$$v = \frac{V_{max} \cdot [Na^+]}{K_m + [Na^+]}$$

The curve approximates a rectangular hyperbola (Figure 2). At high Na^+concentration the transport rate approaches a limiting value that is termed V_{max} by analogy with steady-state enzyme kinetics.

The apparent affinity (K_m) for Li^+ is 20 times higher than that for Na^+ on both sides of the membrane. In normotensive controls, for Li^+ it is 0.5 mM inside and 1.5 mM outside, while that of Na^+ is 9 and 25 mM, respectively (7). Na^+stimulates unidirectional movement of lithium on the trans side and inhibits it on the cis side. LNE is inhibited by high external Mg^{++} concentration, phloretin, and PCMBS (10); it is partially inhibited by bumetanide, and it is insensitive to ouabain, DIDS, furosemide, amiloride, removal of chloride and bicarbonate, and cell swelling at physiological cell pH.

Detailed analysis of the kinetic properties of $Li^+/Na^+$and Na^+/Li^+ exchange in human red cells is consistent with a consecutive "ping-pong" model of the transport reaction (11). According to the model, Li^+ and Na^+bind consecutively on the opposite sides of the membrane. Exchange follows binding on both sides. The rate-limiting step of the overall reaction is cation translocation, without effect of cation binding on affinity on the other side of the membrane.

Theoretically, LNE could be measured as 1) Li^+ efflux promoted by external Na^+(LNE); 2) Li^+ influx promoted by internal Na^+; 3) Na^+efflux promoted by

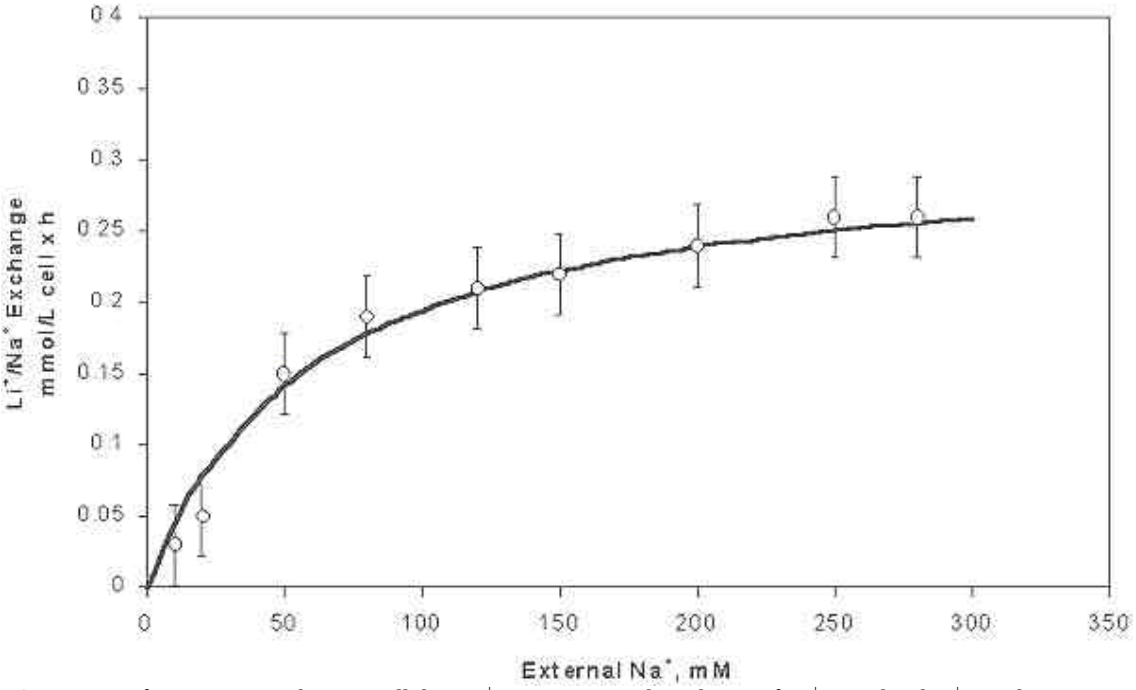

Figure 2. Kinetics of NLE. Raising the extracellular Na^+concentration, the velocity of Na^+-stimulated Li^+ translocation increases according to a Michaelis-Menten kinetic. Only at Na^+concentration > 200 mM it is possible to calculate the maximal velocity of translocation.

external Li^+; or 4) Na^+influx promoted by internal Li^+. However, the transporter is asymmetric for Li^+ and Na^+. The Na^+-stimulated Li^+ flux (LNE) is two to threefold faster than Li^+-stimulated Na^+flux (Na^+/Li^+ exchange) and thus more precisely measurable. Moreover, Li^+ is easily measured with atomic absorption spectrophotometry, is not present normally in biological fluids, and contamination is unlikely. Extracellular Na^+ may inhibit Li^+ influx and its removal may be incomplete. The best method is therefore the measurement of Na^+-stimulated Li^+ efflux (LNE) from lithium loaded cells.

There are three main methods for loading the erythrocytes with Li^+ (12). The classic LiCl loading is the most physiological method and it takes only 3 hours. The Li_2CO_3 has the advantage of taking only 30 min: the Li^+ enters the cell via the HCO^{3-}/Cl^- exchanger as a $LiCO^{3-}$ ion in exchange for Cl^-. The nystatin method was developed by Canessa *et al.* With nystatin, an antifungal drug that penetrates the plasma membranes, the intra- and extracellular osmolarity can be raised to 600 mosm/l, so that extracellular concentration of Na^+up to 300 mmol/l can be used and K_m value can be measured more accurately.

The method for the clinical measurement of LNE has been carefully standardized (13). To perform such measurement, all other transport pathways for Li^+ should be inhibited, including the Na^+pump (with ouabain), $LiCO_3^-$ pathway (with bicarbonate-free solutions), and Na^+-K^+-Cl^- cotransport (with furosemide). Briefly, the red cells are washed with Na^+-free solution to remove extracellular $Na^+$and are Li^+-loaded by incubation in isotonic Li^+ medium. Li^+ loading can be achieved quickly with cold incubation in the ionophore nystatin or with 3-hour incubation at 37° C. Alternatively, Li^+ loading can be achieved with short incubation in lithium bicarbonate (14). After loading, the red cells are washed to remove any extracellular Li^+, bicarbonate, or nystatin, that may be present. Li^+-loaded cells are divided into $Na^+$and Na^+-free media. The efflux of Li^+ is measured over time. It is linear up to 1 hour, and the difference between efflux in the presence and in the absence of Na^+represents the Na^+-stimulated Li^+ efflux.

LNE was also measured as the phloretin-sensitive Li^+ influx, from Li^+ media (15). However, this method cannot standardize intracellular Na^+concentration, which can differ from one patient to another and is very close to the apparent affinity for internal $Na^+$of the transporter (9 mM).

The effects of the age of red cells on LNE are conflicting: LNE has been reported to decrease (16) and to increase with the age of red cells due to an increase of a phloretin-insensitive component (17).

4. LNE IN THE POPULATION AT LARGE

Various laboratories have investigated the distribution of LNE in the population (18-28). It has been shown to be unimodally distributed but skewed to the right. Such distribution results from superimposition of two normal distributions, one with a mean value around 0.24 mmol/L cell x h (70% of the population), the other with a mean value of 0.40 mmol/L cell x h (30% of the population).

Table 1. Li^+/Na^+Exchange (mmol/l cell x h) in normotensives and essential hypertensives

		NORMOTENSIVES			HYPERTENSIVES			
Author[ref]	Publication Year	N	Mean	SD	N	Mean	SD	P
Canessa[9]	1980	26	0.24	0.02	36	0.55	0.02	<.001
Canali[45]	1981	46	0.25	0.09	15	0.33	0.11	<.001
Cusi[46]	1981	12	0.25	0.03	18	0.32	0.02	<.05
Adragna[47]	1982	16	0.27	0.02	22	0.53	0.36	<.005
Clegg[48]	1982	38	0.28	0.09	75	0.53	0.24	<.005
Ibsen[14]	1982	16	0.42	0.10	17	0.68	0.27	<.01
Woods[49]	1982	9	0.17	0.02	16	0.35	0.02	<.001
Cooper[50]	1983	37	0.28	0.10	18	0.36	0.15	<.01
Fujita[51]	1983	35	0.33	0.02	40	0.48	0.03	<.02
Levy[52]	1983	22	0.36	0.03	52	0.48	0.02	<.001
Trevisan[32]	1983	64	0.29	0.08	70	0.35	0.13	<.05
Williams[30]	1983	511	0.26	0.05	54	0.32	0.10	<.001
Canessa[53]	1984	16	0.28	0.12	22	0.52	0.14	<.005
Weder[54]	1984	57	0.27	0.01	29	0.37	0.02	<.05
Corrocher[55]	1985	78	0.19	0.08	64	0.32	0.16	<.001
Hunt[56]	1985	35	0.26	0.12	27	0.36	0.14	<.01
Beuckelmann[57]	1986	10	0.25	0.05	10	0.36	0.13	<.05
Fallo[58]	1986	15	0.26	0.01	15	0.40	0.03	<.01
Hunt[59]	1986	1276	0.27	0.11	143	0.34	0.14	<.001
Morgan[60]	1986	367	0.40	0.01	125	0.45	0.02	<0.05
Gless[61]	1986	39	0.23	0.02	20	0.30	0.03	<.025
McDonald[62]	1987	37	0.28	nr	18	0.36	nr	<.05
Turner[63]	1987	39	0.31	0.07	104	0.44	0.15	<.001
Weder[64]	1987	23	0.29	0.12	20	0.36	0.14	NS
Yap[65]	1989	30	0.34	0.02	50	0.36	0.03	NS
Semplicini[66]	1989	21	0.26	0.10	41	0.35	0.16	<.001
Weinberger[67]	1989	nr	0.21	0.10	nr	0.31	0.10	<.01
Rutherford[23]	1990	17	0.28	0.02	39	0.34	0.01	<.02
Siebers[24]	1990	14	0.39	0.10	14	0.43	0.10	NS
Hunt[68]	1990	1419	0.27	0.11	39	0.27	0.12	NS
Turner[69]	1991	835	0.29	0.11	169	0.35	0.15	<.01
Andronico[70]	1993	14	0.25	0.02	32	0.33	0.02	<.01
Chi[71]	1996	10	0.21	0.06	10	0.32	0.09	<.05
Muriana[72]	1996	12	0.26	0.08	12	0.30	0.04	<.01
Herlitz[73]	1996	17	0.26	0.02	17	0.34	0.03	<.01
Delva[74]	1996	15	0.23	0.03	18	0.34	0.03	<.04
Andronico[75]	1998	32	0.27	0.01	90	0.33	0.01	<.01
weighted mean			0.28			0.38		

abbreviations: N: number of subjects, SD: standard error, nr: not reported, ref: reference

LNE is higher in males than in females (22-26, 29-31) and is related to body weight and body mass index (22, 31, 32).

LNE is higher in whites than in blacks and higher in subjects of Chinese descent then in either Europeans or non Chinese Asians (31, 33). It is ten times slower in cord blood than in adulthood (34, 35), but later no change with aging was found by most, if not all, studies (36).

LNE was investigated in many studies, some of which were not representative of the general population. Moreover, mostly, LNE was treated as an independent variable, with blood pressure as the dependent one. To verify the independent effects of other potentially confounding variables on LNE in the population, we have measured LNE in a random sample of the general population and have confirmed that the mean value is higher in males compared to females, is significantly and independently correlated to blood pressure and alcohol consumption in the whole group, to body mass index in males, and, negatively, to urinary Ca^{2+} excretion in females (22).

5. HERITABILITY OF LNE

Large samples of kindreds have been investigated to define the mode of inheritance of LNE, since bimodality could be either genetic or non-genetic. The coefficients of correlation for LNE in families are larger among first-degree relatives than between genetically unrelated individuals (spouses) (37). A major gene with a polygenic component or an environmental factor was hypothesized (38), since a transversal study showed that LNE was elevated in subjects with MN blood group antigen, and this locus was associated with increased blood pressure (39). Recently, the study of the LNE variability in baboon pedigrees has suggested a link to chromosome 5, the homologue of human chromosome 4 (40).

In siblings with essential hypertension, LNE was influenced by various environmental factors, such as plasma lipids, and only a part of its variance could be explained by hereditable factors (41). On the contrary, in insulin-dependent diabetic twins, raised LNE seemed to be inherited rather than a consequence of overt diabetes (42).

Furthermore, in a large prospective study, variations of BMI, alcohol intake, plasma glucose, and lipids were directly related to LNE in the follow-up, supporting the concept that only a small part of LNE variance can be explained by hereditability, at least in hypertensive patients (43).

Up to now, no single gene product mediating LNE or explaining the association between NLE and hypertension has been identified. However, recently, a link between NLE activity and chromosome 15q26 has been suggested (44).

6. LNE AND ESSENTIAL HYPERTENSION

After the first report by Canessa *et al.* (9), many other laboratories have confirmed the increase of red-cell LNE in patients with essential hypertension compared with normotensive controls, with few exceptions (14, 23, 24, 30, 32, 45-75) (Table 1). Furthermore, red blood-cell LNE was also increased in hypertensive patients *in vivo* after oral administration of Lithium (76).

An increased velocity of cation translocation has been reported by most laboratories at 150 mMol external sodium concentration, which is close to the maximal velocity of translocation. It has been reported that the affinity for $Na^+$is increased at the outside (23) and decreased at the inside ion-binding site in patients with high V_{max} (65), while the affinity for Li^+ on the inside is unchanged in hypertensives compsared to normotensives (24). On the contrary, other studies in our laboratory have suggested that the affinity for sodium is decreased (77). These contrasting results can be explained on the basis of different experimental procedures and different methods used to calculate LNE.

Finally, elevated LNE was demonstrated not only in the red blood cells of hypertensive patients, but also in human skin fibroblasts of essential hypertensives. However, the kinetic parameters of LNE in fibroblasts were different than those in red blood cells and there was no correlation between LNE in red blood cells and LNE in fibroblasts (78).

It is now well established that LNE is not increased in all hypertensive patients and that only a subset of 40 to 50% has a LNE greater than 0.40 mmol/L cell x h, the upper normal limit used in most laboratories. Population studies have shown that the subgroup with the highest LNE has the largest proportion of hypertensives (25, 28). Among hypertensives, LNE is higher in severe and drug-resistant patients (79, 80). It is also higher in hypertensive patients who do not remain normotensive with nutritional interventions, including salt and caloric restriction, and therefore require drug therapy, than in the patients who remain normotensive with simple dietetic measures after stopping drug administration (81).

It is well known that sodium restriction may reduce blood pressure in salt-sensitive patients. Salt restriction, however, did not affect LNE (82, 83).

LNE in patients with essential hypertension shows a peculiar temperature dependence on Arrhenius plots. Li^+ efflux in Na^+ medium and LNE increase with the temperature of the medium, but the increase is not linear. A change in slope ("break") of the line is evident around 20^o C in hypertensives and around 30^o C in normotensives (52).

LNE is increased in the normotensive offspring of hypertensive parents, who are prone to develop hypertension later in life. However, only the patients in whom familiar segregation analysis documents the existence of a recessive major gene for high LNE show increased probability of hypertension in a 10-year follow-up (84). This underlines that increased LNE is not only influenced by the genetic background but also by the environment.

Two studies demonstrate LNE activity as a marker for the onset of hypertension: the Olivetti prospective study showed that LNE per se was a predictor of subsequent hypertension in middle aged men with high-normal blood pressure (85), while the Gubbio study showed that the people who became hypertensive during the 6 years follow up had increased LNE at the start (relative risk 1.63, 95% CI 1.36-1.94) (43, 86). It has therefore been proposed that LNE could be a potential tool to predict the risk of developing hypertension later in life. The mechanism that increases LNE in hypertensives has not yet been discovered. The increased V_{max} supports the hypothesis that hypertensive patients have an increased number of translocators in the membranes. Recently, it has been shown that LNE activity can be modulated by thiol proteins in the membranes of red blood cells and that one of this membrane thiol proteins appears to be tropomyiosin (87-89). Thus, a regulatory protein may lead to an increase of LNE in hypertensive patients.

7. LNE IN SECONDARY HYPERTENSION AND OTHER CLINICAL CONDITIONS

LNE seems to be increased only among essential hypertensives. It was evaluated among patients with primary aldosteronism, estrogen-induced hypertension, polycystic kidney disease, renovascular hypertension, chronic glomerulonephritis, lupus erythematosus, and end-stage renal disease with negative results (9, 36, 90-93). In pigs, however, it has been shown that deoxycosterone acetate treatment may simultaneously increase blood pressure and red-cell LNE (94).

It was reported that LNE is acutely reduced by hemodialysis and ultrafiltration in blacks with end-stage renal disease, attributable to removal of a dialyzable plasma factor (95). These data were confirmed in some (96) but not in other studies (90, 97).

An increase of LNE has been reported during the third trimester of normotensive and hypertensive pregnancy (98, 34), with oral contraceptives (99), Alzheimer's dementia (100), hypothyroidism (101), and Bartter's syndrome (101, 103).

LNE is reduced in hyperthyroid patients (61, 101, 104), normal in patients with idiopathic hypercalciuria and kidney stones (105), and in those with florid psoriasis (106), conditions which are often associated with hypertension.

Chronic alcohol consumption may cause hypertension through uncertain mechanisms. It may induce alterations of Na^+transport in the kidney and smooth muscle cells, directly or through alterations of blood lipids. A positive correlation was in fact observed between alcohol intake and LNE (22,81), and *in vitro* experiments have shown that alcohol may increase LNE (107). LNE is increased in chronic alcoholics but it decreases with abstinence (108).

8. LNE, BODY WEGHT, AND BLOOD LIPIDS

Overweight and obese subjects have increased LNE compared with lean subjects, independent of the presence of hypertension (25,30,32,35,59,69,109). In view of the strong correlation between body weight and blood pressure, this underlines the importance of correcting LNE for body weight when studying the relationship between blood pressure and LNE. Actually, in a sample of the general population, LNE was more strongly correlated to body weight than to blood pressure. After correction for weight, the correlation between LNE and pressure was no longer significant, at least in males (22).

According to a small prospective study of overweight normoglycemic subjects, a weight reduction was associated with a reduction in LNE after 6 months of behavioral dietary intervention. The change in countertransport activity was correlated with the change of body mass index (110).

Overfeeding and reduced physical activity are the main causes of obesity in industrialized populations. It has been shown that an increased physical activity may reduce LNE (111). Since it corrects insulin resistance, reduces total cholesterol and blood pressure, and increases HDL cholesterol, this underlines again the relationships between LNE, insulin resistance, and blood lipids.

The association with blood lipids is even stronger than the association with obesity. LNE is increased in hyperlipidemic patients compared with subjects with normal plasma lipids in many (55, 59, 109, 112, 113) but not all studies (114). LNE was positively correlated to serum total and free cholesterol (55, 112), triglycerides (55, 59, 112), and LDL cholesterol (65) and negatively correlated to HDL (59, 112). It is well known that plasma lipids are in equilibrium with cell membrane lipids and that the lipid composition of the membrane influences ion transport. LNE was in fact positively correlated to the saturated and negatively to the polyunsaturated fatty-acid content of the red-cell membrane (55, 112). The increase of V_{max} of LNE in hyperlipidemic patients may be due to differences in lipid organization in the deep hydrophobic regions of the membrane, which may affect the turnover rate of the transporter, as shown by study of membrane fluidity (115).

In patients with severe hyperlipidemia, extracorporeal LDL apheresis acutely reduced LNE (116). A strong positive correlation between LNE V_{max} and red cell membrane phosphatidilcholine content was observed in patients with elevated levels of triglyceride-rich lipoproteins (117). The transport rate of LNE was reduced when the percentage of endogenous phosphatidilcholine was reduced *in vitro* (118).

The amount of membrane phospholipids containing arachidonic acid and linoleic acid can contribute to the interindividual variability of LNE. However, treatment with the HMGCoA reductase inhibitor lovastatin significantly reduced serum total and LDL cholesterol, and increased HDL cholesterol but did not influence LNE (119). Moreover, *in vitro* enrichment of the cell membrane with cholesteryl-hemisuccinate did not affect LNE (120) nor did *in vivo* reduction of dietary saturated fats and supplementation with oleic (121) and linoleic acid-rich diets (122, 123) modify LNE, despite an increase in unsaturated fatty acid content of the red cell membrane.

Therefore, it seems that the correlation between plasma lipids and LNE is neither strong nor constant. It may be produced not by the lipid composition of the cell membrane but by the cause of hyperlipidemia itself. It is well known that hypertension and hyperlipidemia are often associated with overweight, reduced glucose tolerance, and diabetes. In normotensive individuals with mild fasting hypercholesterolemia, the major significant univariate correlates of LNE were fasting serum triglycerides, HDL cholesterol, and the ratio of fasting glucose to insulin (124). It has been proposed that the underlying mechanisms are insulin resistance and hyperinsulinemia (125). Hyperinsulinemia stimulates sodium reabsorption in the kidney, activates the sympathetic nervous system, and favors cardiovascular remodeling (see below). Hyperinsulinemia could simultaneously cause hyperlipidemia, and hypertension and activate LNE (see below), thereby creating a correlation between these parameters.

9. LNE, URIC ACID, AND RENAL PROXIMAL Na^+REAPSORPTION

In our experience with a large group of essential hypertensives, serum uric acid is significantly correlated to LNE, thus confirming previous observations (126).

The largest proportion of uric acid filtered through the glomerulus is reabsorbed with water along the proximal tubule. It is also partly reabsorbed in the ascending limb of Henle's loop and in the collecting ducts and secreted at the proximal tubule. In agreement with the hypothesis that LNE is an operating mode of the Na^+/H^+

exchange (see below), the increased serum level of uric acid in subjects with high LNE may be attributed to an increased reabsorption of uric acid along the proximal tubule, in parallel to Na^+ and water reabsorption driven by the activity of Na^+/H^+ exchange (126,127). However, it is also possible that high serum uric acid is secondary to the plurimetabolic syndrome (also named syndrome X) (128) a common finding in patients with high LNE (see below) and characterized by obesity, hypertension, reduced glucose tolerance, hyperinsulinemia, hyperlipidemia, and hyperuricemia. Following the suggestion that LNE is an operational mode of Na^+/H^+ exchange, it appears possible that the correlation between LNE and hypertension is mediated by increased proximal Na^+ reabsorption, Na^+ retention, plasma volume expansion, and increased cardiac output. Na^+/H^+ exchange is in fact responsible for the isotonic reabsorption of Na^+ and bicarbonate in the proximal tubule. A correlation between LNE and renal fractional reabsorption of Li^+, an estimate of proximal Na^+ reabsorption, was reported by Weder (129), but not by others (130, 131). However, a subgroup of hypertensive patients with high LNE showed increased proximal Li^+ reabsorption compared with hypertensives who had normal LNE (132). It is therefore possible that the relationship between LNE and Li^+ reabsorption in the kidney is not a continuous one and that increased LNE reflects proximal tubule Na^+ retention only in some patients.

10. LNE, ACTIVITY OF THE RENIN-ANGIOTENSIN SYSTEM, AND ANTIHYPERTENSIVE DRUG TREATMENT

The effects of antihypertensive drugs on LNE have scarcely been investigated (133-135). LNE was not affected by diuretics, but it was negatively correlated to serum potassium and increased in patients with diuretic- and laxative-induced hypokalemia (99,112,133). This may reflect overactivity of the renin-angiotensin system in patients with high LNE (see below), which is one of the predisposing factors to diuretic-induced hypokalemia. Hypertensives with high LNE are less sensitive to dietetic measures, such as salt and caloric restriction, and are more prone to develop hypertension when drug therapy is interrupted (81).

The first description of a positive correlation between plasma renin activity and LNE in patients with essential hypertension was provided by Brugnara *et al.* (36). Their findings were confirmed by others (79,136). More recently, Redgrave *et al*. have shown that non-modulating essential hypertensives have increased LNE (137).

Non-modulation is a clinical entity characterized by strong family history for hypertension, blunted vascular, renin, and aldosterone response to sodium load and angiotensin II infusion, and reduced ability to handle a sodium load, which favors salt sensitivity. In this subset of hypertensive patients, LNE was increased in comparison with that found in modulating hypertensives, and enalapril treatment decreased LNE to values found in normotensives (138).

Canrenone, atenolol, and captopril did not affect LNE (134), while the S2 inhibitor ketanserin, *in vivo* and *in vitro*, significantly increased it (135).

According to recent data from our laboratory, hypertensive patients with high LNE have also glomerular hyperfiltration, and higher atrial natriuretic peptide plasma levels than hypertensives with normal LNE (139). Treatment with ACE-I reduced glomerular filtration rate, plasma ANP levels and proximal tubule Na^+ reabsorption in the formers but not in the latters (139). This observation suggest that abnormalities of Na^+ transport may contribute to the pathogenesis of glomerular hyperfiltration in hypertensives with high NLE.

11. LNE, PERIPHERAL HEMODYNAMICS, AND PREGNANCY

According to Fujita *et al.* (51) and Weder *et al.* (28, 64), LNE is positively correlated to total peripheral resistances. On the contrary, in our experience, patients with insulin-dependent diabetes (IDDM) and some treated essential hypertensives with high LNE (see below) have reduced peripheral resistance and increased cardiac output, consistent with sodium and water retention (140).

During pregnancy LNE increases, but unlike the case with hypertensive patients, total vascular resistance is decreased. LNE was similar in normotensive and hypertensive pregnancy, suggesting no link between LNE and pregnancy-induced hypertension (141, 142).

12. LNE, INSULIN RESISTANCE, AND NON-INSULIN-DEPENDENT DIABETES MELLITUS

Clinical and epidemiological data show that hypertension and diabetes are often associated (143). The prevalence of hypertension among patients with non-insulin-dependent diabetes (NIDDM) is increased, only in part because of obesity and overweight. NIDDM, reduced glucose tolerance, and hyperinsulinemia are more frequent among hypertensives than among the general population. Moreover, some hypertensives with normal glucose tolerance are insulin resistant and hyperinsulinemic (125). Insulin resistance in patients with essential hypertension is pathway- and tissue-selective since it involves only glucose conversion into glycogen in the skeletal muscle (144). Hyperinsulinemia, a compensatory effect to overcome tissue resistance, may cause hypertension by salt and water retention, activate the sympathetic nervous system, and affect cation transport. In view of the association of hypertension and diabetes, a link is likely also between diabetes, reduced glucose tolerance, and insulin resistance on one side and LNE on the other.

In vitro, physiological concentration of insulin augmented the maximal transport rate (V_{max}) of Na^+/H^+ exchange (145) and LNE in red blood cells of normotensive subjects (146). An increased K_m for external Na^+ was found in hyperinsulinemic hypertensive patients in comparison with normoinsulinemic subjects (77). The main determinant of V_{max} was blood pressure; the main

determinants of K_m were blood pressure and insulin levels (77). These results are still matter of debate, since some groups could find a relation between insulin levels and LNE neither *in vivo* (147) nor *in vitro* (148). In other studies, LNE was correlated with the whole body glucose clearance rate, an index of insulin resistance, but not with insulin levels (73, 149). After treatment with metformin or metoprolol, neither LNE nor glucose disposal rate were affected (150). Therefore, it is still uncertain whether LNE may be considered a cellular marker for insulin resistance.

When measured in NIDDM patients, LNE was higher in patients with hypertension than in those with normal blood pressure. Moreover, LNE was higher in patients with macroalbuminuria than with microalbuminuria, and higher in patients with microalbuminuria than with normoalbuminuria (149-157). Andronico *et al.* studied the relationship among microalbuminuria, LNE, and insulin sensitivity, and showed that hypertensive patients with microalbuminuria had a lower insulin sensitivity index and exhibited a positive correlation between LNE and microalbuminuria (75).

13. LNE AND INSULIN-DEPENDENT DIABETES MELLITUS

Among patients with insulin-dependent diabetes (IDDM) and hypertension but without diabetic nephropathy, we have reported an increased activity of the red-cell Li^+/Na^+ and Na^+/H^+ exchange compared with that seen in normotensive IDDM (158). Larger studies have confirmed that LNE is increased in hypertensive IDDM compared to normotensive IDDM and have also shown that LNE is increased in IDDM compared with non diabetic subjects (159-161). The association between LNE and IDDM is independent from plasma renin activity, aldosterone levels and atrial natriuretic peptide plasma levels (162).

While studying patients with IDDM in good metabolic control, we were puzzled by a positive correlation between LNE and insulin daily dose. Since insulin requirement is a rough measure of insulin sensitivity, we assessed insulin sensitivity during hyperinsulinemic euglycemic clamp in patients with IDDM and found that LNE was significantly inversely correlated to glucose disposal. Similar studies were seen also in patients with essential hypertension. Hypertensives with high Li^+/Na^+exchange have a 40% reduction of whole body glucose disposal during euglycemic hyperinsulinemic clamp, indicating reduced insulin sensitivity (159).

In patients with IDDM and high LNE, the reduced insulin sensitivity could cause poor metabolic control and favor the development of diabetic nephropathy. The patients with IDDM who develop diabetic nephropathy in fact have higher LNE than the patients free of this renal complication. Quite a large number of studies were carried out in diabetic patients with and without nephropathy, and the results are univocal (160, 161, 163, 164).

LNE can be considered a cellular marker of diabetic nephropathy. Moreover, in a study carried out in 159 normotensive normoalbuminuric IDDM patients, those who exhibited higher LNE had an increased risk of developing microalbuminuria with an odds ratio of 4.2 (163).

The association of LNE with kidney disease is independent of serum triglycerides, total cholesterol, and glycemic control (165). Hyperactivity of Na^+/H^+ exchange in the kidney, reflected by high LNE in the red cells (see below), could stimulate cell growth, mesangial expansion, and glomerular hyperfiltration, thereby causing glomerulosclerosis, accounting for the risk of developing diabetic nephropathy seen in these patients (166).

Treatment with ACE inhibitor reduces progression of diabetic nephropathy. In parallel, ACE inhibition reduced LNE in nephropatic IDDM patients (167) and corrected glomerular hyperfiltration in essential hypertensives (139), confirming the link between LNE and nephropathy.

Finally, increased LNE has been documented in IDDM patients with retinopathy in comparison with IDDM patients without retinopathy (168).

14. LNE AND CARDIOVASCULAR "REMODELING"

The cardiovascular system in the hypertensive patient is remodeled to normalize wall stress. In the heart, the typical response is concentric left ventricle hypertrophy (LVH); in the resistance arteries, the wall thickness and the wall/lumen ratio are increased. It has been proposed that such "remodeling" of the cardiovascular system is due not only due to hemodynamic overload but also to growth factor stimulation and increased cellular response favored by abnormal cation transport (169). It is well known that cardiac hypertrophy cannot be accounted for by increased afterload alone. Genetic factors may be of relevance, since normotensive offspring of hypertensives with LVH have increased cardiac mass compared to normotensives offspring of hypertensive parents with no LVH (170).

Na^+/H^+ exchange plays a permissive role on cellular growth (171). After the suggestion was made that LNE was a functioning mode of the Na^+/H^+ exchange, we looked for a correlation between LVH and LNE. We compared hypertensives who had LNE levels higher than 0.4 mmol/L cell x h with hypertensives who had normal LNE but were comparable for other clinical and demographic variables (132). The former had greater interventricular septum, posterior, and relative wall thickness than the latter on echocardiography. Moreover, the former had greater kidney volume, glomerular filtration rate, and proximal sodium reabsorption, which supports the hypothesis of common cardiac and kidney abnormalities in some hypertensive patients.

An increased prevalence of LVH on electrocardiogram was also reported by Yap (65). Weder *et*

al. did not observe increased left ventricular mass on echocardiogram in patients with high LNE (28). However, they did report a reduced left ventricular internal diameter and increased relative wall thickness, which is an early indicator of cardiac remodeling.

We have also studied the relationships between LNE and cardiac "remodeling" in other clinical situations. In a group of predominantly normotensives male with IDDM, we observed a significant independent correlation between LNE and interventricular septum, posterior, and relative wall thickness (140). In a large family affected by dominant autosomic familial hypertrophic cardiomyopathy, we observed a significant correlation between LNE and interventricular septum thickness, relative wall thickness, and indexes of diastolic dysfunction. LNE was not greater in the affected members than in the unaffected. We therefore think that LNE exerts a permissive and aggravating role in the clinical manifestation of the disease. Recently, we demonstrated that the T allele of GNB3 gene, which seems to be associated with hypertension and increased Na^+/H^+ exchange, is also associated with increased left ventricular mass in young hypertensives who had never undergone treatment for hypertension (172).

15. LNE AND Na^+/H^+ EXCHANGE

It was first proposed that LNE reflects Na^+/Na^+ exchange. Subsequently it was independently hypothesized by Funder (173) and Aronson (174) that LNE represents a mode of functioning of the cell membrane Na^+/H^+ exchanger. Na^+/H^+ exchange is a membrane function involved in the regulation of cell volume, and cell pH, in the cell response to hormones, mitogens, and growth factors, and in the renal reabsorption of $Na^+$and bicarbonate (175). Increased Li^+/Na^+ could therefore reflect abnormal kinetic properties of the Na^+/H^+ exchanger, which could in theory account for the relationship between LNE and hypertension.

Examination of the effects of extracellular sodium and intracellular protons on LNE may elucidate the relationships between this exchange mode and the Na^+/H^+ exchanger. In the absence of external Na^+, cytoplasmic acidification (from internal pH 7.2 to 6.0) stimulates unidirectional Li^+ efflux (as uncoupled Li^+ efflux or Li^+-H^+ outward cotransport). In the presence of external Na^+, cytoplasmic acidification stimulates Li^+ efflux to a smaller extent, which suggests that LNE is partially inhibited, probably because in these experimental conditions the transporter is exchanging external Na^+ for internal H^+ (Na^+/H^+ exchange) and not for internal Li^+. Under these experimental conditions Li^+ efflux in Na^+ medium becomes amiloride-sensitive.

After the proposal that LNE may represent an operational mode of Na^+/H^+ exchange, various attempts were made to confirm this hypothesis and to show heterogeneity of Na^+/H^+ exchange, which may account for the variability of LNE. Technically, the problem is made difficult by the fact that Na^+/H^+ exchange is a "housekeeping" function largely diffused among all the eukaryotic cells. The problem was therefore approached indirectly, by comparing the kinetic properties of the two transport modes and the effects of inhibitors. In sarcolemmal vesicles from the bovine superior mesenteric artery, Na^+/H^+ and Na^+/Li^+ exchange are probably mediated by the same transport system, as demonstrated by inhibition studies (both are inhibited by ethylisopropylamiloride) (176, 177).

Striking kinetic similarities were shown between human and rabbit red cells (178). It was demonstrated that both are asymmetrical, and have a higher affinity for H^+ and Li^+ than for Na^+. Na^+/H^+ exchange is only partially sensitive to amiloride, while LNE is insensitive (179). However, some amiloride sensitivity of the LNE can be elicited by proton activation.

Using nuclear magnetic resonance and $^7Li^+$, Na^+/Li^+ exchange was inhibited by pH gradient, presumably because of the competition between Li^+ and H for transport in the same protein; moreover amiloride had a similar inhibitory effect on both $Na^+$and Li^+ binding site to red blood cell membrane (180).

Various of evidence suggests that high LNE does not simply reflect increased maximal velocity of translocation of the Na^+/H^+ exchange. In man, LNE is more than 100 times slower than Na^+/H^+ exchange and their maximal velocities are only weakly correlated (66).

Both red blood-cell Na^+/H^+ and Li^+/Na^+ exchange rates are higher in hypertensive than in normotensive individuals. Na^+/H^+ exchange and Li^+/Na^+ exchange were significantly and positively correlated in red blood cells from hypertensive patients with insulin-dependent diabetes mellitus (66). However, kinetic heterogeneity of the red-cell Na^+/H^+ exchange has been shown in patients with essential hypertension (181). Hypertensives classified as "high LNE" according to red-cell LNE above the mean of the study group showed higher blood pressure and significantly different kinetic properties of the red cell Na^+/H^+ exchange but similar V_{max}. These data suggest that an increased LNE is more likely to reflect abnormal kinetic properties of Na^+/H^+ exchange (reduced affinity for internal H+ and low Hill's number) than an increased number of transport units (182). These kinetic abnormalities may facilitate Li^+ movement across the membrane at physiological cell pH (7.2).

Ng *et al.* showed that red blood-cell LNE was strongly correlated to the level of NHE-3 protein and was negatively related to level of NHE-1 protein expressed in the proximal tubules (183). Siffert *et al.* injected NHE-1 cRNA in Xenopus laevis oocytes, and found that expression of the NHE-1 induces the expression of LNE (184).

As a whole, this body of evidence provides evidence that LNE is mediated by the cell membrane Na^+/H^+ exchange. Increased activity of the cell membrane Na^+/H^+ exchange could contribute to the development of hypertension and to its complications by facilitating vascular smooth muscle cell contraction and growth, cardiac contractility and hypertrophy, and, finally, salt and

water retention by the kidney (175). However, we must admit that, to date, the definite proof is still missing.

16. PERSPECTIVES

Among the various alterations of cell membrane cation transport reported in hypertension, the increase of red-cell LNE has been most widely investigated and confirmed. Moreover, it raised interest in the study of cation transport in hypertension.

The pathophysiological significance of this finding is still incomplete. The transport protein and its gene have not been identified. Moreover, we do not even know whether abnormal NLE activity is primary or secondary to other alterations of the cell metabolism. Our long-standing hypothesis is that the increase of LNE reflects abnormal kinetic properties of the Na^+/H^+ exchange, either structural or functional (related to the phosphorylation of the transporter and to cytoplasmic calcium) (185, 186).

It seems unlikely that the alterations of NLE are secondary to heterogeneity of Na^+/H^+ exchange, since Lifton could not show mutation of Na^+/H^+ exchange gene in subjects with high NLE (187). Other genes could be involved, and this is still under investigation.

Altered phosphorylation of the transporter secondary to protein kinase activation or phosphatase inactivation is another possibility. In fact, Angiotensin II, insulin, and growth factors activate Na^+/H^+ exchange through direct or indirect activation of membrane protein kinases (185, 188).

Calcium activates Na^+/H^+ exchange (189) and LNE (190) while Na^+/H^+ exchange on its own modulates free cytoplasmic calcium (191). It is therefore likely that the high Li^+/Na^+ and Na^+/H^+ exchange noted in many hypertensive patients reflects a genetically determined alteration of the phosphorylation state of the cell membrane and/or increased cytoplasmic calcium.

Regardless of the pathophysiological relevance of the LNE for the development of hypertension, its measurement could be of clinical use. Its association with severe and drug-resistant hypertension, insulin resistance, vascular and cardiac hypertrophy, hyperlipidemia and obesity, positive family history for hypertension, and for major cardiovascular accidents suggests that high LNE could be a biochemical marker for increased cardiovascular risk. This possibility still awaits further inquiry.

Finally, NLE is an intermediate phenotype of hypertension and its associated metabolic abnormalities, often collectively identified as “the metabolic syndrome”. Therefore, it could be a useful tool for genetic association studies in cardiovascular medicine.

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Abbreviatiations: LNE: Li^+/Na^+exchange; BMI: body mass index; NIDDM: non insulin dependent diabetes mellitus; IDDM: insulin dependent diabetes mellitus; LVH: left ventricle hypertrophy; V_{max}: maximal velocity of translocation; K_m: affinity constant for extracellular Na^+

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