

Erythrocyte sodium/lithium countertransport and renal lithium clearance in a random sample of untreated middle-aged men

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SUMMARY

1. It has been proposed that the enhanced erythrocyte Na^+/Li^+ countertransport observed in many patients with essential hypertension could be a marker of abnormal renal proximal tubular function. We thus investigated the relationship of blood pressure and Na^+/Li^+ countertransport to an index of proximal tubular function such as renal Li^+ clearance.

2. The study was carried out in a sample of 299 untreated male subjects (aged 21–59 years) randomly selected from a population at work. Na^+/Li^+ countertransport was measured in a representative sub-group of 176 men.

3. We did not detect statistically significant correlation of either blood pressure or Na^+/Li^+ countertransport (V_{max}) with fractional excretion of Li^+ , while confirming the existence of a significant continuous association of blood pressure and body mass index with Na^+/Li^+ countertransport ($P < 0.01$).

4. A sub-sample of 57 participants belonging to the lowest or the highest quintiles of Na^+/Li^+ countertransport distribution repeated the Li^+ clearance study after moderate Na^+ restriction.

5. Although fractional excretions of Na^+ and Li^+ were reduced on the low Na^+ diet (both $P < 0.001$), they did not differ significantly between groups.

6. Our results are at variance with the findings of a recent case-control study in a young age group and suggest that further studies are necessary before a conclusion can be drawn as to the suitability of Na^+/Li^+

countertransport as a marker of Na^+ reabsorption in the renal proximal tubule.

Key words: blood pressure, lithium clearance, sodium/lithium countertransport.

Abbreviations: BMI, body mass index; CNT, countertransport; DBP, diastolic blood pressure; FE_{Li} , fractional excretion of Li^+ ; FE_{Na} , fractional excretion of Na^+ ; GFR, glomerular filtration rate; Mops, 4-morpholinepropanesulphonic acid; SBP, systolic blood pressure.

INTRODUCTION

Enhanced erythrocyte Na^+/Li^+ countertransport (CNT) has been found to be associated with reduced renal fractional excretion of Li^+ (FE_{Li}), in young patients with arterial hypertension [1]; a similar relationship has been described in the offspring of hypertensive patients [2]. As Li^+ clearance is a putative index of fluid and Na^+ handling in the proximal tubule [3], these data seem to support the hypothesis that the increased activity of Na^+/Li^+ CNT *in vitro* observed in the erythrocytes of patients with essential hypertension [4–15] would be a marker of enhanced Na^+ and fluid reabsorption in the kidney proximal tubule of these same patients [16]. As a continuous relationship between blood pressure and the maximal velocity (V_{max}) of Na^+/Li^+ CNT has been observed in several population-based surveys [17–19], we sought to determine whether a statistically significant association existed between Na^+/Li^+ CNT and renal FE_{Li} in a representative sample of the untreated adult male population. It was felt that this approach could provide additional information with respect to a case-control design as it largely eliminates the problems related to subject selec-

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tion and allows exploration of a putative biological correlation throughout a broad range of distribution of the variables of interest.

SUBJECTS AND METHODS

This investigation was part of a nationwide survey of the prevalence of cardiovascular risk factors in Italy, sponsored by the National Research Council (ATS-RF2) and started in 1976–1977 [20–22]. It consisted of two consecutive studies both carried out at the Olivetti factory in a suburban area of Naples, where 1200 male workers are employed.

Study 1

Between April 1987 and July 1987, a sample of 360 men was randomly selected from the total population of 1200 male workers. Thirty-eight subjects were not considered for this study because they were receiving treatment with antihypertensive medication. Of the remaining 322 subjects, complete data were available for 299 who are the subject of the present report.

The examinations were carried out in the morning, with the subjects fasting, in a quiet and comfortable room within the factory premises, between 08.00 hours and 11.00 hours. Body weight and height were measured with a standard beam balance scale and an attached ruler, the subjects wearing light indoor clothing and no shoes. The body mass index (BMI) was calculated as the weight/height ratio (kg/m^2). Blood pressure was measured after the subject had been sitting upright for 10 min. Systolic (SBP) and fifth phase diastolic (DBP) pressures were measured three times 2 min apart with a random zero sphygmomanometer (Gelman Hawksley Ltd.) [23] by well-trained observers who had attended blood pressure training sessions for standardization of the reading procedure. The first reading was discarded and the average of the last two measurements for SBP and DBP was recorded and used in the analysis.

On the day before the visit, the subjects consumed their evening meal at no later than 19.00 hours and took a lithium carbonate capsule (300 mg; Carbolithium, IFI), delivering 8.1 mmol of elemental Li, at 22.00 hours with 400 ml of water. On the morning of the study, a fasting timed urine collection was performed; the subjects ingested 400 ml of water at the beginning and at the mid-point of the collection. The volume and length of the collection were recorded and specimens were taken for determination of Na^+ , Li^+ and creatinine. Average collection time was 214 ± 49 min (mean \pm SD) and collection volume 282 ± 162 ml. At the mid-point of the urine collection, a blood sample was obtained by venepuncture without stasis, for determination of Na^+ , Li^+ , creatinine and, in a randomly selected sub-group of 176 subjects, of Na^+/Li^+ CNT. During the test the subjects were ambulant and were allowed to carry out their usual activities within the factory premises; they were instructed to remain fasting and to abstain from alcohol or caffeine-

containing beverages, from smoking and from vigorous exercise throughout the study period.

Study 2

As it has been suggested [1] that the strength of the biological correlation between Na^+/Li^+ CNT and proximal tubule Na^+ reabsorption could be underestimated due to the fact that Na^+/Li^+ CNT *in vitro* is measured at maximal velocity whereas Na^+ reabsorption is not, it was decided to repeat the measurements of both Na^+/Li^+ CNT and FE_{Li} in a sub-sample of study 1 participants after 3 days of moderate dietary Na^+ restriction, i.e. in a setting where proximal Na^+ reabsorption was stimulated to a greater degree. This sub-sample consisted of two sub-groups, namely the subjects in the highest quintile of the Na^+/Li^+ CNT distribution in study 1 (Na^+/Li^+ CNT $> 368 \mu\text{mol h}^{-1} \text{l}^{-1}$ of cells, $n = 40$) and those in the lowest quintile (Na^+/Li^+ CNT $< 252 \mu\text{mol h}^{-1} \text{l}^{-1}$ of cells, $n = 39$). The use of quintiles to identify two groups of individuals with widely different values of Na^+/Li^+ CNT seemed more sound than the alternative choice of defining arbitrary cut-off points for this variable, since there is no criteria other than a statistical one whereby a particular value of Na^+/Li^+ CNT could be judged as 'abnormally' high or low. The use of the two lowest quintiles as a control group for the highest quintile was advantageous in providing a larger control group, thus reducing the risk that a difference between the two groups could be due to an abnormality peculiar to the individuals at the low extreme of the distribution rather than to those with higher Na^+/Li^+ CNT.

Fifty-seven of the 79 men invited accepted to participate and completed this second part of the study. They were prescribed for 3 days a balanced 2000 kcal (8368 kJ) diet with a nominal Na^+ content of 2 g and were invited not to add salt at table and possibly in the preparation of food. They were instructed to collect 24 h urine samples both on the day before they started on the low Na^+ diet and on the third day of the diet. In the morning of the fourth day of the diet fractional excretion of Na^+ (FE_{Na}) and FE_{Li} were determined using identical procedures to those in study 1. The average collection time for the fasting urine collection obtained in the morning was 225 ± 22 min (mean \pm SD) and the average volume was 265 ± 150 ml.

The V_{max} of erythrocyte Na^+/Li^+ CNT was determined by the method of Canessa & Tostesou [23a] with modifications as previously described [24]. Briefly, 4 ml of heparinized blood was centrifuged at 2000 g for 10 min at 4°C. The packed erythrocytes were repeatedly washed with ice-cold choline chloride solution [150 mmol/l choline chloride, 10 mmol/l Tris-4-morpholinepropane-sulphonic acid (Mops) (pH 7.4), 1 mmol/l MgCl_2]. Washed cells (1 ml) were incubated for 3 h at 37°C with a Li^+ -loading solution [150 mmol/l LiCl, 10 mmol/l glucose, 10 mmol/l Tris-Mops (pH 7.4)]. The loaded cells were washed five times with ice-cold choline solution to remove extracellular Li^+ . Aliquots of loaded erythrocytes were then incubated at 37°C in one of two media: Na^+ -

efflux media, containing 150 mmol/l NaCl, 1 mmol/l MgCl₂, 10 mmol/l Tris-Mops (pH 7.4), 0.1 mmol/l ouabain and 10 mmol/l glucose, or choline chloride media, containing 150 mmol/l choline chloride in place of NaCl. Triplicate samples were removed from the incubation at 0 and 60 min and centrifuged at 2000 g at 4°C for 10 min. The Li⁺ concentration was determined in the supernatants by atomic absorption spectrophotometry (Perkin-Elmer model 300, Norwalk, CT, U.S.A.); the difference between the rate of efflux in NaCl versus choline chloride media was taken as the Na⁺-dependent maximal Li⁺ efflux rate.

Blind duplicate measurements, submitted every day to the laboratory on a one-in-ten basis throughout the study, yielded a technical error of 8.3%. The mean day-to-day coefficient of variation, determined by repeated measurements (at least eight) in the same subject on different days over the course of the study, was 9.5%.

Serum and urinary creatinine concentrations were measured by the picric acid colorimetric method, serum and urinary Li⁺ by atomic absorption spectrophotometry, and serum and urinary Na⁺ by an ion-selective electrode (Beckman, EA2). Throughout this paper, unless otherwise stated, the Na⁺ and Li⁺ clearances are expressed as fractional excretions (FE_{Na}, FE_{Li}), i.e. as the ratio of the clearance of the given substance to the clearance of creatinine (used as an estimate of the glomerular filtration rate, GFR) × 100. Preliminary studies [25] of plasma Li⁺ kinetics, performed under experimental conditions similar to those of the present study, had shown linearity ($r = -0.99$) of the logarithmic serum Li⁺ concentration with time between 9 and 17 h after a 300 mg dose of lithium carbonate, a finding which allowed the determination of Li⁺ clearance using a single value of serum Li⁺ concentration measured at the mid-point of the urine collection. A methodological assessment of the variability of FE_{Li}, determined by multiple measurements repeated

several days apart in free-living subjects on an unrestricted diet, has provided intra- and inter-individual coefficients of variation of 8.5% and 14.7%, respectively, with a ratio of intra-individual to inter-individual variance of 0.33; this value is low enough to allow reasonably good characterization of an individual in a population even with a single measurement [25].

Results are expressed as means ± SD, unless otherwise stated. The statistical analysis was performed by standard methods [26] using a Commodore PC-10 computer system and the Northwestern University Statistical Package for the Social Sciences (SPSS-PC).

RESULTS

Study 1

Complete data were available for 299 subjects, apart from Na⁺/Li⁺ CNT. Table 1 gives the mean values, SD values and 5th and 95th percentile values for the variables investigated in the whole study population ($n = 299$) and in the sub-group in which Na⁺/Li⁺ CNT was measured ($n = 176$). It is apparent that the latter sub-group was representative of the whole study population with respect to all the variables specified. Ninety-five per cent of the sample were 35–55-year-old men; 21% had a DBP above 95 mmHg (12.66 kPa) but only four subjects had a DBP of more than 110 mmHg (14.7 kPa). The mean value and SD of FE_{Li} were similar to those found previously in healthy volunteers [25].

Na⁺/Li⁺ CNT was significantly and directly associated with SBP ($r = 0.20$, $P < 0.01$), BMI ($r = 0.19$, $P < 0.05$) and DBP ($r = 0.16$, $P < 0.05$). It was not correlated with FE_{Li} ($r = 0.01$), FE_{Na} ($r = 0.02$) and GFR ($r = 0.01$). FE_{Li} was also unrelated to either SBP ($r = 0.01$) or DBP ($r = 0.07$).

Table 2 shows a comparison of the subjects in the top quintile ($n = 40$) with those in the two lower quintiles ($n = 72$) of the Na⁺/Li⁺ CNT distribution, with regard to

Table 1. Characteristics of the whole study population and of the sub-group in whom erythrocyte Na⁺/Li⁺ CNT was measured

	Whole study population ($n = 299$)				Na ⁺ /Li ⁺ CNT subgroup ($n = 176$)			
	Mean	SD	Percentile		Mean	SD	Percentile	
			5th	95th			5th	95th
Age (years)	46.0	6.1	38	55	46.0	6.6	38	55
Height (cm)	168.4	6.1	159	178	168.5	5.8	159	179
Weight (kg)	73.8	9.8	59	91	74.2	9.9	59	90
BMI (kg/m ²)	26.0	3.0	21	31	26.1	3.0	21	31
SBP (mmHg) (kPa)	126.0 (16.79)	15.7 (2.09)	104 (13.86)	152 (20.26)	126.9 (16.91)	16.8 (2.24)	104 (13.86)	157 (20.93)
DBP (mmHg) (kPa)	86.7 (11.56)	10.2 (1.36)	70 (9.33)	105 (13.99)	87.1 (11.61)	10.6 (1.41)	71 (9.46)	106 (14.13)
Serum creatinine (μmol/l)	98.0	10.0	85	112	98.0	10.0	85	112
GFR (ml min ⁻¹ m ⁻²)	52.2	11.1	33	70	52.2	11.1	33	70
Erythrocyte Na ⁺ /Li ⁺ CNT (μmol h ⁻¹ l ⁻¹ of cells)	—	—	—	—	298	95	150	470
FE _{Na} (%)	1.10	0.51	0.48	1.88	1.12	0.42	0.48	2.00
FE _{Li} (%)	24.7	5.6	17.3	35.1	24.5	5.9	15.9	36.1

Table 2. Variables selected by quintile of erythrocyte Na⁺/Li⁺ CNTResults are means \pm SD. Statistical significance: * $P < 0.05$, ** $P < 0.025$.

	Na ⁺ /Li ⁺ CNT quintile	
	I+II (108–251 $\mu\text{mol h}^{-1}\text{l}^{-1}$ of cells)	V (368–598 $\mu\text{mol h}^{-1}\text{l}^{-1}$ of cells)
No.	72	40
Age (years)	45.5 \pm 6.3	45.4 \pm 6.7
Height (cm)	169.0 \pm 6.2	167.6 \pm 5.7
Weight (kg)	73.9 \pm 9.5	76.8 \pm 11.8
BMI (kg/m ²)	25.8 \pm 2.7	27.3 \pm 3.8**
SBP (mmHg) (kPa)	123.5 \pm 16.1 (16.46 \pm 2.14)	131.3 \pm 18.8** (17.50 \pm 2.50)
DBP (mmHg) (kPa)	85.4 \pm 8.6 (11.38 \pm 1.14)	89.0 \pm 10.7* (11.86 \pm 1.42)
Serum creatinine ($\mu\text{mol/l}$)	96.0 \pm 9.0	99.0 \pm 11.0
GFR (ml min ⁻¹ m ⁻²)	53.3 \pm 10.6	53.4 \pm 10.2
FE _{Na} (%)	1.12 \pm 0.44	1.17 \pm 0.93
FE _{Li} (%)	24.5 \pm 6.2	24.6 \pm 5.3

the other variables under study. In accordance with correlation analysis, significant differences were found in SBP ($P < 0.025$), BMI ($P < 0.025$) and DBP ($P < 0.05$). The ranges of SBP were 111–198 mmHg (14.80–26.40 kPa) for the highest quintile and 101–153 mmHg (13.47–20.40 kPa) for the two lowest quintiles; the ranges of DBP were, respectively, 73–124 mmHg (9.73–16.3 kPa) and 73–107 mmHg (9.73–14.27 kPa).

No differences between the two groups were found for age, weight, height, serum creatinine, FE_{Na} and FE_{Li}.

Study 2

The two sub-groups of subjects with, respectively, high ($n = 29$) and low ($n = 28$) Na⁺/Li⁺ CNT participating in this second study were comparable with regard to age (45.2 \pm 7.3 vs 43.4 \pm 7.2 years), BMI (27.5 \pm 3.8 vs 25.8 \pm 2.3 kg/m²) and GFR (53.8 \pm 10.7 vs 55.9 \pm 14.2 ml min⁻¹ m⁻²) (Fig. 1). The former had, however, higher blood pressures [SBP = 132.6 \pm 16.9 mmHg (17.67 \pm 2.25 kPa) vs 122.0 \pm 14.7 mmHg (16.26 \pm 1.96 kPa), $P < 0.02$; DBP = 91.0 \pm 9.8 mmHg (12.13 \pm 1.31 kPa) vs 84.2 \pm 7.2 mmHg (11.22 \pm 0.96 kPa), $P < 0.005$]. As shown in Fig. 1, both groups reduced their Na⁺ intake to a similar extent; in turn, FE_{Na} was similarly reduced on the low Na⁺ diet in the two groups, whereas GFR was substantially unchanged. FE_{Li} fell significantly in both groups on the low Na⁺ diet; nevertheless, there was no significant difference between groups on the low Na⁺ diet (18.8 \pm 4.9 vs 20.4 \pm 6.0%) or between those on their customary diet (23.7 \pm 5.2 vs 25.6 \pm 5.2%).

DISCUSSION

The statistically significant direct associations of Na⁺/Li⁺ CNT with blood pressure and BMI observed in this study

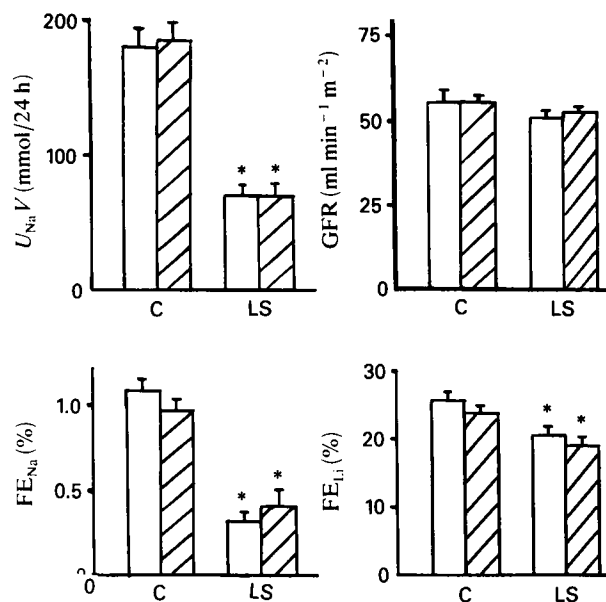


Fig. 1. Twenty-four hour urinary sodium excretion ($U_{\text{Na}}V$), GFR, FE_{Na} and FE_{Li} in subjects with low ($n = 28$; □) or high ($n = 29$; ▨) Na⁺/Li⁺ CNT on their usual diet (C) and on a low Na⁺ diet (LS). Results are expressed as means \pm SEM. Statistical significance: * $P < 0.001$ compared with usual diet.

are in line with previous observations [17–19]; however, the lack of significant correlations of the FE_{Li} with blood pressure and Na⁺/Li⁺ CNT is at variance with the results of a case-control study recently published [1]. The protocol for the measurement of Li⁺ clearance in our study was very similar to that reported by Weder [1], but the two studies are otherwise different in several important respects. First, while that of Weder [1] was a case-control comparison of young hypertensive patients ($n = 14$) with age-matched normotensive volunteers ($n = 31$), our study was a population-based cross-sectional investigation of a random sample of middle-aged men. Secondly, the subjects admitted to our study had never received antihypertensive treatment; this permitted the exclusion of any possible carry-over effect of blood pressure-lowering drugs, particularly diuretics, on FE_{Li}. Individuals with hypertension of a more severe degree were, however, under-represented in our study population as the majority of these were found to be already on pharmacological treatment, a consequence of the increased awareness among both patients and doctors of hypertension and the need for therapy. Each of these points of difference may have contributed to the discrepant results between the two studies.

During our Li⁺ clearance studies the subjects were ambulant and their diet was not standardized; this was also the case in the study mentioned above [1]. Both these factors could affect proximal tubular function; however, we have previously found that in a sample of normal volunteers, under similar experimental conditions, the risk of misclassifying individuals within the population

from which they derive is relatively low even with a single clearance measurement, the ratio of the intra- to inter-individual variance for FE_{Li} being only 0.33 [25].

It has also been observed that, whereas Na⁺/Li⁺ CNT is measured *in vitro* at maximal velocity, proximal tubular reabsorption of Na⁺ is not [1]; thus it could be argued that relatively subtle differences in kidney tubular function could go unrecognized under normal circumstances, e.g. in free-living subjects on their customary Na⁺-rich diets. To test this hypothesis, in study 2 we repeated the Li⁺ clearance measurements in two sub-groups of individuals, taken from the highest and the lowest quintiles of the Na⁺/Li⁺ CNT distribution, during moderate dietary Na⁺ restriction, i.e. a condition where tubular Na⁺ reabsorption is stimulated. Li⁺ clearance does not lose its value as a selective index of proximal tubule Na⁺ handling under these conditions; although it has been reported that on very low Na⁺ intakes some distal tubular Li⁺ reabsorption does occur [27], this does not appear to be the case for moderate Na⁺ restriction effected in our study [28, 29].

As expected, the reduction in Na⁺ intake was associated with a significant fall in Li⁺ clearance, indicating more active Na⁺ and fluid reabsorption in the proximal tubule; this phenomenon, however, occurred to a similar degree in both groups, despite their large difference in maximal velocity of Na⁺/Li⁺ CNT.

Therefore, the present study of a large sample of untreated male workers did not demonstrate statistical associations of Na⁺/Li⁺ CNT and blood pressure with FE_{Li}, a putative index of proximal tubular Na⁺ and water handling. One possible conclusion might be that no biological correlation exists between Na⁺/Li⁺ CNT and water and Na⁺ handling in the proximal tubule, at least across a population sample with the characteristics of the one we studied. The alternative possibility, however, that under the conditions of this study, the Li⁺ clearance was inadequate for detection of such a relationship cannot, in principle, be ruled out; it is of interest in this regard to note the finding of Feig *et al.* [30], who detected a significant difference in the Na⁺/H⁺ exchange *in vitro* by lymphocytes from spontaneously hypertensive and Wistar-Kyoto rats, but were unable to find any difference when Na⁺ in the system was replaced by Li⁺.

It is apparent from the present study that it may not be safe to extrapolate to the activity of the Na⁺/H⁺ exchange system of the renal tubular epithelia from the erythrocyte Na⁺/Li⁺ CNT *in vitro* and that further studies are needed to investigate a possible relationship between this Na⁺ transport system and kidney tubular function.

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