The concomitants of elevated erythrocyte sodium—lithium countertransport activity in diabetic nephropathy: a critical assessment

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Abstract. Elevated erythrocyte sodium—lithium countertransport activity is an intermediate phenotype of essential hypertension among Caucasians, and may also associate with kidney disease in type 1 (insulin-dependent) diabetes mellitus. Evidence supporting the hypothesis that an inherited predisposition to essential hypertension may thus partly identify with the genetic background of susceptibility to diabetic nephropathy is, however, controversial. This review discusses the possible points of controversy, with emphasis upon the need to standardize the manifest heterogeneity in the current techniques of measurement, as well as upon the clinical concomitants and interpretation of elevated sodium—lithium countertransport activity in type 1 diabetes mellitus. Large family studies may be required in order to single out the independent contributions of genes and environment to sodium—lithium countertransport activity in type 1 diabetes mellitus. However, the original hypothesis that genes underlying elevated sodium—lithium countertransport in essential hypertension and in diabetic nephropathy may also reflect in part a predisposition to diabetic kidney disease cannot be rejected on the basis of current evidence.

Key words: Diabetic nephropathy — Hypertension — Microalbuminuria — Na⁺/Li⁺ countertransport — Type 1 diabetes mellitus

Introduction

Evidence that the onset of clinical proteinuria in type 1 (insulin-dependent) diabetic patients can be explained partly by a predisposing genetic background mainly derives from the incidence pattern of proteinuria in longitudinal cohort studies [1, 2]. The cumulative incidence of proteinuria attains a plateau when duration of diabetes approaches 25–30 years, by which time only about 30%–40% patients have developed this complication. Thus, surviving longer than 30 years with type 1 diabetes without developing proteinuria appears to be associated with a strongly reduced risk of kidney disease, suggesting that the metabolic abnormalities of diabetes may represent a necessary but insufficient explanation for the pathogenesis of nephropathy, and that genetic factors may be involved in conferring susceptibility to diabetic kidney disease. This hypothesis has been more directly supported by the observation that siblings with type 1 diabetes mellitus often share renal destiny [3], but the nature of the predisposing genes eludes identification.

The finding that parental blood pressure may be higher among type 1 diabetic patients with proteinuria than among normoalbuminuric patients of similar age and duration of diabetes initially suggested that an inherited susceptibility to essential hypertension may partly explain the risk of diabetic nephropathy [4]. Furthermore, two studies simultaneously and independently associated elevated erythrocyte sodium—lithium countertransport activity (Na⁺/Li⁺ CT) in type 1 diabetic patients with nephropathy [5, 6], and were soon followed by the finding of similarly elevated Na⁺/Li⁺ CT activity in their parents [7] as well as in patients with microalbuminuria [8].

Elevated erythrocyte Na⁺/Li⁺ CT is a known concomitant of essential hypertension [9], with a bimodal distribution pattern in the general population [10, 11]. Heritability estimates among Caucasian pedigrees suggest that about 80% of inter-individual variability in Na⁺/Li⁺ CT can be explained by a major autosomal gene with polygenic effects, while only 20% residual variability seems to be accounted for by environmental components [12]. These characteristics confer on Na⁺/Li⁺ CT the properties of an intermediate phenotype of essential hypertension among non-diabetic individuals, and underlie the suggestion that an inherited predisposition to essential hypertension may partly identify with the genetic component in the susceptibility to diabetic kidney disease [5–7]. This hypothesis is also supported by prelimi-
nary observations in our patients [13–15]. At variance with this hypothesis, three investigations failed to document elevated Na⁺/Li⁺ CT either in type 1 diabetic patients with nephropathy [16, 17] or in their parents [18], and suggested that initial findings could be biased by as yet experimentally unproven major environmental components.

However, both methodological and conceptual considerations seem to indicate that the original hypothesis cannot be rejected on the basis of these data. Our view of the present controversy is briefly outlined here, and some of the major issues discussed with special reference to those metabolic abnormalities of type 1 diabetes of putative environmental relevance to Na⁺/Li⁺ CT. The possible relevance of Na⁺/Li⁺ CT to sodium-hydrogen (Na⁺/H⁺) exchange and to the pathophysiology of arterial hypertension and diabetic nephropathy has been recently and extensively reviewed by other authors [19–21], and will not be examined here.

**Measurement of erythrocyte Na⁺/Li⁺ CT: the need for a standardized technique**

Evidence that type 1 diabetic patients with nephropathy of any degree may indeed present with elevated Na⁺/Li⁺ CT now rests upon the cumulative observation of over 150 cases versus as many well-selected controls at low risk of nephropathy (as suggested by normoalbuminuria) but of similar age, gender and duration of diabetes [5, 6, 8, 14] – including a study where statistical significance was just missed [18]. Na⁺/Li⁺ CT was also found to be elevated in hypertensive type 1 diabetic patients without proteinuria, though the possible effect of microalbuminuria was not sought in this independent study [22]. Median Na⁺/Li⁺ CT was approximately 50%–80% higher among patients with nephropathy than among patients at lower renal risk in these studies.

On the other hand, evidence that Na⁺/Li⁺ CT may be largely independent of the presence of nephropathy among type 1 diabetic patients presently accounts for a substantially lower and unbalanced number of observations, i.e. 72 cases and 30 controls [16, 17].

There are differences in the selection criteria and in the clinical features of patients between studies, but these are relatively small and certainly cannot explain such divergent findings. However, a definite heterogeneity in the techniques used to measure Na⁺/Li⁺ CT appears to parallel the heterogeneity in results. All of the studies reporting that Na⁺/Li⁺ CT may be frankly elevated in diabetic nephropathy used a basically similar technique [5–7, 14], relying on the measurement of Li⁺ efflux rates in the supernatant of erythrocyte suspensions at low haematocrit by atomic absorption after a relatively short incubation time [9]. Identical techniques were also employed in the general population and pedigree studies in which the genetic component and clinical concomitants of Na⁺/Li⁺ CT were originally described [10, 12]. In contrast, studies failing to observe significantly higher Na⁺/Li⁺ CT in diabetic nephropathy relied upon methods different in several respects from the original technique.

Some of these differences are: the choice of lithium carbonate rather than lithium chloride to load erythrocytes with lithium, and/or the study of Li⁺ efflux either in erythrocyte suspensions at relatively high haematocrit (12%–16% vs 3%–4%) or after prolonged incubation times from 30 min up to 120 min, and/or the detection of Li⁺ concentration by ordinary emission flame photometry rather than by atomic absorption [16–18]. To our knowledge, no exhaustive methodological study has accurately compared and validated such measurements to investigate whether any bias in the techniques exists and is relevant.

Anecdotal evaluations in our laboratory, where Na⁺/Li⁺ CT measurements are routinely done using the classic technique, suggest that linearity of Li⁺ efflux may only be apparent when plotted versus incubation time (Figs. 1, 2). Li⁺ efflux rate is geometrically represented by the slope of the regression line fitting Li⁺ concentration in efflux buffers against incubation time, and routine calculations rest upon duplicate measurements of Li⁺ concentration at three different incubation times. In these preliminary experiments we attempted to model Li⁺ efflux as a function of incubation time by measuring Li⁺ concentration in usual efflux buffers every 10 min up to total incubation time of 90 min, thus allowing observation of 10 sequential points in Li⁺ efflux both in sodium and in magnesium media. Deviation from linearity may appear very small in conventional plots of Li⁺ concentration versus incubation time (Fig. 1), but is manifest and significant when changes in Li⁺ concentration (i.e. point-by-point calculated Li⁺ efflux rates) are plotted, again versus incubation time (Fig. 2). Indeed, this curvilinear efflux pattern could be identified from initial scatter plots, where efflux measurements at 0, 15 and 30 min did not fit the same line even though the coefficient of correlation approached 1, forcing data into a linear regression

![Fig. 1. Study of lithium (Li⁺) efflux from Li⁺-loaded erythrocytes from 11 healthy individuals in sodium (filled circles) and in magnesium-sucrose (open circles) buffers. Each point represents mean±SE Li⁺ concentration after the corresponding incubation time. The fit of mean values was highly significant at linear regression analysis (sodium buffer: \( y = 4.32 + 0.291 \times, r^2 = 0.998 \); magnesium-sucrose buffer: \( y = 4.51 + 0.15 \times, r^2 = 0.984 \), but intercepts were significantly higher than mean Li⁺ concentration, as directly measured at 0 min (\( P < 0.01 \) in both efflux buffers).](image-url)