Novel strategies in hypertension research have to consider that ‘essential’ hypertension is not a pathological entity. Recent efforts to define subgroups of patients with essential hypertension have focused on ion transport abnormalities and this approach appears promising in terms of identifying candidate genes which may contribute to the development of high blood pressure. A considerable body of work has been devoted to the Na+/H+ exchanger isoform 1 (NHE-1), a membrane ion transport protein which is ubiquitously expressed and which serves intracellular pH regulation, cell volume control, and transepithelial Na+ transport (for review see [1]). Recent studies have established that the NHE-1, besides mediating Na+/H+ exchange, can also work in the mode of a Na+/Li+ countertransporter [2]. This property is important in view of earlier findings which demonstrated that enhanced Na+/Li+ countertransport is an important genetic trait in essential hypertension.

A variety of studies has confirmed that the activity of the NHE-1 is enhanced in erythrocytes, leukocytes, platelets, and in skeletal muscle of patients with essential hypertension (for review see [3]) and it appears that this abnormality may exist in almost 50% of all patients with essential hypertension (for review see [4]). This ‘enhanced NHE-1 phenotype’ is apparently associated with an increased body mass index, insulin resistance, left ventricular hypertrophy, renal Na+ retention, low plasma renin, reduced suppression of plasma renin activity and aldosterone upon saline infusion, and an increased risk of diabetic nephropathy in patients with Type 1 diabetes mellitus [4].

These findings have raised the important question regarding the mechanisms underlying the enhanced NHE-1 activity in essential hypertension. Since this ion transport, as stated above, is enhanced only in a certain group of patients with essential hypertension, it appeared very unlikely that this abnormality is just another consequence of elevated blood pressure itself. Nevertheless, multiple mechanisms could contribute to its abnormal behaviour and the factors which had to be considered could be grouped into ‘systemic’, ‘genetic’, and ‘intracellular’ [4]. Recent studies have confirmed that in vivo NHE-1 activity is not static, but regulated by influences which could be termed ‘systemic’. These factors include metabolic acidosis [5] as well as increased consumption of sodium chloride [4] which both increase NHE-1 activity and/or NHE-1 mRNA expression. Furthermore, ‘genetic’ reasons, e.g. mutations in the NHE-1 gene or an overexpression of the NHE-1 in essential hypertension, had to be taken into consideration. Finally, because NHE-1 activity is under the control of multiple intracellular signal transduction pathways including activation by different protein kinases and Ca2+-calmodulin [1], specific abnormalities in such signal transduction pathways in essential hypertension (‘intracellular’ reasons) had to be thought of.

Rosskopf et al. established a novel approach to study the functional abnormalities of the NHE-1 in essential hypertension [6]. These authors carefully selected two collectives, one comprising normotensive control subjects with normal NHE-1 activity in blood cells and absence of a family history of hypertension, and a second one, comprising patients with essential hypertension who displayed enhanced NHE-1 activity and who reported of a genetic background of this disorder. After this selection procedure, lymphocytes of these collectives were immortalized by Epstein-Barr virus to establish permanent cultures of B lymphoblasts. The idea of this approach is a rather simple one: If the enhanced NHE-1 activity in essential hypertension is merely a reflection of an altered ‘hypertensive milieu’, this abnormality should vanish after prolonged cell culture, because under these conditions all these potential confounding influences (‘systemic’ factors) can be ruled out. On the other hand, if the enhanced NHE-1 activity persisted even after prolonged cell culture, this abnormality had to be regarded as being somehow under genetic control. It could be demonstrated that these lymphoblast cell lines retained their initial properties of normal or enhanced NHE-1 activity [6]. Furthermore, ‘hypertensive’ cell lines with enhanced NHE-1 activity differed from their ‘normotensive’ counterparts in terms of increased DNA synthesis and an accelerated proliferation [6,7]. Thus, the first cell culture model for a certain phenotype of essential hypertension was established.

Subsequent studies focused on analyzing the molecu-
lar reasons underlying these distinct features of 'hypertensive' cell lines. Mutations in the NHE-1 gene could be ruled out by direct comparative sequencing of cDNAs encoding for the NHE-1 protein in 'normotensive' and 'hypertensive' cell lines [6]. Furthermore, Rosskopf et al. excluded an overexpression of NHE-1 mRNA in 'hypertensive' cell lines [6], and recent Western blot analysis by Ng et al. ruled out an overexpression of the NHE-1 protein or a different post-translational processing in a similar cell culture model [8]. These and other studies [9] make 'genetic' changes in the NHE-1 protein itself or its promoter extremely unlikely.

Subsequent studies, hence, had to focus on an abnormal intracellular signal transduction in 'hypertensive' cell lines with special emphasis on those pathways, which ultimately control both cell proliferation as well as NHE-1 activity. It could be demonstrated that agonist-stimulated 'hypertensive' cell lines displayed increased rises in the cytosolic free Ca\(^{2+}\) concentration which were accompanied by an increased formation of the intracellular second messenger inositol-1,4,5-trisphosphate, although phospholipase C activities were almost identical in 'normotensive' and 'hypertensive' cell lines [10]. Interestingly, this enhanced cellular reactivity of 'hypertensive' cell lines was restricted to stimulation by agonists which exert their action via G protein-coupled receptors, whereas signal transduction mediated by e.g. the surface IgM receptor was unchanged [10]. To characterize the G proteins involved, experiments were conducted on cells pretreated with pertussis toxin, which selectively blocks signal transduction via G proteins of the G\(_s\)/G\(_\alpha\) class. Surprisingly, receptor-stimulated signal transduction was virtually identical in 'normotensive' and 'hypertensive' cell lines after pertussis toxin treatment, which suggested that signal transduction via receptors coupled to pertussis toxin-sensitive G proteins is selectively enhanced in essential hypertension. This hypothesis could be confirmed in experiments in which the activation of pertussis toxin-sensitive G proteins was quantified by measurements of receptor-mediated, pertussis toxin-sensitive binding of \([\text{35S}]\text{GTP}\gamma\text{S}\) to 'normotensive' and 'hypertensive' cell lines. In fact, 'hypertensive' cell lines displayed an enhanced binding of \([\text{35S}]\text{GTP}\gamma\text{S}\) and this phenomenon was not only observed upon receptor stimulation with a natural agonist (platelet-activating factor) but also upon direct, receptor-independent G protein stimulation by mastoparan, a wasp venom peptide which mimics the conformation of an activated G protein-coupled receptor [10].

Collectively, these data suggest that essential hypertension in the group of patients with enhanced NHE-1 activity could result from a genetically fixed enhanced activation of pertussis toxin-sensitive G proteins. Although this evidence is derived from immortalized lymphoblasts, i.e. cells of the immune system which do not contribute themselves to the pathogenesis of essential hypertension, it must be emphasized that this class of G proteins is ubiquitously expressed, including cells of the cardiovascular system (vascular smooth muscle cells, endothelial cells, cardiomyocytes, and platelets).

These novel developments appear important in terms of two issues: First, unravelling primary defects in essential hypertension now appears achievable by means of the established cell lines, and future investigations will have to focus on the regulation, expression, and molecular structure of the pertussis toxin-sensitive G proteins involved. Secondly, although the molecular basis of the enhanced G protein activation remains to be elucidated, these findings allow some novel speculations regarding the pathogenesis of essential hypertension. It has long been controversial whether essential hypertension develops on the basis of an increased vasoconstriction, an enhanced proliferation of vascular smooth muscle cells in resistance vessels, or both. The 'G protein concept of essential hypertension' would be ideally suitable to integrate these opposing views, since pertussis toxin-sensitive G proteins integrate signals from receptors which mediate vasoconstriction as well as cell proliferation. Furthermore, this concept would allow to interpret left ventricular hypertrophy, which at least partially develops independently of the magnitude of blood pressure elevation, as only one precipitator of a genetically fixed enhanced proliferation tendency in essential hypertension. On the other hand, these findings could shed a new light on the pending controversy regarding the contribution of genes versus environmental factors in the process leading to essential hypertension. An enhanced activation of pertussis toxin-sensitive G proteins could be the primary genetic basis for essential hypertension. However, lifestyle can be predicted to exert important modulatory influences on such a hypothetical, predisposing genetic background. Multiple exogenous factors including salt and alcohol intake, smoking, and mental stress can be expected to determine the frequency by which the respective signal transduction pathways are 'in use', thereby potentially determining the time of onset and/or severity of manifest blood pressure elevation.

References


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*Nephrol Dial Transplant* 1995: Editorial Comments
Apoptosis: will cell death add life to nephrology?

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Nephrology evolved to preserve life. Remarkably, fundamental scientific work in the field of apoptosis, a "programmed" and physiological form of cell death [1,2], is yielding new insights into the pathogenesis of renal disease, and may lead to new therapies [3]. Apoptosis is profoundly different from necrosis, the "accidental" type of cell death which occurs in fields of contiguous cells exposed to noxious stimuli such as hypoxia or chemical toxins. Instead of swelling and then undergoing disintegration, cells dying by apoptosis shrink and remain intact until they are rapidly ingested by neighbouring phagocytes. By contrast with necrosis, this protects tissues from the potentially toxic contents of dying cells and prevents inflammation and further tissue injury. Apoptotic cells, which are usually scattered among healthy cells, are identified by their highly characteristic pattern of nuclear chromatin condensation consequent upon organized cleavage of nuclear DNA by endonucleases. In histological sections conventional light-microscopy, supplemented by electron-microscopy, is often sufficient to detect and count cells undergoing apoptosis, but the condensed chromatin of such cells can be highlighted with fluorescent dyes, while the fragmented DNA allows easy detection by nick-end labelling with marked nucleotide bases. However, as yet there are no reliable molecular markers suitable for confident immunohistochemical detection of apoptosis.

The nephrologist needs to appreciate two key features of cell death by apoptosis. The first is that the speed, efficiency, and capacity of this cell clearance mechanism render apoptosis histologically inconspicuous. Time-lapse studies show that the morphological changes of apoptosis occur in minutes. Furthermore, phagocytosis and degradation are so fast that apoptotic cells only remain histologically detectable for between 0.5 and 2 h in most tissues, the so-called clearance time. Thus a few apoptotic cells may represent the barely visible tip of an iceberg of cell death and clearance. For example, in self-limited mesangial proliferative nephritis induced in rats by Thyl.1 antibody [4], we found an increase in apoptosis from only 0.01% of glomerular cells in healthy animals to 0.25% at the peak of cell death. Since our calculations suggest an apoptotic cell 'clearance time' of about 1.4 h in the model, it indicates that >4% of glomerular cells can be cleared each day, a large fraction of the ~25% increase in cell number seen at the peak of glomerular hypercellularity. Indeed, recent data on human postinfectious glomerulonephritis suggests that cells can be cleared at ten time this rate [5]. However, perhaps the most dramatic example is offered by the thymus, in which up to 99% of thymocytes are believed to fail in the race for selection. This vast burden of unwanted cells is eliminated by apoptosis and destroyed in 'tangible body' macrophages. Widespread apoptosis can lead to tissues literally being eaten away before our eyes.

The second key feature of apoptosis is that whether a cell survives or dies depends upon a balance of influences [2]. On the one hand there is compelling evidence that virtually all cell types rely on exogenous survival factors to ward off otherwise inevitable apoptosis [6]. Insufficient survival factor, be it hormone, cytokine, or extracellular matrix, leads to suboptimal engagement of cell-surface receptors and then, by poorly understood mechanisms, to cell death by default. For example, erythroid cell precursors in the bone marrow undergo apoptosis if deprived of erythropoietin. The susceptibility of cells to apoptosis can also be reduced by expression of endogenous survival genes, such as those of the bcl-2 family [7]. On the other hand certain gene products can drive the 'suicide' programme of apoptosis. These include cell-surface receptors such as Fas, cytoplasmic proteases including...