G proteins and hypertension: An alternative candidate gene approach

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G proteins and hypertension: An alternative candidate gene approach. Hypertension affects approximately 20 to 30% of individuals in industrialized countries, and is commonly believed to develop on the basis of both genetic and environmental factors. The identification of genes susceptible to the most frequent form of hypertension, commonly referred to as “essential” hypertension, is hampered by the fact that blood pressure is a poorly defined phenotype that is modulated by multiple factors, such as gender, race, body mass etc., and that the definition of hypertension depends on a rather arbitrarily chosen cut-off value. Hence, more progress has been made in the identification of genes responsible for rare autosomal dominant forms of hypertension, such as Liddle’s disease. This review focuses on an experimental approach that attempts to define candidate genes for essential hypertension using immortalized cells from well characterized normotensive and hypertensive subjects. From the presently available results, one attractive speculation is that an increased intracellular signal transduction caused by an enhanced reactivity of Gα-type G proteins represents a genetically fixed trait that renders affected individuals susceptible to essential hypertension.

It is generally agreed that essential hypertension is a multifactorial polygenic disorder [1, 2]. Current research efforts aim at identifying those genes that increase the susceptibility for hypertension either alone or in combination with other genes and/or environmental factors.

Several quite different experimental strategies exist to identify such “hypertension genes.” One involves the examination of rare autosomal dominant forms of hypertension, such as Liddle’s disease, which is caused by mutations in the β or γ subunits of the epithelial sodium channel [3, 4]. Schuster et al focused their investigations on a Turkish family with severe autosomal dominant hypertension that cosegregates 100% with brachydactyly and maps to chromosome 12p [5]. Interestingly, this form of hypertension, despite very early onset and steep increase in blood pressure, resembles the common form of essential hypertension [6], and identification of the responsible gene(s) in that Turkish kindred can therefore be expected to be of major importance for understanding the genetic basis of essential hypertension. Other strategies rely predominantly on genetic linkage analysis, which has led to the identification of a common M235T variant in the gene encoding for angiotensinogen [7].

Animal models of hypertension are very useful for the identification of hypertension genes, as illustrated by the recent findings of Cusi et al [8]. These authors could identify a polymorphism in the α-adducin gene that is tightly associated with salt sensitivity in patients with essential hypertension. Originally, this protein was identified as a possible candidate gene in the Milan hypertensive strain of rats, and mutations within the adducin genes were shown to be associated with blood pressure variations in these animals [9].

Finally, one method that remains very useful in defining genes susceptible to hypertension consists of the “classical” candidate gene approach. This method depends on the careful characterization of intermediate phenotypes in essential hypertension, that is, traits that are obviously inherited and tightly linked to, but not necessarily directly involved in the pathogenesis of essential hypertension.

This review will focus on our attempts to identify novel hypertension candidate genes.

NA+/H+ EXCHANGER ACTIVITY AS AN INTERMEDIATE PHENOTYPE IN ESSENTIAL HYPERTENSION

The Na+/H+ exchanger isoform-1 (NHE-1) is a ubiquitously expressed ion transport protein that mediates electroneutral exchange of extracellular Na+ ions against intracellular protons, thereby contributing to intracellular pH homeostasis. Most likely this protein also mediates Na+/Li+ countertransport [10, 11], which constitutes an established genetic marker for hypertension [12, 13]. Multiple studies have shown that the activity of the Na+/H+ exchanger isoform-1 (NHE-1) is enhanced in a group of patients with essential hypertension, and this enhancement of NHE activity was found in all cells and tissues investigated, such as erythrocytes, platelets, lymphocytes, and even skeletal muscle in vivo [reviewed in 14–16]. However, the activity of the NHE-1 is known to be increased in vitro by phosphorylation and Ca2+-calmodulin-mediated reactions upon cell activation via G protein-coupled receptors and receptors with tyrosine kinase activity [17, 18]. Furthermore, NHE-1 activity in vivo is enhanced by multiple influences, such as metabolic acidosis [19, 20], oral glucose uptake [21], acute saline infusion [22, 23], and chronic NaCl loading [24], to name but a few. Thus, it has been impossible to draw definitive conclusions regarding the ultimate cause of enhanced NHE-1 activity in essential hypertension, and it is conceivable that this...
ENHANCED ACTIVATION OF G PROTEINS IN “HYPERTENSIVE” B LYMPHOBLAST CELL LINES

Upon stimulation of B lymphoblasts with platelet-activating factor (PAF), there were distinct increases in intracellular \(\text{Ca}^{2+}\) in cells from hypertensive subjects with high NHE-1 activity compared to cells from normotensive controls with normal NHE-1 activity [29]. These enhanced \(\text{Ca}^{2+}\) signals resulted from an enhanced PAF-evoked formation of inositol-1,4,5-trisphosphate, that is, the second messenger that releases \(\text{Ca}^{2+}\) from intracellular stores. Furthermore, PAF-stimulated proliferation of “hypertensive” lymphoblasts was significantly enhanced, although the PAF receptor expression was not significantly different between cell lines established from normotensive and hypertensive subjects [29]. Previous studies had shown that the PAF receptor of EBV-immortalized B lymphoblasts couples to pertussis toxin (PTX)-sensitive Gi as well as to PTX-insensitive G proteins [30]. Upon preincubation of “normotensive” and “hypertensive” cell lines with PTX there was a strong, albeit not complete, inhibition of PAF-evoked \(\text{Ca}^{2+}\) signals, suggesting the involvement of both \(G_i\) as well as \(G_{V11}\) type G proteins. Most remarkable, however, was the finding that the observed large difference between normotensive and hypertensive cell lines in terms of \(\text{Ca}^{2+}\) signals was completely blunted upon treatment with PTX. This strongly suggested that signal transduction via PTX-sensitive G proteins was selectively enhanced in “hypertensive” cell lines. By quantification of PAF-stimulated \([^{35}\text{S}]\text{GTPyS}\) binding to permeabilized B lymphoblasts we could actually demonstrate an enhanced activation of PTX-sensitive \(G_i\)-type G proteins in “hypertensive” B lymphoblasts [30]. This observation was strengthened in experiments in which the peptide mastoparan-7 was applied, the latter inducing a direct, receptor-independent activation of PTX-sensitive G proteins [31]. Mastoparan-7-stimulated \(G_i\)-protein activation was, on the average, enhanced twofold in lymphoblasts from hypertensive individuals [30].

Since these above findings were obtained in cell lines established from highly selected normotensive and hypertensive individuals, the question arose of whether enhanced G protein activation is a rare phenomenon that may contribute to the pathogenesis of essential hypertension in few patients only, or whether this abnormality can be frequently observed in essential hypertension. We therefore immortalized lymphocytes from independently selected hypertensive and normotensive individuals who had not been characterized previously in terms of NHE-1 activity. Larger PAF-evoked \(\text{Ca}^{2+}\) signals mediated by \(G_i\) proteins were more frequent in cells from hypertensive individuals compared to normotensive controls, and were independent of donor age, gender, or antihypertensive treatment (Fig. 1). From these studies we concluded that enhanced G protein activation could constitute a pathogenetically important mechanism in 27 to 43% of individuals with essential hypertension [32].

STUDIES ON SKIN FIBROBLASTS

The model of immortalized lymphoblasts allows alterations in signal transduction pathways that are ubiquitously expressed to be studied, such as receptor-G protein coupling, and the rapid growth of such cell lines provides a rich source of material for biochemical studies. Nevertheless, this model also has some inherent limitations. Although enhanced G protein activation in “hypertensive” cell lines was consistently found in repeated experiments conducted during a period of two years, we could not definitively rule out that this abnormality could potentially arise from an experimental artifact. It was still possible that the immortalization process induced by the EBV genome was different in cells from hypertensive and normotensive individuals and could, finally, result in the establishment of different cellular
phenotypes. Furthermore, B lymphoblasts lack receptors that are important for blood pressure regulation or control of proliferation in the cardiovascular system. These limitations led us to establish cultures of primary skin fibroblasts from these previously characterized individuals. We hypothesized that fibroblasts from hypertensive individuals would display an increased DNA synthesis, enhanced calcium signals and inositol phosphate formation upon the stimulation of receptors that predominantly couple to PTX-sensitive G proteins. In fact, skin fibroblasts from hypertensive individuals displayed an enhanced PTX-sensitive incorporation of [3H]thymidine upon stimulation with hormones including norepinephrine (via \(\alpha_2\)-adrenergic receptors), angiotensin II, and thrombin activate receptors coupled to Gi proteins. Activated \(\alpha_i\) and \(\beta_G\) subunits influence a variety of intracellular second messenger systems including ion channels, adenyl cyclase, and the ras-rat-MAP-kinase (MAPK) pathway. Furthermore, \(\beta_G\) subunits can directly activate isoforms of phospholipase \(C\) (PLC), thereby inducing activation of protein kinase \(C\) (PKC) and an elevation of the intracellular calcium concentration. Enhanced activation of G proteins is predicted to cause a pronounced activation of these pathways, which may ultimately result in increased cell proliferation.

**CONCLUSIONS AND PERSPECTIVES**

Although the ultimate reasons for enhanced G protein signaling in patients with essential hypertension remain to be defined, the observations in this article fit well with those of others. Increased intracellular signal transduction was frequently observed in platelets from patients with essential hypertension and, for example,

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**Fig. 2. Signal transduction pathways activated upon stimulation of G\(_i\) proteins.** A variety of hormones including norepinephrine (via \(\alpha_2\)-adrenergic receptors), angiotensin II, and thrombin activate receptors coupled to Gi proteins. Activated \(\alpha_i\) and \(\beta_G\) subunits influence a variety of intracellular second messenger systems including ion channels, adenyl cyclase, and the ras-rat-MAP-kinase (MAPK) pathway. Furthermore, \(\beta_G\) subunits can directly activate isoforms of phospholipase \(C\) (PLC), thereby inducing activation of protein kinase \(C\) (PKC) and an elevation of the intracellular calcium concentration. Enhanced activation of G proteins is predicted to cause a pronounced activation of these pathways, which may ultimately result in increased cell proliferation.
increased Ca\(^{2+}\) signals and enhanced activation of protein kinase C were frequently reported [38, 39]. Interestingly, such differences were not observed when platelets were stimulated with vasopressin [40], which primarily activates PTX-insensitive G proteins in platelets. The “G protein hypotheses of essential hypertension” is also in complete agreement with the commonly held notion suggesting that high blood pressure results from an interaction between inherited and environmental factors. An attractive speculation is that enhanced Gi protein reactivity alone is insufficient to result in essential hypertension in the absence of excessive stimulation of the affected signal transduction pathways. However, environmental factors such as emotional stress could drastically increase hormonal output, thereby contributing significantly to the development of high blood pressure on the genetic background of abnormal signal transduction.

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APPENDIX

Abbreviations used in this article are: EBV, Epstein Barr virus; G protein, guanine nucleotide-binding protein; LPA, lysophosphatidic acid; NHE-1, sodium-proton exchanger isoform-1; PTX, pertussis toxin; SPP, sphingosine-1-phosphate.

NOTE ADDED IN PROOF

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