
Preface

Hypertension is thought to affect about one quarter of the population in the western world. Worldwide, the numbers of patients will continue to increase as less affluent countries follow this trend and adopt first world dietary habits and lifestyles. Hypertension is known to increase the risk of stroke, myocardial infarction, renal failure, and cardiac failure, and treatment is known to reduce these risks. We still do not have a complete understanding of essential hypertension, let alone a cure. Since the clinical importance of hypertension became fully appreciated in the last century, research in the field has continued to accelerate. New methods in molecular biology have arrived at an exponentially accelerating rate since the seminal discoveries of nucleotide sequencing and recombinant DNA technology of the 1970s. Modern molecular biology provides a bridge of understanding from the chemical through to the clinical. The availability of such new information as that derived from the Human Genome project and the accessibility of that information via the Internet have revolutionized medical research. The application and availability of new molecular techniques and knowledge mean that our understanding of the pathogenesis of hypertension and the potential for improved clinical intervention have never been greater. We hope that the process detailed in *Hypertension: Methods and Protocols* will help researchers to develop a greater understanding of hypertension and uncover new potential targets for antihypertensive therapy.

Hypertension: Methods and Protocols provides many new and essential methods that span the entire spectrum of modern molecular biology. The authors offer an elite collection of knowledge and experience assembled from the four corners of the world. The accessible format of the protocols makes them easy to perform, even by beginners in the field. The breadth of protocols provided will attract experienced scientists interested in pursuing new avenues of research in hypertension.

The first part deals with animal models of hypertension and describes methods of producing congenic, consomic, transgenic, and knockout models of hypertension. The final chapter in this section describes a method for the telemetric measurement of blood pressure in such animals.

Free radical-mediated damage plays an important role in many chronic diseases, including hypertension. The second part of this book describes methods of assessing the role of free radicals in hypertension that are applicable to all forms of endothelial dysfunction. The quantitative method of chemiluminescence and the qualitative method of dihydroethidine staining combined with wire myography assessment provide methods of assessing hypertension and other forms of endothelial dysfunction at both a molecular and a physiological level.

As our molecular understanding improved, the emphasis in hypertension research moved from rare monogenetic disorders to understanding the complex contribution of genetics to essential hypertension. Much work has gone into the pursuit of the genes

associated with hypertension. Part III contains several methods to aid in the genetic dissection of hypertension, such as the candidate gene approach, genome wide scans, SNP analysis, and microarray analysis. One of the most promising aspects of human genetic research is the search for SNPs associated with hypertension. Several methods of SNP analysis, such as Taqman, WAVE, SSCP, and mass spectrometry, are provided. Genotyping may allow stratification of patients according to risk, but will also guide therapy one day. Pharmacogenetics and pharmacogenomics will become increasingly important and are also discussed. Functional genomics using microarray analysis will lead to the discovery of new genetic pathways, and thus to greater insights into the genetic input in hypertension and the patients' likely response to treatment.

Part IV describes two very different protein techniques. The first chapter looks at the analysis of a small number of related yet relevant amino acids. The roles of arginine and ADMA have become increasingly associated with endothelial dysfunction and hypertension. The HPLC analysis of ADMA and other arginine analogs is discussed. The second chapter discusses a proteomics procedure for the large-scale analysis of protein expression. Proteomics holds great promise for the field of cardiovascular biology; benefits will include the identification of marker proteins, dissection of signaling pathways affected by the underlying perturbation, and drug-target identification and validation. This chapter focuses on a few examples of how the powerful combination of two-dimensional gel electrophoresis, bioinformatics, and mass spectrometric analysis can be applied to the study of hypertension.

Gene transfer of specific genetic material to either overexpress or inhibit gene expression is a useful scientific tool and a potential source for the future long-term treatment of hypertension. The last decade has shown many exciting developments in this field, so Part V includes a comprehensive section on gene transfer. Several nonviral methods are described including nonviral techniques using polymers, liposomes, and antisense agents. Post-transcriptional inhibition using siRNA is also described. One of the most popular viral vectors used in hypertension research is the adenoviral vector; therefore two chapters of adenoviral methods are presented: one describing the generation of large-capacity, sustained-expression vectors and another describing the practical application of adenoviral vectors to reduce blood pressure in an animal model using superoxide dismutase gene transfer. The liver and spleen absorb the vast majority of systemically injected viral vectors, reducing their effectiveness. The possibility of selectively targeting vectors to the endothelium of the systemic vasculature or other target organs would have major implications. Selectively targeted vectors would improve the efficacy of gene transfer, thereby reducing the viral dose required and improving the safety profile. The chapter on *in vivo* biopanning using phage display describes a remarkable technique for isolating targeting peptides, which could enable *in vivo* organ- or tissue-specific binding of gene transfer vectors.

Stem cells research may have the potential to supply replacement cells for the cells damaged by hypertensive remodeling or from the complications of hypertension, such as myocardial infarction. These previously unimaginable prospects may soon be reality. Part VI describes a practical method for the derivation of cardiomyocytes from embryonic stem cells.

In recent years a veritable avalanche of accessible technology and information has become available through the Internet. The information technology revolution has been of enormous benefit to molecular biology research and promises even more. Embryonic areas of research—such as microarrays, proteomics, and pharmacogenomics—would simply not exist without the help of modern IT systems. Although many chapters contain relevant IT information, Part VII is dedicated solely to bioinformatics, with an excellent introduction to bioinformatic resources for pharmacogenomics and a more practical chapter describing the application of *in silico* strategies to identify nuclear matrix attachment regions.

I would like to thank my family, Dr. Catherine Cleary, and Dr. William Fennell for their patience, advice, and support. This work would not have been possible without the contributions of our series editor John M. Walker and of course our publisher, Humana Press. Finally I would especially like to thank my coeditor, Andrew H. Baker for his extensive efforts and guidance in this project.

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Hypertension

Methods and Protocols

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2005, XVI, 502 p. 98 illus., Hardcover

ISBN: 978-1-58829-323-7

A product of Humana Press