REVIEW

Developmental windows and environment as important factors in the expression of genetic information: a cardiovascular physiologist’s view

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ABSTRACT

Genetic studies in humans and rodent models should help to identify altered genes important in the development of cardiovascular diseases, such as hypertension. Despite the considerable research effort, it is still difficult to identify all of the genes involved in altered blood pressure regulation thereby leading to essential hypertension. We should keep in mind that genetic hypertension and other cardiovascular diseases might develop as a consequence of early errors in well-co-ordinated systems regulating cardiovascular homoeostasis. If these early abnormalities in the ontogenetic cascade of expression of genetic information occur in critical periods of development (developmental windows), they can adversely modify subsequent development of the cardiovascular system. The consideration that hypertension and/or other cardiovascular diseases are late consequences of abnormal ontogeny of the cardiovascular system could explain why so many complex interactions among genes and environmental factors play such a significant role in the pathogenesis of these diseases. The detailed description and precise time resolution of major developmental events occurring during particular stages of ontogeny in healthy individuals (including advanced knowledge of gene expression) could facilitate the detection of abnormalities crucial for the development of cardiovascular alterations characteristic of the respective diseases. Transient gene switch-on or switch-off in specific developmental windows might be a useful approach for in vivo modelling of pathological processes. This should help to elucidate the mechanisms underlying cardiovascular diseases (including hypertension) and to develop strategies to prevent the development of such diseases.

INTRODUCTION

The incidence of polygenic multifactorial diseases (such as hypertension, obesity, insulin resistance, Type II diabetes and atherosclerosis) is increasing in the developed and developing worlds. Although these diseases usually become a medical problem in late adulthood, their roots can be traced back to earlier stages of the ontogeny [1–3]. The aims of this review are to outline (i) how the environmental factors could interfere with the expression

Key words: cardiovascular phenotype development, developmental window, genetic determinant, key environmental stimulus, ontogenesis.

Abbreviations: 11βHSD2, 11β-hydroxysteroid dehydrogenase type 2; ACE, angiotensin-converting enzyme; AT1, angiotensin II type 1; BP, blood pressure; QTL, quantitative trait locus; SHR, spontaneously hypertensive rat; SHRSP, stroke-prone SHR; VSMC, vascular smooth muscle cell; WKY, Wistar–Kyoto.

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of normal and abnormal genetic information during the early ontogeny, (ii) how these changes modify development of the cardiovascular system, (iii) how such induced modifications can mask the true impact of genetic abnormality on the phenotype of interest, and (iv) what should be done to decode the complex pathogenetic gene–environment interactions during development of major cardiovascular diseases.

ONTGENETIC ASPECTS OF CARDIOVASCULAR DISEASE DEVELOPMENT: BASIC PRINCIPLES

It should be stressed that genetic information (irrespective of whether normal or abnormal) is programmed to be expressed in particular stage(s) of ontogenetic development (ranging from embryogenesis to senescence). The expression of particular genetic information at a given ontogenetic stage is influenced by several factors. First, it should appear in concert with simultaneous expression of many other genes scheduled for the same ontogenetic stage. Secondly, it should cope with the physiological demands of the developing organism which readily compensates for missing or inappropriate products of gene expression. Thirdly, it is often modified by environmental factors specific for the corresponding stage of development. The environmental factors include not only toxic substances, nutritional loads or deficiencies and pharmacological interventions, but they also concern many natural cues such as changes in intrauterine environment, suckling quality, maternal care, diet after weaning, electrolyte intake, hormonal pattern etc. [4]. These three aspects represent the physiological basis of gene–gene and gene–environment interactions which are crucial, especially during early stages of ontogenetic development. Such principles are also valid for the ontogeny of the cardiovascular system, ranging from embryonic angiogenesis [5] to the postnatal development of baroreflex control [6].

There is no doubt that genetic factors play a key role in the pathogenesis of cardiovascular and metabolic diseases, irrespective of the fact that their contribution is only 30–50%. [7]. However, several possibilities should be considered as to how the environment might influence genetic predisposition to such diseases [4,8]. First, the expression of abnormal genetic information (coding for altered structure and function of certain proteins, such as ion channels, receptors, enzymes etc.) results in minimal changes in the immature organism, but during subsequent maturation these changes progress to manifest diseases under the modifying influence of the environment. Different environmental factors aggravate or ameliorate various aspects of the pathophysiological process during disease progression. The variable influence of the environment on such a process is combined with the complex compensatory responses of the organism, leading to adaptation of the individual to both its genome abnormality and the actual living circumstances. Secondly, the environmental conditions might alter the development of the organism prior to the expression of the altered genetic information, so that the susceptibility of the organism to the deleterious effects of abnormal gene(s) expression is modified. In contrast with the previous situation, the response to primary stimuli need not be as uniform as one would expect. Thirdly, the primary gene abnormality is silent until its expression is elicited by endogenous (expression of other abnormal genes or hormonal imbalance) or exogenous factors (diet or stress) characteristic for the particular stage of development (e.g. puberty, menopause or senescence). Finally, the expression of a primary gene abnormality might occur early in ontogeny, but the elicited change remains hidden unless exogenous and/or endogenous factors make it a part of the pathogenetic mechanisms of the disease. Any of the above possibilities might also be true for the cardiovascular system [9–12].

The present review focuses on environmental factors that can modify expression of genetic information during ontogeny, namely in critical developmental periods which are characterized by exceptional sensitivity to environmental factors or endogenous stimuli. These periods (also known as developmental windows) can be defined as relatively short stages in pre- and postnatal development characterized by the high density of maturation processes associated with the developmental transformation of structure and function of a given system, e.g. cardiovascular, neural, renal, etc. As some of these processes may be vulnerable, further development of the cardiovascular system might be modified by factors derived from the environment or resulting from genetic defects [8]. The temporal dimension is crucial for understanding ontogeny, the rate of which is given by the density of developmental events that are usually irreversible. Once the organism has passed to the next developmental stage, it can only compensate for the errors that have occurred in previous stage(s). It would always be desirable to describe all of the steps in the adaptation of the organism to primary defect(s), because some disclosed abnormalities might represent the compensation of certain earlier events. In this review, we pay special attention to the development of pathological phenotypes (for details, also see [4]). The impact of distinct environmental factors during ontogeny of individuals carrying abnormal genetic information might differ substantially from that observed in healthy controls. The identification of important critical developmental periods and corresponding environmental or endogenous cues will, therefore, facilitate precise therapeutic targeting of the altered development of the cardiovascular apparatus resulting from genetic defects.

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ENVIRONMENTAL FACTORS AS ADDITIONAL CUES FOR THE DEVELOPMENT OF THE CARDIOVASCULAR SYSTEM

How is the expression of genetic information modified during normal ontogeny?

It is evident that the high plasticity of the developing organism represents an ‘ideal terrain’ for induction of long-term or permanent modifications by the action of environmental stimuli. This may also apply to common variations in the living conditions which are an integral part of the normal development of each individual. Characteristic examples in the rat are shown in Figure 1. Thus the expression of genetic information during ontogeny is carried out in concert with the influence of the existing environment.

There is little doubt that altered fetal nutrition, due to changes in maternal diet or in uterine blood flow, affect fetal development. Since a part of the induced changes is mediated by enhanced access of glucocorticoids to the fetus, it is not surprising that the long-term consequences affect tissues and organs, including those of the cardiovascular system. Smaller birth weight is, therefore, accompanied by abnormalities predisposing to increased incidence of cardiovascular and/or metabolic diseases [13]. This is also true for rats subjected to maternal protein malnutrition [14], intrauterine growth retardation [15], exposure to exogenous glucocorticoids [16] etc. It should be kept in mind that even minor changes in fetal nutrition might modify metabolic programming of the organism [17] and alter its susceptibility to other environmental factors acting at later stages of ontogeny.

During the perinatal period in the rat, there is a relatively short critical period during which endogenous or exogenous androgens may imprint the hypothalamus, changing its cyclic pattern (typical for females) into the male non-cycling one [18,19]. This change, together with its hormonal consequences, is then responsible for the sex-dependent pattern of salt intake in adult rats [20]. Similarly, neonatal gonadectomy modifies the development of spontaneous hypertension [21,22] and pulmonary hypertension induced by exposure to hypoxia in adulthood [23]. Although these effects are usually ascribed to an altered hormonal pattern at the time of hypertension development, other possibilities should be considered. VSMCs (vascular smooth muscle cells) isolated from male rats show signs of enhanced growth compared with those from females [24,25], and this gender-dependent phenomenon has been observed even in VSMCs isolated from neonatal rats [26]. Hence the possibility of early steroidal imprinting should also be considered.

After birth, both maternal care and milk supply are the principal factors affecting further development of the newborn during the suckling period (first 2 postnatal weeks in the rat; for more details, see [4]). Cross-fostering of SHR (spontaneously hypertensive rat) pups to WKY (Wistar–Kyoto) dams revealed the essential importance of the above factors for the subsequent development of high BP (blood pressure) [27]. Maternal experience that can modify hyperactive behaviour of primiparous SHR dams [28] might also be of importance, because differences in BP development of SHR pups born in the first and subsequent deliveries of the same dam were reported [29]. Profound long-term changes can be induced by overnutrition (small litters) or undernutrition (large litters) in rat pups during the suckling period [30,31]. It is important to note that this period is characterized by major maturation of the sympathetic nervous system [32,33]. Altered milk composition in terms of electrolytes (potassium and calcium), fatty acid spectrum and protein content [34,35], together with decreased efficiency of mother-to-pup milk transfer [36,37], were reported to influence the development of hypertension in SHRs. The effect of nutritional interventions in suckling period might be surprising. For example, the antihypertensive effect of high calcium intake is almost specific for suckling and weanling rats [38–40], whereas the application of a high-calcium diet to adult animals has either no effect on BP or even exaggerates the development of salt hypertension [41,42]. On the other hand,
increased potassium intake lowers the BP of genetically hypertensive rats [43,44] and reduces the susceptibility of animals to hypertensive effects of excess salt ingestion [45,46] throughout life. This illustrates that certain environmental factors (e.g. nutrition) exert considerably different effects in the early stages of ontogeny than in mature animals (for review, see [4]).

The impact of environmental (especially nutritional) factors becomes particularly significant during the weaning period in which the offspring spontaneously replace maternal milk by an available diet and drinking fluid. In the rat, this process occurs during the third and fourth postnatal weeks, but it can be severely impaired by premature weaning. Further alterations can be induced by the composition of the diet offered to weanlings. It is important to point out that premature weaning at the age of 21–23 days, when the consumption of maternal milk is highest, represents an abrupt dietary change from high-fat consumption (maternal milk) to high-carbohydrate intake (normal rat chow). Careful studies by Coates et al. [47], Hahn et al. [48] and Hahn and Srubiski [49] demonstrated that such interventions induce long-term modifications of lipid metabolism, which are manifested by altered responsiveness of adult animals to high-fat or high-cholesterol diets [50].

The juvenile period, which covers pre-puberty and puberty (4–10 weeks of age in the rat), is characterized by major BP elevation in normotensive rats as well as by a rapid BP rise both in genetically hypertensive rats or rats subjected to high-salt intake (for details, see [4]). It is also a developmental period in which antihypertensive treatment (especially with drugs interfering with the renin–angiotensin system) is most effective and has long-term cardiovascular effects. This is true not only for SHRs [11,51], but also for salt hypertensive Dahl rats [52]. Since chronic captopril treatment of immature rats lowers BP by attenuating its sympathetic component [53,54], the elevated sympathetic output appears to be preset just at the juvenile period.

The above examples illustrate how some complex environmental factors acting throughout defined developmental periods (maternal nutrition, litter size, milk transfer, diet at weaning or salt intake in pre-puberty) can affect the resulting phenotype in adulthood by modifications in the expression of genetic information in youth. The action of environmental factors becomes effective in the corresponding critical developmental periods during which the susceptibility of the organism to such factors is enhanced. These critical periods (developmental windows) usually coincide with the ontogenetic periods of intensive developmental processes associated with fundamental structural and functional transformation of individual systems, e.g. cardiovascular, endocrine, gastrointestinal systems etc. [8]. Knowledge of the molecular mechanisms by which minor early stimuli modify the expression of genetic information during subsequent development might be the desired key for decoding pathways leading from the primary genetic change to the resulting phenotype of interest in the adult organism.

Could epigenetic inheritance explain gene–environment interactions in the pathogenesis of cardiovascular diseases?

One would expect that the environmental impact on genetic information is realized via DNA damage, i.e. gene mutations. However, changes in gene expression that are not linked to alterations in DNA sequences have recently been described. They represent an epigenetic inheritance. The term ‘epigenetics’ comprises all meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself [55].

The association between lower birth weight and subsequent development of impaired glucose tolerance, Type II diabetes and cardiovascular disorders, including hypertension, has been recognized [13,56,57]. Although the molecular mechanisms of this process are not fully understood, one of the mechanisms involved in this long-term modulation of gene expression might include altered DNA methylation [58]. The period during which the cellular methylation pattern is established is characterized by a high susceptibility to various fetal (maternal) insults, so that gene silencing through altered DNA methylation might be a good candidate mechanism for pre-natal programming. Several recent studies support this hypothesis [59–61]. It has also been demonstrated [62] that epigenetic mechanisms could provide a tentative explanation for the highly cell-specific expression of 11βHSD2 (11β-hydroxysteroid dehydrogenase type 2), which protects mineralocorticoid receptors from glucocorticoid action. Moreover, changes in methylation patterns might explain the inter-individual differences in HSD11B2 expression in mineralocorticoid target tissues, thus modulating BP. The magnitude of these changes appears to be clinically important, because it has been shown that moderate changes in 11βHSD2 activity significantly affected BP in normal volunteers [63]. Alikhani-Koopaei et al. [62] hypothesized that epigenetic modifications of genes important for BP regulation, such as HSD11B2, are linked to the development of hypertension.

DEVELOPMENT AND THE ENVIRONMENT AS CONFOUNDING FACTORS IN GENETIC RESEARCH OF THE CARDIOVASCULAR SYSTEM

Progress in understanding the genetic basis of hypertension

There is growing evidence that complex interactions among multiple genes and environmental factors play
an important role in determining an individual’s risk of common diseases. Gibson [64] stated that gene–gene interactions and gene–environmental interactions must be ubiquitous, given the complexities of intermolecular interactions that are necessary to regulate gene expression and the hierarchical complexity of quantitative traits. Moreover, Dominiczak and co-workers [65] predicted that “the classical genomic paradigms, including the central dogma of gene → protein → functions as well as our increasing ability to study gene/gene and protein/protein interactions, will increasingly dominate mechanistic studies in hypertension and all other complex polygenic traits”. Advances in molecular markers (especially microsatellite repeats) and recently developed computer analysis programs have accelerated the identification of chromosomal regions associated with hypertension, e.g. QTLs (quantitative trait loci), containing genes responsible for high BP or other phenotypes of interest. However, the small contributions of individual genes and the heterogeneity of patients make genetic studies of essential hypertension difficult. Until recently, only a few genes were found to play a role in rare monogenic (Mendelian) forms of hypertension in humans [65,66]. Although the majority of mutations detected that affect BP are in the coding sequences of these genes, it seems likely that the sequence variants affecting gene regulation and splicing of its products also play an important role in determining disease susceptibility. It is still unclear whether inheritance of hypertension susceptibility in humans is due to variations occurring in a small subset of genes with major effects or in a large number of genes with small effects. Recent heritability estimates of BP indicate that the genetic contribution to BP variation ranges from 30–50% [7]. Hypertension develops as a consequence of errors in a well-co-ordinated cascade of molecular, biochemical and genetic processes which regulate BP. The identification of susceptibility genes for common complex multifactorial diseases has largely been unsuccessful, although the standards for gene identification of these complex traits [67] as well as novel integrative approaches to the identification of candidate genes in hypertension [68] have been proposed recently.

Several rat and mouse models of genetic hypertension with different aetiologies have been developed (for reviews, see [69,70]). These models provide an opportunity to investigate the genetic determinants of different types of hypertension, but one has to be cautious in extrapolating results obtained in animal models to human essential hypertension. Studies in animal models indicate a diversity of genes influencing BP, suggesting that a larger number of genes are involved in hypertension development.

It should be kept in mind that it is not enough to identify a trait difference in two different inbred strains. Even if such a difference is physiologically relevant to BP regulation, it is always difficult to ascertain that this trait difference is due to a primary genetic cause of BP increase. The observed BP phenotype of a particular inbred strain is the sum of the contributions of many genes which were fixed during inbreeding. Currently, the genetic approaches, such as segregating populations, congenic or consomic strains, recombinant inbred strains, transgenic or knockout animals, are used for evaluating the true importance of phenotypic differences between two inbred strains [68,71].

Recent progress in molecular biology and genomic techniques and its integration with physiological techniques started the era of functional and/or physiological genomics [72,73]. This is a multidisciplinary approach to facilitate gene identification and to study their function. It also includes the detection of sequence variations and characterization of aberrations in gene function(s) leading to the disease. As only a small proportion of the many sequence variations in human genomes will probably have a functional impact, identification of the decisive subset of sequence variants will be a major challenge of the next decade. Proteomics represents a more recent addition to functional genomics, because proteins are the final products for the realization of genetic information. Recently, proteomics has been introduced for analysing differential protein expression and cellular protein composition even in cardiovascular medicine. Proteomics allows for the examination of global alterations in protein expression in the diseased tissues and should provide new insights into cellular mechanisms involved in cardiovascular dysfunctions [74–76].

As mentioned by Cowley [77], the progress in genomics, proteomics, pharmacogenomics etc. depends on a “deep understanding of physiological functions with maximal application of all of the available tools of modern biology”. However, despite the great potential of the above approaches, it is still difficult to identify all of the genes involved in the regulatory pathways responsible for the development of BP dysregulation leading to essential hypertension.

How can environment and age modify the expression of abnormal genetic information?

The interaction between genes and the environment is defined as co-participation in the same causal mechanisms leading to a given disease [78]. This interaction was also defined by Ottman [79] as “a different effect of an environmental exposure on disease risk in persons with different genotypes” or, alternatively, “a different effect of a genotype on disease risk in persons with different environmental exposure”. However, it is still unclear how genetic and environmental factors combine their influence on the level of risk. The contribution of genetic and environmental factors to pathogenetic mechanisms may differ among families or clinically.
defined subsets of the disease. Even within a single family, the effect of a susceptibility genotype might be variable because of the modifying effects of other genes or environmental factors. Therefore the use of inbred strains, which are kept under constant conditions, should minimize this problem. However, individual phenotypic differences within particular inbred strains could not be explained by differences in genetic information, but they might be related to the influence of particular environmental factors on the expression of genes and/or on the modification of resulting proteins. Several models of the relationship between a high-risk genotype and an environmental exposure have already been suggested [79].

The human genome is changing at a much slower rate compared with changes in risk lifestyle occurring since the agricultural revolution 10000 years ago [80]. Accumulating evidence suggests that the discrepancies between our Paleolithic genome and contemporary diet and lifestyle could play a significant role in the ongoing epidemics of obesity, hypertension, diabetes, atherosclerosis and the metabolic syndrome. The change from wild unprocessed food to a diet with a high-fat, -sugar and -salt content represent a typical example.

Salt intake is one of the major environmental factors that influences BP development in an age-dependent manner (for reviews, see [4,81]). A genetic predisposition is the most important factor for salt-hypertension development, as has been demonstrated by the selection of salt-sensitive and salt-resistant Dahl or Sabra rat strains [82–84]. However, it has been demonstrated that a high-salt intake level in young and adolescent rats is a decisive factor, enhancing the susceptibility of adult rats to salt hypertension [85]. The important BP effects of elevated salt intake in the post-weaning period are in contrast with a much smaller influence of high-salt intake in rat mothers during pregnancy or lactation on subsequent BP development in their offspring[86,87]. On the other hand, the susceptibility to hypertensive effects of high-salt intake decreases with progressing age of the animals, so that the BP response of 6-month-old salt-sensitive Dahl rats to a high-salt intake was substantially attenuated compared with young animals [88,89]. This supports the assumption that pre-puberty might be a critical period for the induction of self-sustaining salt-dependent hypertension in the rat (for review, see [10,81]).

This supports the idea that environmental factors modify gene expression according to the stage of ontogeny [90]. In fact, the impact of several BP QTLs fluctuated substantially with age of the animals. In male F2 rats derived from SHRSP (stroke-prone SHRs) and normotensive WKY rats, the LOD scores at the peaks of three QTLs on chromosomes 1, 3 and 4 appeared greater in the early developing stages, but decreased later [91]. Samani et al. [92] reported similar results in F2 hybrids derived from SHRs and WKY rats. The effect of QTL for BP on chromosome 2 was seen throughout from 12–25 weeks of age, whereas the effect of QTL on chromosome 13 was maximal at 20 weeks of age, but it disappeared at 25 weeks of age. These age-dependent changes in the significance of the linkage of particular QTLs for BP could be the result of either epistatic factors or environmental influences. The data above emphasize the importance of age at which phenotypes are measured or tissue samples for gene expression are obtained. This factor should be taken into account when the effects of individual QTLs on a particular trait with significant age-related changes are being analysed. Using growth data, Schork et al. [93] proposed that the effects of QTLs could be largely affected by the age at which phenotype measurements are taken. Moreover, it was demonstrated in male SHRSP × WKY F2 rats [91] that QTL for BP on chromosome 10 only exerted a significant linkage after 7 months of salt loading, i.e. at 12 months of age. It remains to be determined whether the changing impact of this QTL depends more on the duration of high-salt intake or on the age at which the BP effects of chronic salt loading was studied. This could also be very important for future genetic studies of hypertension and other multifactorial disorders in human populations.

**Does age influence the effects of therapeutic interventions?**

Age is not only important for hypertension development, but also for its treatment. Knowledge of the exact age for the most effective intervention(s) is important not only for pharmacological intervention (see below), but also for future gene therapy of cardiovascular diseases. For example, gene therapy of 5-day-old SHRs, resulting in the long-term expression of an AT1 (angiotensin II type 1) receptor antisense transcript, successfully prevented the development of this type of genetic hypertension [94,95], but the effect of such treatment in adult animals was only temporary [96].

The same is true for chronic treatment of SHRs with ACE (angiotensin-converting enzyme) inhibitors or AT1 receptor antagonists, which have different consequences according to the stage of ontogeny in which they are applied (for a review, see [4]). It is evident that the early short-term administration of these drugs in the juvenile period (pre-puberty and/or puberty) not only attenuates the development of hypertension, but also causes long-term BP reduction persisting for several months after withdrawal of the antihypertensive drugs [11,51,97]. When the same drugs are given to SHRs older than 20 weeks of age, there is also a considerable fall in BP during active antihypertensive treatment, but no long-term BP reduction was observed after drug withdrawal [11,98]. On the basis of the findings by Harrap et al. [11] and Adams et al. [97], “a time window for efficient
short-term treatment of spontaneous hypertension” was formulated. This window coincides well with “the critical developmental period for spontaneous hypertension” described earlier by Albrecht [9]. It should be noted that pre-puberty was also suggested to be a critical period for the induction of severe salt-dependent hypertension in the rat [10].

The mechanisms responsible for long-term BP reduction induced by transient antihypertensive treatment in young SHRs are far from being clear, but structural changes in the resistance vessels, altered kidney function or centrally mediated alterations of sympathetic outflow and baroreflex efficiency have been suggested to be responsible for the late effects of early antihypertensive treatment (for details, see [4]). Although recent data strongly support the key role of the kidney in maintaining the BP reduction after withdrawal of ACE inhibitors [99], the contribution of angiotensin II-dependent central nervous system mechanisms leading to enhanced sympathetic tone [53,100] deserves special attention. Our recent experiments have indicated that the BP decrease in young SHRs treated with captopril during the critical juvenile period (4–10 weeks of age) was based entirely on a reduction in the sympathetic BP component, whereas there was no change in angiotensin-dependent vasoconstriction [54]. This was accompanied by major attenuation of the structural changes in resistance vessels which persisted for at least 20 weeks after withdrawal of this antihypertensive treatment [101]. In contrast, captopril treatment of adult SHRs elicited a less pronounced BP reduction, which disappeared soon after drug withdrawal. The same was also true for captopril-induced changes in the sympathetic BP component and structural remodelling of resistance vessels [101].

Major effects of antihypertensive treatment in the juvenile period were described not only in SHRs [11,51], but also in salt hypertensive Dahl rats treated with the AT\textsubscript{1} receptor blocker candesartan [52]. The common denominator of the two apparently contrasting forms of experimental hypertension might be the altered role of the central renin–angiotensin system in the control of sympathetic tone, because both forms of hypertension are characterized by enhanced sympathetic output [53,102–104]. Since chronic captopril treatment of immature rats lowers BP by attenuating its sympathetic component [53,54,101], elevated sympathetic output appears to be preset just at the juvenile period.

The data above suggest that the juvenile period might be critical for the induction of functional and structural alterations of resistance vessels characteristic for spontaneous and/or salt-dependent hypertension. Although these changes are based upon a genetic susceptibility to hypertension, they could be more prone to modifications induced by environmental factors (including nutrition or pharmacotherapy) influencing the organism just during this period.

**PERSPECTIVES OF THE JOINT EFFORT OF PHYSIOLOGISTS AND MOLECULAR BIOLOGISTS**

Several questions could be raised for discussion between physiologists and geneticists. What should be done beyond the human genome? Is our current methodology adequate? What more can we learn from animal studies? How can the results from animals be effectively applied to humans? Could the environment modify the genome? What is the exact critical developmental period (window) for prevention of hypertension? etc.

It is evident that the search for genetic and environmental determinants of multifactorial diseases (including hypertension, obesity, atherosclerosis etc.) is not easy, as documented by decades of extensive effort by many investigators. Although there is extensive knowledge about potential candidate genes, as well as the pathophysiology of established forms of such diseases, enormous difficulties are still faced in tracing the development of these diseases back to their origin. One of the complications in our models concerns the fact that ontogenetic changes of the phenotype overlap with its pathophysiological alterations occurring during disease development, with SHRs being a characteristic example. It is challenging to decode such a complicated spatio-temporal dynamic arrangement, because it is difficult to perform the necessary investigations (ranging from gene expression and biochemistry in various tissues up to organ function) at all stages of disease development. In addition, most of the methods used for estimation of intermediate phenotypes do not permit continuous measurement of changes emerging during disease development.

For years, physiologists have been trying to unravel the very early alterations in the physiological mechanisms important for abnormal development of given phenotypes, e.g. BP or plasma cholesterol. Meanwhile, geneticists have concentrated their efforts on searching for abnormal genes responsible for phenotype peculiarities in diseased animals or humans. Such a strategy becomes much less effective if the disease is a consequence of inappropriate regulation of normal unaltered gene(s). If the initial trigger of disease development depends on a transient alteration in gene function occurring during the early critical period (developmental window; for reviews, see [4,8]) that considerably precedes the time of phenotype determination (usually performed in animals or subjects with obvious disease), we can hardly find the true cause of the disease of interest. The respective gene is unchanged, its actual function is normal, the original pathophysiological event is definitely lost, but the development of a particular system (cardiovascular, renal, sympathetic, central nervous etc.) has already been substantially modified. It seems that we know a lot about the genome as well as about the physiology of the
mature organism in several mammalian species, but our knowledge about the realization of genomic information in the physiology of the developing organism is still insufficient. If we knew how the genes operate in normal ontogeny (especially during particular critical periods), we would probably be closer to decoding the primary causes of genetic hypertension, metabolic syndrome, dyslipidaemia, obesity etc. Better said, we should fill the gap between the genotype and phenotype of interest by intermediate phenotypes which should, however, be perceived in their full developmental (ontogenetic) dimension.

Fetal ‘programming’ of cardiovascular and metabolic diseases [1,105] is the most striking example of how important the developmental approach is for studying chronic multifactorial diseases which are based upon enhanced genetic susceptibility to particular environmental factors. In this case, there is a gap of months or even decades (depending on the species investigated) between the initial event and the manifestation of the disease. If epigenetic ‘gene silencing’ was the underlying mechanism [55], the first signs of dysfunction might become apparent at a stage of ontogeny when the environmental challenge or intrinsic demands require a proper function of the respective gene.

It is evident that the combinations of detailed genome scanning, large-scale utilization of mRNA and protein microarrays and determination of multiple intermediate phenotypes (physiological, morphological, biochemical etc.) in adult animals with established forms of cardiovascular diseases can hardly yield the desired information about what is happening in immature animals at the time when the initial (primary) stimulus started the cascade of pathophysiological events leading to the development of the disease of interest. To answer the principal questions, such as (i) which genes were important at the beginning of the pathophysiological process? (ii) when did mRNA and protein expression begin to be abnormal? (iii) which were the primary phenotypic changes in disease development? or (iv) which mechanisms should be therapeutically targeted before the disease had developed?, it would be desirable to investigate the cardiovascular system at earlier stages of ontogeny. The time schedule of critical developmental periods provides a useful tool for orientation to the stages of major transformation of the cardiovascular system. Physiologists should provide reliable data on the very early onset of functional and structural abnormalities of particular cardiovascular parameters. Molecular geneticists should search for changes in the expression of relevant genes just prior to the appearance of such functional abnormalities. Transient pharmacological or molecular biological interventions during respective critical developmental periods will show how important the disclosed mechanisms for the development of a particular cardiovascular abnormality and/or disease might be. A necessary prerequisite for this type of research is the use of such animal models in which the adult phenotypes of interest can be anticipated, i.e. recombinant inbred congenic or consomic strains, rather than classical F1 hybrids or backcross populations. In this respect, Prague recombinant inbred strains [106] are a good example. On the other hand, great care should be taken with regard to the rational use of transgenic or knockout animals, because the genetic manipulation might alter specific features of ontogenetic development (e.g. cardiovascular or renal system), such that the secondary changes elicited by compensatory mechanisms might obscure alterations due to primary defects. Perhaps the models based upon ‘gene switch-on/switch-off’ or transient gene disabling by antisense application might be helpful if rationally applied in particular critical developmental periods.

CONCLUSIONS

In this review, we have proposed some ways in which physiologists and molecular biologists can collaborate to achieve the decisive breakthrough in cardiovascular research. We believe that not only an understanding of all of the regulatory mechanisms and their genetic basis is required, but that the precise timing and definition of all of the developmental events participating in the relevant pathophysiological processes should help to solve the pathogenetic mechanisms of cardiovascular diseases. Accordingly, the concept of critical developmental periods (windows) represents a useful tool for future research. Nevertheless, future success is primarily dependent on changing our way of thinking of ‘physiological genetics’ or ‘genetic physiology’, rather than on refining our physiological or genetic methods.

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