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PERSONAL VIEW

The Phenotypic Patterns of Essential Hypertension Are the Key to Identifying "High Blood Pressure" Genes

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Summary

The genes that cause or increase susceptibility to essential hypertension (EH) and related animal models remain unknown. Their identification is unlikely to be realized with current genetic approaches, because of ambiguities in the genotype-phenotype relationships in these polygenic disorders. In turn, the phenotype is not just an aggregate of traits, but needs to be related to specific components of the circulatory control system at different stages of EH. Hence, clues about important genes must come through the phenotype, reversing the order of current approaches. A recent systems analysis has highlighted major differences in circulatory control in the two main syndromes of EH: 1) stress-and-salt-related EH (SSR-EH) – a constrictor hypertension with low blood volume; 2) hypertensive obesity – SSR-EH plus obesity. Each is initiated through sensitization of central synapses linking the cerebral cortex to the hypothalamic defense area. Several mechanisms are probably involved, including cerebellar effects on baroreflexes. The result is a sustained increase in sympathetic neural activity at stimulus levels that have no effect in normal subjects. Subsequent progression of EH is largely through interactions with non-neural mechanisms, including changes in concentration of vascular autacoids (e.g. nitric oxide) and the amplifying effect of structural changes in large resistance vessels. The rising vasoconstriction increases heterogeneity of blood flow, causing rarefaction (decreased microvascular density) and deterioration of vital organs. SSR-EH also increases food intake in response to stress, but only 40% of these individuals develop hypertensive obesity. Their brain ignores the adiposity signals that normally reduce eating. Hyperinsulinemia masks the sympathetic vasoconstriction through its dilator action, raises blood volume, whilst renal nephropathy and other diabetic complications are common. In each syndrome the neural and non-neural determinants of hypertension provide targets for identifying high BP genes. Reading the genome from the phenotype will require new approaches, such as those used in developmental genetics. In addition, transgenic technology may help verify hypotheses and examine whether an observed effect is through single or multiple mechanisms. To obtain answers will require substantial collaborative efforts between physiologists and geneticists.

Key words

Genotype-phenotype relationship • Integrative Biology • Neural and non-neural mechanisms • Stress, high salt, obesity • Systems analysis and phenotype

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Introduction

Little is known about the genes responsible for the rise in blood pressure (BP) in essential hypertension (EH) and in inbred strains of rats with genetic hypertension, apart from the polygenic nature of their inheritance (Lifton and Jeunemaitre 1993, Luft 1998, Rapp 2000, Pravenec *et al.* 2003, Cowley 2006). Similar difficulty in identifying causal genes has also been found in non-cardiovascular polygenic disorders such as multiple sclerosis (Baranzini *et al.* 2010, Barash *et al.* 2010). In contrast, the causal genes have been successfully identified in several rare types of monogenic hypertension (Lifton 1996). In these, each hypertension phenotype consists of a well defined pathophysiological

abnormality that provided clues about the function of the causal genes.

The uncertainty about BP genes in EH may be compounded by the greater complexity in the regulation of gene function that has come to light in the last 10-15 years (Barash *et al.* 2010, Collins 2010, Feero *et al.* 2010, Varmus 2010). The old view of one gene → one protein no longer applies, in view of the great disparity between the number of mammalian genes (~25,000) and the number of proteins (~100,000). It suggests that most genes manufacture more than one protein. In addition, several mechanisms regulating gene transcription have been discovered, including small non-coding RNA molecules, epigenetic phenomena and tissue-specific determinants of alternative splicing. All the above factors are likely to increase the ambiguity of the relationship between genotype and phenotype in polygenic disorders.

For over 25 years geneticists have made an intensive effort to discover the genes important in EH and the various types of genetic hypertension in the rat. Early studies searched for linkages between EH (and animal models), and various mutant "candidate" genes, identified through markers related to pathways contributing to circulatory regulation. The markers selected were related to the renin-angiotensin-aldosterone system, the adrenergic pathways, vascular structure and several metabolic pathways (Lifton and Jeunmaitre 1993, Cowley 2006). None were strongly (or reproducibly) related to hypertension. Since the mid-1990s, genome-wide association studies have been employed, using markers distributed throughout the genome, in accord with the widespread distribution of blood pressure (BP)-related quantitative trait loci (QTLs) (Pravenec *et al.* 2003, Cowley 2006). Some studies reported that in subjects with EH, BP-related QTLs were preferentially located on certain chromosomes (Cowley 2006). However, the findings have not been uniform, and in most instances little is known about the function of the genes of a particular QTL, i.e. their phenotypes. As Venter (2010) has stated "*The generation of genomic data will have little value without corresponding phenotypic information about individuals' observable characteristics and computational tools for linking the two*." Defining the hypertension phenotype needs to be in the context of components of the control system responsible for the alteration in function.

The linkage and association studies suggest that a large number of genes affect the level of BP. This accords with George Pickering's original conclusion, that many genes, each with only a small effect, determine inheritance of BP (both high and low) in the general population (Pickering G. W. 1961, 1968). Probably most of the polymorphisms relate to minor variation in cardiovascular structure and function, that differ only slightly between hypertensives and normotensives. Importantly, this does not preclude their co-existence with a relatively small number of mutants important in the pathogenesis of EH. The linkage and association studies make no distinction between the two sets of genes and provide little useful information about the mechanisms that elevate BP, because of their focus on the BP phenotype.

George Pickering regarded EH as a "*quantitative deviation in blood pressure from the norm*", and rejected the view that EH was caused by "*a unique and specific fault that distinguishes those with the disease from those without it*" (Pickering G. W. 1968). These conclusions are due to overemphasis on the BP phenotype, which continues to this day.

BP is influenced by all that goes on in the circulation, which makes it an ideal clinical variable for diagnosing EH, for risk assessment and for determining the role of lifestyle, diet and other environmental factors. However, its multifactorial nature provides no clues about underlying mechanisms, which require variables that give specific information about which component of the circulatory control system is affected. To deliver this type of information has been the aim of much of the hypertension research performed over the last 80 years. This has resulted in a number of theories about the pathogenesis of EH (Folkow 1982, Swales, 1994, Laragh and Brenner 1995). Many of them have promoted single mechanisms including renal dysfunction, high salt intake, obesity, sympathetic overactivity and vascular structural changes, without regard to interactions between them, or when a given factor comes into play. Since each explains only part of the story, many still regard EH as a disorder of unknown cause.

What has long been required is a comprehensive analysis of the working of the cardiovascular control systems in EH and in the normal circulation. I have tried to do this in my recent book on the causes of EH (Korner 2007) and this essay summarizes some of its conclusions. The two main syndromes of EH are 1) stress-and-salt related EH (SSR-EH) in non-obese individuals, a constrictor hypertension with subnormal blood volume, and 2) hypertensive obesity, where obesity is superimposed on SSR-EH and blood volume is raised.

Smoking and other dietary factors may affect BP in each syndrome. Each syndrome is initiated through neural mechanisms, whilst non-neural mechanisms largely account for the progression from mild to severe EH. Major functional differences in the operation of the control system between those susceptible to EH and normotensives define critical elements of the phenotype, and suggest targets for genetic analysis. As Noble (2006) has expressed it "*The genome needs to be read through the phenotype, not the other way about*". This is the opposite to the approaches currently employed.

Some features of the cardiovascular control system

Genetic factors account for 30 % of the BP variance in the population, whilst 70 % are environmentally determined (Pickering G. W. 1961, Ward 1995). The causal genes are thought to increase susceptibility to environmental factors including life style. This accords with findings in the borderline hypertensive rat (BHR; i.e. the F_1 hybrid SHR×WKY of the spontaneously hypertensive rat and Wistar Kyoto rat), in which the BP is normal in a favorable environment, whilst adverse environmental conditions are necessary in order to develop hypertension (Lawler *et al.* 1981). In contrast, in SHR, with twice as many "high BP" genes as in BHR, hypertension develops in virtually every environment. Similarly, the other parent strain, the WKY, remains normotensive in most environments. Thus, the BHR is a good model for EH, since in both environmental "reinforcement" is necessary to develop hypertension.

The circulation behaves like a non-linear adaptive control system, where parameters are actively altered to maintain system performance once operating conditions exceed defined limits (Korner 2007). Special sensors and test stimuli provide information about moment-to-moment performance, whilst a computer calculates the magnitude of the required parameter adjustments. Adaptive systems are multivariate and have a hierarchical configuration, memory and logic circuits, much as in the central nervous system (CNS).

A disturbance usually activates several sensory inputs to the CNS and affects the activity of a network of "cardiovascular" neurons which translate the afferent information into autonomic and hormonal outputs. The outputs often depend not only on the immediate sensory input, but also on analogous events stored in memory.

The activity of central sympathetic and parasympathetic vagal motoneurons is regulated by premotor neurons that release "fast" (rapidly acting) excitatory or inhibitory transmitters at their synapses, e.g. glutamate or γ-amino butyric acid (GABA) (Dampney 1994, Pilowsky and Goodchild 2002). In turn, premotor activity is modulated by groups of "slow" transmitter neurons that carry information from various brain regions about changes within the body and environment. Their release of slow transmitters, such as monoamines and peptides, modulates the responsiveness of the fast transmitter neurons (Greengard 2001). The slow transmitter neurons are the parameter adjusters of the central autonomic neurons. There are also peripheral parameter adjustments, e.g. through the development of functional and structural changes in the resistance vasculature and heart in hypertension, which enhance their responses (see below) (Korner *et al.* 2010).

Environmental causes of early neural changes

This section briefly refers to the mechanisms that initiate SSR-EH and hypertensive obesity and some of the genetic rat models of hypertension. Evidence that stress initiates EH comes from several case-control studies in humans (Schnall *et al.* 1990, Staessen *et al.* 1994, Poulter and Sever 1994, Timio *et al.* 1997).

Stress-and-salt-related EH

Stress arises when the subject has serious doubts about coping with an environmental challenge (Karasek *et al.* 1981, Frankenhaeuser 1987, Pickering T. G. 1994). Stress is sensed through thalamocortical and limbic neurons, and raises the activity of dopaminergic (DA) neurons which stimulate a pathway linking the prefrontal cortex to neurons in the hypothalamus and midbrain (Le Moal and Simon 1991). This evokes the defense response, where BP increases as a result of sympathetic α-adrenoceptor-mediated vasoconstriction in renal, gastrointestinal and skin beds and vasodilatation in skeletal muscle. In the latter β-adrenoceptor-mediated vasodilatation masks the vasoconstrictor effect of increased sympathetic neural activity (SNA). Sympathetic stimulation of cardiac β-ARs also provides chronotropic and inotropic support to the heart.

Chronic stress elicits the defense response in both normotensives and those susceptible to EH. But in the EH group, particularly if they are sedentary or have

little social support, DA synapses become sensitized (strengthened). This explains why the defense response is already evident under resting conditions in persons with mild EH, whilst the same environment has no effect in normal subjects (Brod 1960, 1963). When the period of stress is over, BP and SNA return more slowly to pre-stress values in those with EH compared to normotensives.

In persons with mild SSR-EH acute stressors elicit rises in regional vascular resistance or SNA that are about double the responses of normotensives (Brod *et al.* 1959, Hollenberg *et al.* 1981, Somers *et al.* 1988). The true enhancement is attenuated by baroreflexes and forebrain inhibitory mechanisms, such as the lung inflation reflex which moderates the constrictor effects of arterial hypoxic stress (Daly *et al.* 1967, Korner *et al.* 1969). Eliminating this during hypoxia by brief voluntary apnoea, unmasks the true increase in SNA in mild EH, which is 10-15 times the response in normotensives (Somers *et al.* 1988). This indicates considerable sensitization of synaptic transmission.

Characteristically sensitization is induced in hippocampal memory synapses through increased serotonergic (5-HT) neuron activity associated with learning (Kandel 2001). This produces long-term potentiation (LTP) of excitatory postsynaptic potentials (EPSPs), so that with repeated stimulation the response threshold is reduced progressively. In the short-term glutamate release per nerve impulse rises, whilst in the long-term there is additional growth of new synaptic endings.

The capacity for almost permanent excitation of the defense pathway is very close to a "*unique and specific fault*", suggesting that George Pickering was wrong. The synaptic changes are partially antagonized by regular submaximal exercise training, as discussed below (Jennings *et al.* 1986, 1991, Nelson *et al.* 1986, Meredith *et al.* 1990).

Exercise training as a physiological stress model

Exercise is a self-imposed challenge and the circulatory changes are a variant of the defense response. Exercise training provides insights into some of the brain's long-term operations in this and probably other types of stress. The training program consisted of 30-min periods of regular exercise training (three to seven times a week) at either submaximal or near-maximal work intensity, which was expressed as a percentage of each subject's maximum work capacity W_{max}. With submaximal exercise (50-60 % of W_{max}), resting BP and SNA fall progressively between successive training sessions in both humans and SHR, reaching a plateau in 10-14 days (Tipton *et al.* 1983, 1984, Jennings *et al.* 1986, Meredith *et al.* 1990, 1991). In humans the change occurs in both normotensives and those with EH. On the other hand, when training at 75-85 % of W_{max} resting BP does not fall between sessions and often increases (Tipton1983, Kingwell and Jennings 1993). Clearly, what happens during exercise determines the long-term resting BP between training sessions.

The autonomic drive during exercise is provided by 1) "central command" from the cerebral cortex, 2) muscle "chemoreceptors" from active muscle, and 3) interactions of 1) and 2) with neurons subserving baroreflexes (Ludbrook and Graham 1985, Miki *et al.* 2003). The cerebellar Purkinje cells receive input from central command *via* the climbing fibers and from muscle chemoreceptors *via* the mossy-parallel fiber system. Korner (2007) has suggested that a comparison of the magnitude and direction of these inputs at the Purkinje synapses alters baroreflex properties in the long-term, contributing to the changes in resting BP and SNA between training sessions.

Almost everyone can cope with submaximal levels of exercise. Central command activity declines during each session, whilst that from muscle chemoreceptors increases with accumulation of anaerobic metabolites. The disparity between the two sets of signals produces long-term potentiation of excitatory postsynaptic potentials at the synapses linking mossy-parallel fibers with the GABAergic Purkinje neurons (Ito *et al.* 1982, Ito 1984). This increases Purkinje neuron inhibition of the deep cerebellar nuclei, reducing gain and range of sympathetic constrictor baroreflexes, probably through inhibitory bulbar noradrenergic (NA) neurons (Korner *et al.* 1987). This contributes to the fall in BP and SNA between training sessions. However, when training intensity is greater than 75-80 % of W_{max} the inputs from central command and muscle chemoreceptors both increase, since much mental effort is required to maintain the level of exercise during each session. The result is long-term depression (LTD) at Purkinje synapses and disinhibition of deep cerebellar nuclei, with increases in gain and range of constrictor baroreflexes, probably through 5-HT neurons (Korner and Head 1981). In turn, this increases BP and SNA during exercise and between training sessions.

The "memory" of the network for exercise is

long, but not as long as in BHR after repeated exposure to aversive stress. The same probably applies in the responses to psychosocial stress in persons with EH. A similar comparator action of the cerebellum probably participates in other stress-related responses (Bartha and Thompson 1995). It should be noted that LTP and LTD occur not only at Purkinje or hippocampal synapses but also at synapses of other neurons, including DA circuits important in behavior (Malenka and Bear 2004).

At the cellular level, the early phase of LTP depends on greater activation of glutamate receptors, changes in properties of presynaptic proteins, Ca^{2+} entry into the postsynaptic dendritic spines, activation of calcium-calmodulin-dependent protein kinases and phosphorylation of synaptic receptor proteins. The later phase of LTP requires new protein synthesis (Kandel 2001). The mechanisms underlying LTD involve distinctive changes in dendritic Ca^{2+}, dephosphorylation of specific synaptic proteins and post-translational changes in AMPA receptor channels.

High salt intake

Mammals have an intrinsic "hunger for salt", which may be genetically determined (Denton 1982). However, the BP of most normotensives is "resistant" to a high salt intake. This is due to the relative impermeability of the blood-brain barrier to the sodium ion (Simchon *et al.* 1999) and due to the kidney's high capacity for excreting sodium. The latter depends on the maintenance of a high medullary blood flow, which, in turn, requires high rates of NO synthesis (Cowley *et al.* 1995). In contrast, in the highly salt-sensitive Dahl S rats and most substrains of SHR the blood-brain barrier is more permeable to sodium than in inbred normotensive strains, including the salt-resistant Dahl R and Wistar-Kyoto (WKY) rats.

The greater influx of sodium into the cerebrospinal fluid (CSF) increases brain aldosterone synthesis, which enhances sodium transport through an amiloride-sensitive sodium channel. This stimulates neurons that release ouabain-like cardiotonic steroids, which in turn stimulate other neurons to release angiotensin II (Ang II). These project to the hypothalamic defense area and activate the sympathetic nerves (Huang *et al.* 2008, Leenen 2010).

About 20-30 % of normotensive humans are salt-sensitive (Fujita *et al.* 1980, Sullivan 1991), indicating that their BP is not quite as salt-resistant as many inbred normotensive rat strains. As EH increases in severity, the proportion of salt-sensitive individuals rises to almost 100 %. The reason for this is uncertain. The ECF volume in this group is reduced (see below), so that for a given high salt intake plasma concentration of NaCl will be higher than in normotensives. Hence, even a slight increase in the blood-brain barrier's Na^+ permeability will allow a greater influx of sodium from plasma to CSF. In fact, in salt-sensitive persons with EH, a high salt diet raises SNA and elicits the defense response after a latency of 10-14 days (Koolen and Van Brummelen 1984, Fujita *et al.* 1990). This is much longer than in salt-sensitive rats, but the rise in SNA suggests that similar central pathways are involved.

The initial rise in BP in mild SSR-EH is entirely due to an increased cardiac output (CO). This is a part of the defense response and is not due to volume overload. It arises because the vasodilation in skeletal muscle offsets the vasoconstriction in renal and gastrointestinal beds, with the raised cardiac SNA maintaining the elevated cardiac output (Korner 2007).

Hypertensive obesity

Some have regarded the association between hypertension and obesity as due to failure of sympathetic regulation of energy balance (Bray and York 1979). Others consider the thermogenesis-related elevation of SNA in obesity as sufficient to raise BP to the levels found in EH (Landsberg and Young 1978, Landsberg 2001). This is not in accord with the finding that by the time obesity has become chronic, thermogenesis is increased only slightly. Another theory considers that stress causes both EH and obesity (Björntorp 1996, Björntorp *et al.* 2000). This does not explain why about 60 % of obese individuals are nearly normotensive. In my opinion a variant of Björntorp's theory, in accord with observations, is that the excessive consumption of food is a stress-relieving response in all persons with SSR-EH (Kissebah and Krakower 1994), where increased DA neuron activity not only increases food intake (Leibowitz 1984), but also evokes the defense response. However, relief of stress is very transient, contrasting with the long lasting effects of exercise training.

Persons with SSR-EH tend to eat more, but are only slightly overweight, because of the effective way that the system regulating energy balance responds to leptin and other feedback signals from the accumulating fat. However, in about 40 % of persons with SSR-EH the brain ignores these signals, so that the obesity is superimposed (Korner 2007). Their BP is only slightly

greater than in non-obese persons with SSR-EH. The sympathetic pattern is similar to that in SSR-EH, but there is baroreflex-mediated attenuation of the magnitude of SNA changes due to marked elevation of cardiopulmonary load (Grassi *et al.* 1995).

Baroreflexes in essential hypertension

In every type of hypertension baroreflexes undergo similar changes in properties, due to rises in cardiopulmonary load (Korner 2007). This alters the relative magnitude of afferent inputs from arterial, cardiac and pulmonary baroreceptors. Only moderate increases in cardiopulmonary load are required to alter baroreceptor-heart rate reflex properties, which include reduction in gain and heart rate range and attenuation of the vagal plateau at high blood pressures. The changes are due to the modulatory action of central 5-HT neurons (Korner and Head 1981). Similar changes are produced by elevation of plasma Ang II through its inhibitory action on neural transmission in the peripheral vagal ganglia. However, sympathetic constrictor baroreflexes are inhibited only at very high cardiopulmonary loads, e.g. in pronounced hypertensive obesity or during cardiac failure (Grassi *et al.* 1995). In non-obese persons with SSR-EH, gain of these reflexes is enhanced, possibly through cerebellar mechanisms similar to those associated with high intensity exercise training.

SHR hypertension

SHR provide a genetic model of neurally initiated hypertension. A lifelong sympathetic hyper-responsiveness is in large part due to sensitization of the DA neuronal pathway between cortex and hypothalamic defense area. The hypertension is markedly attenuated following 6-hydroxydopamine-mediated destruction of DA neurons in young animals (Van den Buuse *et al.* 1984). The hypothalamic defense neurons are already sensitized in the embryo: grafts of hypothalamic tissue from SHR embryos into 10-week-old WKY elicit a sustained 30 % rise in BP, whilst only small and transient changes develop after grafts from WKY embryos (Eilam *et al.* 1991).

An additional source of the raised BP in SHR is its so-called hypernoradrenergic innervation (Head 1989, 1991). It is due to secretion early in life of large amounts of nerve growth factor by differentiating vascular smooth muscle cells. This increases the number of neurons in sympathetic ganglia with corresponding increases in the number of post-ganglionic fibres innervating the vasculature (Thoenen and Barde 1980, Levi-Montalcini 1987). This has been promoted as an important cause of SHR hypertension, but the magnitude of the effect is difficult to assess by standard physiological methods. However, in a cosegregation study performed in an F_2 population derived from SHR and inbred normotensive rats, the difference in mean arterial pressure (MAP) between homozygotes for the SHR genotype and homozygotes for the normotensive genotype was found to be 8 mm Hg (Kapuscinski *et al.* 1996). This is only about 16 % of MAP difference between the parental strains.

It is not known whether peripheral hyper-noradrenergic changes occur in EH, but analogous increases have been described in the number of nerve cells in peripheral sympathetic ganglia in stressed hypertensive mice deficient in the phosducin gene and in hypertensive humans with a similar genetic deficit (Beetz *et al.* 2009).

Lastly, as SHR hypertension becomes more severe, non-neural mechanisms account for much of the rise in BP and TPR, which is in accord with what happens in EH (see below). However, the initiation by neural factors is a prerequisite for the development of SHR hypertension, since this is prevented completely by sympatho-adrenal inactivation in early life (Lee *et al.* 1991, Korner *et al.* 1993).

Non-neural mechanisms

As EH becomes more severe, SNA remains at levels observed in mild EH, despite progressive further elevation in BP, TPR and regional vascular resistances. It suggests that non-neural mechanisms account for the added rises in BP and vascular resistance. The non-neural mechanisms differ somewhat in the two syndromes of EH, but contribute to the deterioration of vital organs in both. SNA may rise again in older hypertensives with impaired renal function and/or heart failure (Esler *et al.* 1981, 2002, Esler 1995, Grassi 1998, Augustyniak *et al.* 2002).

Stress-and-salt related EH

Endothelial NO production is already impaired during mild EH through cortisol-induced inhibition of NO synthase (NOS), as part of the defense response (Korner 2007). As hypertension progresses, further increases in destruction of NO occur through local increases in thromboxanes and reactive oxygen species (ROS). The changes in concentration of both dilator and constrictor autacoids raise basal constrictor tone beyond levels due to the sympatho-adrenal changes.

The structural changes develop in the large resistance vessels (R_1 vessels, internal radius r_i of 50-200 μm) (Folkow 1982). They include narrowing of r_i, an increase wall thickness/r_i (w/r_i) ratio and often reduction in distensibility. Similar changes also occur in the renal afferent arteriole, despite its small size, whilst in the other small arterioles (R_2 vessels, r_i < 50 μm) structure remains normal. In the R_1 vessels the first two factors confer a mechanical advantage, where a given constrictor or dilator stimulus elicits a greater-than-normal change in vascular resistance (Korner and Angus 1992). The enhancement is due to the altered geometry and is similar for different agonists (Korner and Angus 1992). The amplifier effect is readily demonstrated *in vitro* (Folkow *et al.* 1970, Adams *et al.* 1989, 1990, Korner *et al.* 1992, Agabiti-Rosei *et al.* 1995). Its role is similar *in vivo*, but its action is over a smaller dose range about the resting value (Korner *et al.* 2010). In EH and SHR the amplification factor is about 1.3-1.5 (Folkow 1995, Rizzoni *et al.* 1996). Thus, in SSR-EH the action of the structural amplifier enhances the effects of raised SNA plus autacoid-induced constriction on BP and TPR by a further 30-50 %.

Structural remodeling of vascular smooth muscle (VSM) and other wall constituents occurs in response to local hemodynamic, neuroendocrine and paracrine signals (Berk *et al.* 1987, Dzau and Gibbons 1987, Berk and Alexander 1989, Dzau *et al.* 1994, Bobik and Campbell 1993). For example, *in vitro* elevation of circumferential wall stress induces VSM cell proliferation through locally produced Ang II and plasma derived growth factor (PDGF) (Sudhir *et al.* 1993). *In vivo* these are complemented by neuroendocrine and local signals. The nature of the latter signals is probably different in SSR-EH and renovascular hypertension (Rizzoni *et al.* 1996).

The rising vasoconstriction leads to micro-circulatory rarefaction, through closure of a proportion of the R_2 arterioles and associated capillaries. At first rarefaction is functional (reversible) and but later some of it becomes permanent (Korner and Courtice 1954, Prewitt *et al.* 1982, Hutchins *et al.* 1996, Korner 2007). The vessels that close first are the narrowest and most distensible R_2 vessels in a particular bed (Kostromina *et al.* 1991, Sands *et al.* 1992). Small veins also close in beds with highly distensible veins, i.e. the gastrointestinal and skin beds. Rarefaction contributes to reduction in extravascular fluid and total blood volume in moderate and severe SSR-EH (Tarazi *et al.* 1968, Ulrych *et al.* 1971, Safar *et al.* 1995). The associated precapillary constriction shifts blood into the central cardiopulmonary compartment, whilst the renal action of atrial natriuretic peptide maintains the reduction in total blood volume at about 20 % (Tarazi *et al.* 1968).

Vasoconstriction-induced rarefaction accentuates heterogeneity of blood flow. This is particularly striking in the kidney where intrinsic heterogeneity is already considerable (Sands *et al.* 1992). As hypertension becomes more severe, there is closure of many afferent arterioles to outer and mid-cortical nephrons, with shunting of blood flow to the juxtamedullary nephrons. This is responsible for further elevation of BP and vascular resistance.

Permanent rarefaction develops in response to frequent episodes of functional rarefaction and activation of factors that cause intravascular thrombosis and induce tissue and perivascular inflammation (Johnson *et al.* 2008, Manfredi and Rovere-Quirinia 2010, Zhang *et al.* 2010). The inflammatory response is partly due to release mitochondria from dying tissue, which the immune system mistakes for bacteria.

Tissue integrity is further compromised through increasing impairment in production of NO in conduit arteries. This leads to loss of "ascending vasodilatation" in conduit arteries. This normally helps to meet the blood flow needs of very active tissues without compromising those of less active nearby tissue. Without this dilatation of conduit arteries these needs cannot be met, so that the active tissues "steal" the blood supply of nearby regions. This further accentuates the functional impairment of kidney, myocardium and brain (Reivich *et al.* 1961, Engelmann *et al.* 1987, Ljungman and Granerus 1995, Lambert *et al.* 1996).

Structural changes also develop in the conduit arteries and consist of an increase in w/r_i ratio and reduction in wall distensibility, with the latter particularly striking on a habitual high salt diet (Benetos and Safar 1993, Benetos *et al.* 1995). The raised w/r_i ratio restores circumferential wall stress close to normal and prevents arterial blowout. However, a stiffer conduit artery wall contributes to a preferential increase in systolic compared to diastolic BP in the ascending aorta, increasing the risk of developing left ventricle (LV) failure and stroke (O'Rourke 1982, 2002).

Hypertensive obesity

One of the main accompaniments of obesity is impaired cellular glucose transport. This places a chronic burden on the pancreas that causes hyperinsulinemia and

hyperglycemia and eventually type 2 (non-insulin dependent) diabetes. The impairment of glucose transport is due to numerous factors, including suboptimal function of insulin receptors (Kahn 1995). Presumably the objective is to provide energy through metabolism of fat rather than glucose. One mechanism that depresses insulin receptor function is the release of tumor necrosis factor alpha (TNF-α) by adipocytes.

The vasodilator action of high insulin masks SNA-related vasoconstriction in most beds, so that TPR is lower than in SSR-EH. Indeed, the hyperinsulinemia accounts for most of the hemodynamic changes in obesity, with or without elevation of BP (Hall *et al.* 1992). Factors contributing to the vasodilatation include greater flow-related NO production, stimulation of Na/K-ATPase and increased Ca-ATPase activity (Anderson and Mark 1993, Tack *et al.* 1996). Blood volume is high due to the action of insulin on the proximal convoluted tubules of the kidney, which promotes sodium and water reabsorption (Baum 1987, Kurokawa *et al.* 1992). Salt sensitivity in these subjects tends to be reduced (Tuck 1994).

Because of the wider R_1 vessels in hypertensive obesity the enhancement of vascular resistance by the structural amplifier is smaller than in SSR-EH. In many vascular beds microcirculatory perfusion heterogeneity is also less pronounced, but in the renal bed focal nephropathy develops, partly associated with greater renin-Ang II production and a leakier glomerular membrane causing microalbuminuria. LV failure due to high volume and pressure loads is a common complication, as are numerous lipid abnormalities which account for the high prevalence of coronary, renal and other types of vascular disease.

Family and cosegregation studies

A large number of family studies support the idea that EH has a genetic basis. For example, a history of hypertension in parents increases the chances that their children will be hypertensive (Hunt *et al.* 1991, Burke *et al.* 1998). In the Bergen study the BP of the offspring, measured some 30 years after that of the parents, was found to be highest when both parents were hypertensive and lowest when both were normotensive (Mo *et al.* 1995). Another study found that the rises in renal vascular resistance and plasma renin activity in response to mental stress were greater in persons with mild EH than in normotensives with a negative family history of EH and were of intermediate magnitude in normotensives with at least one hypertensive parent (Hollenberg *et al.* 1981). Parental BP has also been used to identify a "pre-EH" group of subjects: in those with two hypertensive parents exchangeable sodium space and plasma volume were lower and renal vascular resistance was greater than in those with two normotensive parents (Harrap *et al.* 2000). Another association was that the chances of becoming overweight over a 10-year period were greater in persons with EH than in those with normal BP (Kannel *et al.* 1967, Julius *et al.* 2000).

A greater range of traits has been studied in SHR than in humans. Studies in F_2 or backcross populations have shown cosegregation of resting renal SNA with BP (Judy *et al.* 1979), the rise of renal SNA in response to stressful stimuli (Di Bona *et al.* 1996), and the fall of renal SNA following intracerebroventricular guanabenz, as a marker of overall central sympathetic tone (Di Bona *et al.* 1996). In the periphery, the replication rate of VSM cells under controlled *in vitro* conditions is more rapid in SHR than in WKY, suggesting a role for genetic mechanisms (Hadrava *et al.* 1992, Hamet *et al.* 1998). This is also suggested by the inverse correlation between r_i in renal afferent arterioles of 7-week-old rats and the BP of the same animals at the age of 23 weeks (Norrelund *et al.* 1994).

Fetal origins of hypertension

One current controversy about the causes of EH is whether it begins in young or middle aged adults, as appears to be the case clinically, or whether it starts during fetal life, through neuroendocrine reprogramming due to maternal ill health or malnutrition (Barker *et al.* 1992). According to Barker, babies with the lowest birth weight are at greatest risk and there is some support that EH may develop through this mechanism (Law and Barker 1994). However, its role is probably smaller than envisaged in the earlier small scale studies. A large study on 150,000 Swedish army recruits aged 18 years found that the systolic BP difference between the highest and lowest birthweight group was only 1.5 mm Hg, though this was statistically significant (Nilsson *et al.* 1997). It suggests that the effect of fetal ill health on adult BP probably accounts for only a small proportion of adult EH (Korner 2007). Reprogramming may also contribute to a greater increase in salt sensitivity and to various metabolic perturbations, as suggested from the work of Zicha and Kuneš (1999).

In my opinion, EH mostly begins in adult life and, because of the relatively small number of stress-and-salt related genes, environmental reinforcement is needed to raise BP, as in BHR. Both contrast with the childhood onset of hypertension in SHR, with twice the number of "high BP" genes. In an interesting study Harrap (1988) categorized an F_2 population derived from SHR×WKY at 16 weeks of age, by selecting rats in the upper and lower BP quartiles of the population. In fact the "high" and "low" BP rats had identical blood pressures till the age of 12 weeks before the BP diverged. In the parental rats BP already diverged at 5-6 weeks of age. The difference suggests that in the F_2 populations the high-BP rats probably had fewer high-BP genes than SHR, whilst the low-BP rats had more BP genes than WKY.

Renal transplantation

The results of the renal transplantation experiments that were first performed in the mid 1970s have long been regarded as cast-iron evidence that genetic hypertension starts as a developmental renal fault (Rettig and Schmitt 1994, Rettig and Grisk 2005, Korner 2007). Hence the subsequent emphasis on kidney-related genes. We now know from the results obtained when the animals are maintained on a high salt diet, that the hypertension associated with transplanting a normotensive donor kidney into a hypertensive recipient is due to activation of the CNS salt sensitive pathway and not to primary renal dysfunction (Morgan *et al.* 1990, Leenen *et al.* 2002).

Moreover, most transplants have been performed in 9-15 weeks old rats, in which structural narrowing of the R_1 vessels is well developed, so that the transplantation of a kidney from a hypertensive donor is equivalent to transplanting a kidney with renal artery stenosis, which also produces permanent hypertension (Rettig and Schmitt 1994). Giving an angiotensin converting enzyme inhibitor to young animals widens the R_1 vessels and now transplanting the kidney no longer elicits hypertension (Smallegange *et al.* 2003). Thus, factors other than renal tubular dysfunction affect the BP changes following transplantation.

A physiologists perspective of the phenotype: genotype relationship

It is only during embryonic and fetal life that the genes are the organism's paramount regulators. In the newly formed embryo the development of the cardiovascular system and of the other organs represent the execution of the prerecorded instructions of specific genes (Griffiths *et al.* 2000). During the second half of fetal life some of the regulatory functions of the genes are taken over by the placental and fetal hormones and later by the autonomic nervous system, all of which contribute to differentiation and growth of body tissues (Thorburn and Harding 1994, Thornburg and Morton 1994). In adults the great neuroendocrine systems are the main regulators responding to changes in the internal and external environment. Genetic regulation is less evident than in the fetus. Indeed, many genes important in the organism's development have been silenced through DNA methylation and other epigenetic phenomena. Nevertheless, genes remain immensely important for cellular maintenance and for long-term changes in structure and function of individual organs which are mediated through local and neuroendocrine stimuli.

To-date the huge effort of geneticists has delivered colossal advances in genetic technology. However, their attempts to identify the genetic basis of EH and related complex disorders by refining linkage and association studies seems unlikely to succeed in the absence of detailed knowledge about the phenotype. The importance of systems analysis is that it relates phenotypes to functions in identifiable pathways of the control system, rather than considering them as an aggregate of individual traits.

Physiologists have compounded the confusion associated with the definition of the phenotype, by overemphasizing not only BP but by considering individual mechanisms in isolation from the rest of the control system. Thus the kidney's role as the initiator of all hypertension, as suggested in Guyton's theory of hypertension (Guyton 1980), is not in accord with the facts (Korner 2007). Similarly, whilst elevation of SNA initiates EH, it is often overlooked that it accounts for less than half the rise in BP, with the rest due to functional non-neural changes, the structural resistance amplifier and rarefaction.

How then does one read the genome from the phenotype? New approaches are likely to be developed, probably resembling those used in developmental genetics (Davidson 2006). In addition, transgenic technology may help determine whether an observed effect involves single or multiple mechanisms. At least one can be certain of one thing: with the identification of important genetic mechanisms, physiologists will also

alter their horizons on how the control system really works.

For example, enhanced defense pathway responsiveness to stress in SSR-EH appears to be due to sensitization of DA synapses linking cortex and hypothalamus. This may be reinforced by DA neuron-mediated enhancement of sensory input from thalamus to cortex (Carlsson 1988, 2001). In addition, cerebellar mechanisms, such as those discussed in relation to "heavy" exercise training, could also participate by long-term changes in constrictor baroreflex properties. The convergence onto hypothalamic neurons of the modulatory effect of cardiotonic steroids and Ang II in the salt-sensitive neural pathway could also increase the duration of long-term potentiation. The net effect of these various factors could involve many genes.

We also need to confirm the relationship of hypertensive obesity to stress and increase our knowledge of the mechanisms by which the brain ignores a range of signals to curb eating. Several mutations that give rise to binge eating behavior have been identified in humans (Branson *et al.* 2003, Farooqi *et al.* 2003, Bochukova *et al.* 2010). Indeed, even in the nematode worm *Caenorhabditis elegans*, feeding behavior is regulated by genes belonging to the *npy* family (Coates *et al.* 2002, de Bono *et al.* 2002). One single amino acid difference in one of the genes determines whether a worm becomes a greedy solitary eater or a social eater, which eat less but have a better chance of survival from ingested bacterial toxins.

Non-neural mechanisms account for the R_1 vessel and LV structural changes associated with remodeling in EH. The changes in resistance vessel wall geometry and composition occur through induction of genes producing local growth factors such as Ang II and PDGF in conjunction with neuroendocrine and local paracrine signals (Finckenberg *et al.* 2003). Variation in local of availability of some factors (e.g. Ang II) affects wall geometry and the magnitude of the structural amplifier (Korner and Bobik 1995, Raizada *et al.* 2000) and may affect R_2 vessel heterogeneity. As regards functional factors (e.g. local NO concentration), this may depend on inadequate substrate supply and/or insufficient enzymatic function. Another set of interactions results from the superposition of hyperinsulinemia on SSR-EH in hypertensive obesity. A good deal is known about local genetic regulators, but a considerable amount remains to be learned.

Conclusions

Essential hypertension is a multifactorial disease, mostly in young and middle aged adults, that has long awaited an adequate systems analysis. My current hypothesis is that in genetically susceptible individuals stress and high salt intake sensitize the defense pathway. This places the individual into a state-of-permanent readiness to meet a challenge, which might appear to be of evolutionary advantage. This is certainly not the case in the long-term, because of the destructive nature of the mechanisms that come into play as hypertension progresses. These impair the function of vital organs, including kidney, heart and brain. Defining the phenotype in the context of particular control system components provides geneticists with a focus of where and when to search for the main genes important in essential hypertension. To achieve this will require more interaction between physiologists and geneticists, who must become more familiar with each other's "art". The beneficiary of such collaboration will be integrative biology.

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