# Blood pressure: from cells to populations

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The risks of high blood pressure are directly related to the degree of blood pressure elevation: even minor elevation of blood pressure is associated with an increased risk of sustaining a heart attack or stroke. Because blood pressure is a unimodally distributed variable, far more patients lie just above the population mean than at the higher extremes. Although the individual risk is not as great in subjects with the mildest degrees of blood pressure elevation, because of their numerical preponderance most of the excess deaths attributable to hypertension occur in them [1]. This presents a major public health dilemma. The greater part of the population risk of high blood pressure is distributed thinly across a group so large that drug treatment cannot be contemplated. There is an urgent need therefore to define environmental factors which influence the prevalence of hypertension.

### Epidemiological clues

There is no doubt that such factors exist and that they are more influential determinants of an individual's blood pressure than genetic factors. Anthropological and epidemiological studies have identified discrete populations across the world which have low mean blood pressures and do not show the normal rise in blood pressure with age [2]: hypertensive complications are rare in peoples who do not enjoy the debatable benefits of Western civilisation. The absence of hypertension is attributable to environmental rather than genetic factors, since when individuals from these populations migrate to an urban environment their blood pressure rises [3]. The difficulty in interpreting this experiment of nature is that so many environmental factors are radically different in the 'primitive tribesman' and 'Westernised subject'. Most obviously, stress [4,5] and diet [2] have been implicated without any obvious way of distinguishing the relative role of these factors. Nevertheless these data, difficult as they have been to obtain, well-nigh impossible as they are to interpret, hold out the hope of beneficial modification of environmental influences.

There is, however, another natural experiment under rather more controlled conditions. Several groups have now reported significantly lower blood pressures in vegetarians than in omnivores [6]. This is not due to different personalities in such a self-selected group, since normal subjects exposed to a vegetarian diet also show a fall in blood pressure [7]. The dietary component responsible for this fall is unknown, although changes in electrolyte intake are almost certainly not responsible. One possibility is that changes in the nature of dietary fats are involved. Thus in one large study, reduction in total fat intake and an increase in the proportion of unsaturated dietary fats lowered blood pressure [8], whilst another group produced substantial blood pressure lowering in hypertensive patients with unsaturated marine oils [9]. Conversely, when Italians changed from a conventional Mediterranean diet to one rich in saturated fats there was a significant blood pressure rise [10].

### The resistance vessel—structure or function?

So far it has not been possible to identify more precisely the dietary components which influence blood pressure (apart from such factors as obesity and alcohol which act in selected individuals). Salt intake is still very much a matter of controversy. I would like, therefore, to discuss another approach which examines the physiological and biochemical changes associated with blood pressure. Here, we must focus on what was, in man until recently, a fairly inaccessible tissue, the resistance vessel, since chronic hypertension of whatever form is associated with an increase in peripheral resistance. There are two fundamental ways in which this could occur. Vascular smooth muscle tone could be increased: this could be produced by stimulation of smooth muscle receptors by circulating constrictor agonists or neurotransmitters, or by overactivity of the second messenger system within the smooth muscle cells. This system involves the release of free calcium, binding to calmodulin, a receptor protein, which activates an enzyme, myosin light-chain kinase, which in turn initiates activity of the contractile protein. Alternatively, the increase in resistance could be secondary to the increase in vessel wall thickness which occurs consistently in chronic hypertension. The consquences of this for tissue blood flow and blood pressure have been defined in a series of experiments carried out in Göteborg by Björn Folkow and his group [11]. These studies have already become modern classics. As a result of the change in resistance vessel geometry, any constrictor stimulus produces increased resistance when the vessel is maximally stimulated. Any given dose of a pressor agonist such as noradrenaline therefore will produce a greater increase in resistance than normal: there is thus no true hypersensitivity, ie, the amount of pressor agonist needed to produce,

say half maximal contraction, is unchanged. This structurally-produced increased reactivity is difficult to distinguish in man *in vivo* from increased sensitivity, and in the older literature the two quite distinct processes were consistently confused. Thus, the fact that a given dose of noradrenaline consistently produced a greater rise in blood pressure in hypertensives was interpreted as indicating hypersensitivity.

Recently, it has become clear that changes in smooth muscle tone and structure are linked at the cellular level as well. Phosphoinositol is a membrane lipid now known to participate in the second messenger system which produces contraction of smooth muscle when vasoconstrictor agonists such as noradrenaline or angiotensin II bind to their specific receptors. This is achieved through phosphorylation of phosphoinositol and hydrolysis of the products to inositol phosphates which act as water soluble messengers causing the release of free calcium within the cell [12]. The removal of inositol phosphate from the membrane-bound phosphoinositides leaves diacylglycerol within the cell membrane. It acts as an intermediary in quite a different signalling system through activation of the enzyme protein kinase C which, among other actions, regulates the sodium-proton exchange system. This, through induced changes in cell pH, regulates differentiation and growth. Thus, the same system which is responsible for contractility (through intracellular calcium) also regulates growth. Contraction and hypertrophy may not therefore be the distinct processes they were once believed to be.

### Clues from electrolyte transport

Over the past few years, a group of puzzling phenomena in cellular electrolyte transport has been described in essential hypertension [13]. It is now becoming clear that these phenomena throw an important light on both environmental and genetic factors in hypertension.

There is a gradient in sodium between the high concentrations in extracellular fluid and the much lower concentrations within the cell: this is maintained by active sodium extrusion through the sodium-potassium ATPase dependent sodium pump and can be inhibited by ouabain. In addition, both sodium and potassium move across cell membranes by pathways which are not dependent upon sodium-potassium ATPase. Some of this movement of ions is due to diffusion — 'ground permeability' of the membrane. More specific transport pathways also exist. Pairs of sodium and potassium ions accompanied obligatorily by chloride move across the cell membrane in both directions (sodium-potassium co-transport). This pathway is specifically inhibited by loop diuretics, although if too high concentrations are used, more global inhibition is produced. When red cells are preloaded with lithium, one lithium ion is extruded in exchange for one sodium ion by a system which is inhibited by phloretin (lithium-sodium countertransport). Lithium is not of course a physiological ion, but it seems likely that sodium lithium countertransport measures a system which exchanges one internal sodium for one external sodium ion (sodium-sodium countertransport). The physiological role of these two systems is uncertain, although sodium-potassium co-transport may regulate cell volume under some circumstances [14]. Sodium proton exchange, besides regulating intracellular pH, has other specific functions. It has recently become clear that this system participates in the contractile response to humoral stimulation of vascular smooth muscle with noradrenaline [15]. It is specifically inhibited by some analogues of amiloride.

Worldwide clinical interest in these systems was provoked when it was reported that erythrocyte sodium-potassium cotransport was reduced in patients with essential hypertension but normal in patients with secondary hypertension. Indeed, the absence of overlap between measurements in the two groups led the authors of the report to suggest that cotransport assay would provide a diagnostic test for essential hypertension [16]. However, later work qualified the conclusion about co-transport which seemed to be slightly reduced in some hypertensive populations but increased in others [17]. There was more consistency in the results when erythrocyte lithium sodium countertransport was examined: with few exceptions this was found to be increased in patients with essential hypertension [18]. Ironically, the best understood system, the erythrocyte ouabain-sensitive sodium pump, showed no consistent abnormalities in the numerous studies which have been carried out. On the other hand, Hilton and his colleagues examined the leukocyte which has a much more rapid turnover of sodium and showed a small reduction in ouabain-sensitive sodium pumping, a finding which has been confirmed in several laboratories [19]. This is by no means the end of the story of cellular electrolyte handling in hypertension. Erythrocyte calcium binding to the cell membrane is reduced [20,21] and active calcium extrusion reduced [22,23].

The initial response to this wealth of abnormalities was one of bewilderment. One intriguing suggestion was that sodium and water retention in hypertension stimulated the secretion of an inhibitor of active sodium transport [24,25]. Although this explanation is still held by some, it is difficult to see how it can explain such multiple disturbances involving quite different transport pathways. Our own and other groups' work suggested that sodium loading and depletion had exactly the opposite effect on sodium transport to that predicted by the inhibitor hypothesis [26]. It also has to be said that some of the confusion in this field was probably due to studies carried out in poorly matched hypertensive and control populations.

The relevance of changes in blood cells to vascular smooth muscle might well be questioned and frequently was. However, two pieces of evidence supported the view that parallel changes occur in vascular smooth muscle. Rather similar abnormalities of ion fluxes were observed in vascular tissue in models of hypertension in the rat [27] and, more directly, it is possible to show a good correlation between transmembrane sodium fluxes in isolated human vascular smooth muscle and in human leukocytes [28].

### Physico-chemical changes in cell membrane

It seems highly improbable that there should be multiple independent abnormalities of several quite discrete membrane systems. It seems much more probable that there is a global physico-chemical 'disturbance' of cell membrane lipids [29] or, more correctly, a variability in cell membrane function which correlates with tissue function and with blood pressure within what can be regarded as an acceptable biological range. Alterations in the proportion of saturated to unsaturated fatty acids in the acyl side chain of membrane phospholipids influence membrane fluidity and membrane ionic fluxes. Thus, sodium-potassium ATPase requires lipid as an essential co-factor and its activity can be modified by changes in the composition of the fatty acids of these side chains [30]. The importance of the lipid environment for lithium-sodium countertransport has been neatly demonstrated by studying the temperature dependence of the process. When temperature is decreased the physical state of lipids changes from a liquid to a gel phase. This abrupt change can be observed as a break in the temperature-activity curve for lithium-sodium countertransport: in addition the break point is shifted in hypertensives [31].

There is more direct evidence for an association between membrane lipids and blood pressure. The polyunsaturated fatty acid linoleic acid is reduced in the erythrocyte [32] and platelet [33] membranes. Erythrocyte membrane fluidity is increased in hypertensives [34]. However, some of the most important evidence comes not from small laboratory studies but from large scale investigations of unselected populations [35]. In one of the most important of these a negative relationship between blood pressure and lithium-sodium countertransport was again observed. However, there was also a highly significant association between some plasma lipids (high density lipoproteins and triglycerides) and countertransport: indeed lipids, blood pressure and countertransport were interrelated variables.

### Cell membrane and blood pressure

The associations described above tell us nothing about the mechanisms which give rise to them. It is tempting to incriminate alterations in sodium homeostasis, but this seems unlikely when such processes as sodium-sodium countertransport are involved, which exchange one external for one internal sodium ion. One obvious possibility is that the handling of messenger intracellular calcium is altered in hypertensive patients producing an increase in vascular tone. There is some support for the suggestion that membrane lipid composition does influence cell calcium handling. For instance, both calcium binding to the inner surface of the erythrocyte membrane [36] and intracellular calcium [37] are related to the ratio of saturated to polyunsaturated fatty acids in the cell membrane.

It would seem reasonable to test the lipid calcium hypothesis by changing the lipid composition of the cell membrane. Fortunately, dietary fatty acids are incorporated readily into blood cell membranes. Accordingly we gave linoleic acid to a group of normal volunteers. Besides producing a demonstrable increase in erythrocyte membrane linoleic acid, this had a substantial effect upon ouabain resistant sodium fluxes [38] and lowered leukocyte calcium [37]. We could therefore postulate from these studies a sequence of events whereby changes in smooth muscle membrane phospholipids gave rise to increased second messenger calcium and enhanced smooth muscle vascular tone. We now believe such a hypothesis is a gross over-simplification.

The first intimation of this was the fact that, despite quite major lipid induced changes in blood cell intracellular calcium, the drop in blood pressure was minimal or non-existent. In a further unpublished study we loaded our volunteers with as much linoleic acid as they would tolerate—up to 64 capsules, 32 grams a day, in some cases. Although progressive changes in leukocyte sodium fluxes could be produced there was no progressive change in blood pressure. There were two explanations for this disappointing result: either the calcium hypothesis in the form postulated was wrong or changes in leukocytes were not indicative of changes in vascular smooth muscle.

### Hyper-reactivity due to structural change

Our outlook has now been radically altered as a result of a newer and extremely valuable technique for looking directly at the metabolism and characteristics of the human isolated resistance vessel [39,40]. This has been developed as the result of a collaborative venture between the Department of Medicine at Leicester and the Institute of Biophysics in Aarhus, where Mulvany's group had developed a myographic technique for examining small segments (2 mm) of resistance vessels. These can be obtained from any available tissue, but in our studies of hypertensive patients and their relatives, we used subcutaneous blood vessels dissected from a skin biopsy. Two stainless steel wires are inserted through the lumen of the vessel which is suspended in a physiological oxygenated buffer solution. After equilibration, thickness of the vessel media can be measured after which vessels are maintained at a standardised internal diameter. They can then be stimulated to contract by depolarisation, pressor agonists such as noradrenaline or angiotensin II or by calcium (reinstituted in the medium after previous depletion). Resistance vessels from hypertensive subjects showed consistently increased maximal contractility when stimulated in this way. However, there was a marked increase in media thickness so that the wall to lumen ratio was increased by an average of 28 per cent. The increased muscle mass accounted wholly for increased contractility. When the tension generated by the isolated vessels was corrected for increased media mass (media stress) there was no evidence for increased sensitivity to any agonist studied. Further, calcium sensitivity was slightly decreased and relaxation of the vessel after contraction was accelerated in vessels from hypertensives, suggesting that removal or sequestration of intracellular free calcium was rather more efficient. In other words, in hypertension there was no evidence for an overactive calcium second messenger system or indeed for any form of hyperrespon-

siveness, despite claims to the contrary over the preceding three decades. Instead, the conspicuous abnormality is hypertrophy giving rise to a generalised increase in contractility.

It thus seems that the disorders of monovalent and divalent cation handling are markers for a global minor disturbance in the cell membrane which in the chronic phase of essential hypertension is associated with structural rather than functional resistance vessel changes.

From the clinical point of view the importance of resistance vessel hypertrophy in maintaining blood pressure in hypertension is not perhaps all that surprising. When, for instance, antihypertensive drug therapy is stopped blood pressure climbs only slowly and certainly takes much longer than would have been predicted from the pharmacological life of the antihypertensive drug [41].

## The phospho-inositide system

We can only speculate over the nature of the cell membrane based control system which is disturbed. The trophic action of the sympathetic nervous system which may be activated in the early phase of hypertension is one strong candidate [42,43]. There is however an additional possibility which provides a link between the sympathetic nervous system membrane lipids and altered cation handling. The phosphoinositol system acts as a second messenger for the vasoconstrictor effects of the sympathetic nervous system and for some vasoconstrictor agonists such as angiotensin II through regulation of free intracellular calcium. In addition, it is a regulator of growth and hypertrophy through the diacylglycerol/sodium proton exchange pathway. Pioneering studies by Postnov and others have indicated that erythrocyte phosphoinositol turnover may be increased in hypertension [44–46]. It is now possible to measure phosphoinositol turnover in vascular tissue [47]. So far, only studies in the rat have been completed although the technique can be applied to human vascular tissue. These studies have shown increased phosphoinositol turnover in the early phase of genetic hypertension in the rat at a time when sympathetic nervous system activity is increased [47]. Further, the system appears to be important at least in large vessel hypertrophy. Thus, when coarctation is induced by constriction of the aorta, the wall of the proximal part of the vessel exposed to increased pressure thickens: this thickening is preceded by increased phosphoinositol turnover which is not seen in the part of the vessel distal to the coarctation [48]. Much more remains to be learnt of this important cellular system in hypertension.

## Conclusions

Our understanding of the cellular basis of hypertension is undergoing a revolution. There are still enigmas and contradictions to be resolved in trying to identify the sequence of events in a multifactorial condition of great complexity. Yet these matters are by no means academic. There is the very real possibility of identifying dietary components which could account for the wide differences in blood pressure observed in different populations. The recognition of the importance of hypertrophy raises the second possibility which is already being explored. It seems likely that antihypertensive drugs may have specific effects upon structure independent of their blood pressure lowering action. There are already indications in this direction in experimental animals [49]. Information on regression of vascular hypertrophy with treatment of hypertension in man may lead to a refinement of our current rather blunderbuss approach to lowering blood pressure and enable us to select drugs more rationally.

Understanding a multifactorial disorder such as hypertension depends upon a degree of collaboration between different disciplines, including that of the clinician and laboratory scientist. No one individual in this day can be expected to command the expertise to investigate rationally subcellular processes at one extreme and population blood pressure variability at the other. There are great difficulties of establishing such an expensive and fraught joint enterprise. Nevertheless the rewards make such an enterprise well worth attempting.

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# Dearest Betsy

The nice thing about searching archives is that you will find something that is not part of your search. Tucked away among thousands of items in the Essex Record Office is a bundle of late 19th century letters that came from the desk of Mrs Harriet Vaizey. Most of these deal with her husband's estate, some were written by her brother, and one, written a hundred years earlier, was from her grandfather to his wife. Grandfather was Dr Henry Revell Reynolds, the most favoured of George III's many doctors.

Reynolds, born in Nottinghamshire in 1745, after his father's death, was a good physician. From 1773 to 1777 he was on the staff of the Middlesex Hospital and then succeeded his friend and mentor, Dr Huck Saunders, as a physician to St Thomas' Hospital. He was both Censor and Registrar of the College and had the distinction of refusing to print his Harveian Oration of 1776. His long service to the King hastened Reynold's death. For, in 1811, when already ill, he had to stand for two hours, as protocol demanded, to give evidence to the House of Lords on the King's health. He died in the October of that year. Munk wrote of him, 'In the investigation of diseases he was acute yet cautious; in the application of remedies, fertile in resource, yet not rash in experiment; . . . gaining entire ascendancy over the minds of his patients by the rare fascination of his manners, and the confidence with which he inspired them in his skill, and in his zeal to relieve them.'

The letter from Mrs Vaizey's desk was written by Reynolds to his wife at their home in 10 Bedford Row during his first attendance on the King. It reads:

> Dearest Betsy
>
> I was so much engaged this morning that I had not time nor opportunity of writing to you by the King's messenger, but I do not choose to let a day pass without assuring you that I am well, and that I love you and your children dearly. There is very little alteration in His Majesty since yesterday, and there is little prospect of such an alteration soon as will permit us to return to our business in London, and to our domestic comforts. If his Majesty is not worse, or if nothing particular happens, I will come home for a couple of hours tomorrow. . . .I have not time for more, as the King has sent for me. My love to the children. I am, Dearest Betsy, your most affectionate husband,
>
> H. R. Reynolds

That letter says something about its writer, he must have been a very nice man in all his high profile professional life. But what about Mrs Harriet Vaizey's letters from her brother to their mother? The brother, Revell Reynolds' grandson, was to become Sir John Russell Reynolds FRCP FRS, who was elected President of the College in 1893. His letters, told his mother of the successes of his student career.