Session II. Animal Models

Experimental Hypertension

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Several interventions are used to induce hypertension in the experimental animal. The three most used interventions are renal ischemia, mineralocorticoid excess, and genetic manipulation. The sequence of events leading from these initiating manipulations to the elevated arterial pressure is being explored to define the mechanism responsible for hypertension. The following mechanisms are currently extensively evaluated: Pressor and depressor factors of renal origin, neurogenic regulation, circulating humoral factors, vessel wall hypertrophy, and membrane transport abnormality. The experimental models of hypertension hold great promise in providing an understanding of the mechanisms and developing effective treatment in clinical hypertension. (Hypertension 1991;17[suppl I]:I-39–I-44)

With over 30,000,000 cases of hypertension in the United States, opportunity for clinical research is unlimited. Yet a major source in understanding this disease comes from the use of experimental models of hypertension. Evidence for the magnitude of the contribution made by experimental hypertension is seen in the number of articles published in the past 2 years in our two major journals on hypertension. In 1987 and 1988, Hypertension published 118 clinical and 166 experimental articles, and the Journal of Hypertension published 113 clinical and 108 experimental articles. Based on these data, 54% of our information about hypertension is derived from experimental studies.

Historically, “The greatest single advance in the experimental production of the condition resembling that of hypertension in man was a convincing demonstration by Goldblatt, Lynch, Hanzel, and Summerville that persistent hypertension could be produced in dogs” (Sir George Pickering). In 1934, Goldblatt et al showed that partial constriction of the renal artery and removal of the opposite kidney produce persistent hypertension (one-kidney, one clip hypertension [1K1C]) in dogs. Differing from dogs, the constriction of one renal artery in rats results in hypertension regardless of the presence or absence of the contralateral kidney.3

Later that same decade (1939), Kuhlman et al observed that daily subcutaneous injections of deoxycorticosterone acetate (DOCA) produced an increase in blood pressure in unilaterally nephrectomized dogs. These investigators also reported accompanying mineralocorticoid-induced changes that included increased water turnover, hypokalemia, and hypernatremia. The major impetus for the study of DOCA-induced hypertension came in 1943, when Selye et al introduced this model of hypertension in the rat.

The third major model of experimental hypertension was introduced by Smirk and Hall in 1958, when they developed a colony of hypertensive rats by breeding rats with above-average tail blood pressure. This strain of genetically hypertensive rats is now known as the New Zealand strain.

As with mineralocorticoid hypertension, the major impetus for the study of genetic hypertension came later. In 1963, Okamoto and Aoki introduced the spontaneously hypertensive rat (SHR), which is presently the most studied experimental model of hypertension. These investigators commenced their colony by mating a male Wistar-Kyoto (WKY) rat that had elevated blood pressure (145–175 mm Hg) with a female WKY rat that had slightly higher than average blood pressure (130–140 mm Hg). In the F1, and subsequent generations, they conducted brother-sister inbreeding of the siblings selected for having the highest pressures in each litter. After the third generation, these rats, without exception, developed hypertension spontaneously as early as several months after birth. By 1969, they had succeeded in developing an inbred strain of SHR, one in which homozygosity has been achieved in more than 99% of all genetic loci. Theoretically, this occurs after 20 generations of brother-sister inbreeding. The absence of genetic variation among the individuals of an inbred strain makes this strain a powerful tool for a study of the determinants of blood pressure. Blood pressure is a variable determined by both genetic and nongenetic factors. Because there is no genetic variation in an inbred strain, the effects of a specific nongenetic intervention on the variable of blood pressure can be easily dissected.

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pressure are much more readily defined in the inbred strain than in a nonhomogeneous colony of rats.

One example of the problems with an outbred stock of rats in the study of hypertension is found in the report by Meneely and Ball* in 1958, which described the effect of dietary salt content on blood pressure. They observed that in groups of rats fed diets containing 0–10% salt, the average blood pressures correlated well with the salt intake. However, among the group of rats on each dietary level of salt intake, there was a broad variation in blood pressure. Dahl et al9 hypothesized that this variable blood pressure response reflected genetic differences among the individual rats in their interaction with the environmental factor of salt intake. To test this hypothesis, Dahl developed lines of rat that did (DS) and did not (DR) elevate arterial pressures in response to a high salt diet. Rapp and Dene10 developed inbred strains of these rats that are extensively used to clinically investigate important problems related to the mechanism of salt sensitivity.

At least three additional strains of inbred hypertensive rat have been developed along with their inbred normotensive controls. These are the Jerusalem,11 Lyon,12 and Milan13 strains of rat. Mechanisms responsible for the arterial pressure elevations in each of these strains appear to differ and merit investigation because of the diversity of mechanisms probably responsible for essential hypertension in man.

Although most current studies of experimental hypertension are done on renal, mineralocorticoid, or genetic models, the following experimental models of this disease have each added to our knowledge of hypertension: 1) renoprival,14 2) adrenal regeneration,15 3) adrenocorticotropic hormone,16 4) neurogenic,17 5) psychosocial,18 and 6) angiotensin II–salt.19

Too Many Mechanisms

In 1949, Dr. I.H. Page20 introduced investigators in the field of hypertension to the complexity of the physiological mechanisms contributing to the regulation of arterial pressure. His mosaic theory emphasized the interdependence of the several blood pressure regulatory systems. An abnormality in one of these systems causes compensatory changes in the others, so that the investigator of mechanisms of hypertension is confronted with the challenging problem of determining which of the many changes he observes is primary.

Obviously, it would be important to know the cause of hypertension. Studies of experimental hypertension have made some contributions. In investigations of experimental hypertension, the initiating factor and the final process are easily identified. The investigator knows the intervention that he has used to begin the process; the final common event is a hemodynamic change, usually an increase in total peripheral vascular resistance, that causes the increase in arterial pressure. Whereas the investigator-imposed initiating event is clearly defined in renal and mineralocorticoid hypertension, the specific abnormality that initiates genetic hypertension is still a matter of conjecture and differs in the various genetic strains.

Many additional genetic traits to that of high blood pressure have been described as characteristic of genetically hypertensive strains of rat. However, other genetic traits in these strains may be irrelevant coincidences unless a genetic relation to hypertension can be demonstrated. Rapp21 has listed criteria that must be met to establish that an accompanying genetically determined trait may be responsible for the blood pressure elevation of genetic hypertension. They are: 1) A difference in the trait must be demonstrated between the hypertensive and normotensive strains. 2) The trait must follow Mendelian inheritance. 3) The trait must cosegregate with the increment in blood pressure in the F2 generation of a crossbreed between the two strains. 4) There must be some logical link between the trait and blood pressure.

Several phenotypic traits are genetically associated with hypertension: 1) abnormal 18 OH-DOC biosynthesis in DS rats,22 2) contractile response to cobalt in SHR,23 3) increased membrane permeability to potassium in lymphocytes from stroke-prone SHR (SHRSP),24 4) oscillatory response of the tail artery to norepinephrine in SHRSP,25 and 5) vascular smooth muscle sensitivity to calcium in SHR.26

The major unknown for which an answer is being sought not only in genetic hypertension, but also in the renal and mineralocorticoid models, is the sequence of events that leads from the initiating factor to the final common pathway. The current status of this quest is one in which investigators have identified several mechanisms that are promising, but none can be comfortably accepted yet. The possibilities suggested from these investigations follow.

Renal Mechanisms

Strong evidence advanced by many investigators suggest that the kidney is the primary offender in the pathogenesis of hypertension. Historically, Tigerstedt and Bergman27 gave a clue to a role that could be played by the kidney when they observed that the intravenous injection of a saline extract of normal kidney tissue causes a pressor response in the test animal. They called the pressor agent they had extracted "renin."

Hypertension produced by the constriction of one renal artery while the contralateral kidney is left untouched (two-kidney, one clip [2K1C]) is pathogenetically different from that produced by constriction of one renal artery and removal of the contralateral kidney (1K1C).3,26,29 In the first few days after the operation, 1K1C hypertension is due predominantly to sodium retention. This occurrence is associated with decreased activity of the renin–angiotensin system. The characteristics of early-phase 2K1C hypertension are quite different; the unclipped kidney is able to excrete the sodium load imposed by the contralateral ischemic kidney, thus preventing so-
dium overload. This form of experimental hypertension is associated with high plasma renin activity and high aldosterone secretion, which parallel a rise of arterial pressure.20,30,31 Furthermore, in rabbits and rats with 2K1C Goldblatt hypertension, injection of an angiotensin II antibody or infusion of an angiotensin inhibitor causes reduction of blood pressure.32,33 The same doses of an angiotensin antibody or inhibitor failed to lower blood pressure in 1K1C hypertensive animals.

Removal of sodium by peritoneal dialysis decreases blood pressure in 1K1C hypertension, whereas it does not affect blood pressure in 2K1C hypertensive animals.34 A current study by Samani et al35 measured renin messenger RNA (mRNA) content of the rat kidneys in 2K1C hypertension. Four weeks after clipping, renin mRNA levels were sixfold higher in the clipped kidney and eightfold lower in the contralateral kidney than those in the sham-operated control. These observations suggest that renin and angiotensin II have dominant roles in early stages of 2K1C Goldblatt hypertension. Whereas sodium and water retention seem to be the important pathogenetic factors in 1K1C hypertension.

Guyton et al,36 Hall et al,37 and Cowley et al38 have associated impaired water and salt loss by the kidney with the increase in arterial pressure. Blood pressure increase in the presence of normal renal function causes natriuresis, thereby reducing blood volume and lowering arterial pressure. When water and salt excretions by the kidney are impaired, hypertension develops. Grollman, Muirhead, and Vanatta42 postulated a quite different role for the kidney. They observed that anephric animals developed hypertension. This "renoprival" hypertension appeared to result from the loss of a humoral antihypertensive substance of renal origin. Renoprival hypertension and associated cardiovascular injury are prevented by renopapillary transplants that contain the renomedullary interstitial cells.39 These cells have been implicated as the source of the putative renal antihypertensive hormone medullipin.40 Tarazi and Gifford41 suggested that different forms of hypertension may have a renoprival component added after a specific amount of kidney damage has been produced because of hypertension.

Bianchi et al42 used the Milan strain of genetically hypertensive rats to present strong evidence for the primacy of the kidney as a cause of hypertension. When they transplanted kidneys from their normotensive control strain into the hypertensive rat, the pressure of this rat was normalized. Conversely, when they transplanted kidneys from a hypertensive rat into a normotensive control, the pressure of this rat rose to hypertensive levels.

Humoral Agents

Circulating agents in the blood have been extensively evaluated for the roles they might play in hypertension. Of these, the renin-angiotensin system has been studied the most. In some forms of hyper-tension, this system is essential for maintaining elevated arterial pressure; in others, it is not involved. The latter is the case with mineralocorticoid hypertension, where the circulating levels of renin and angiotensin may be very low or absent.

The role of angiotensin in hypertension is not completely understood. For example, although the renin and angiotensin levels in SHR are normal, treatment of these hypertensive animals with a converting enzyme inhibitor that prevents the production of angiotensin II reduces the arterial pressure to normal.43

In 1960, cross-circulation studies were performed to determine whether the renin-angiotensin or other circulating pressor systems were responsible for chronic renal hypertension.44 In control studies that produced an arterial pressure elevation in the test rat by the infusion of renin, we determined that the pressure rose in the control assay rat when the cross-circulatory system was open. We then used a renal hypertensive rat as the test animal and observed no pressure change in the assay rat when the cross-circulation system was open. We concluded that the elevated arterial pressure of renal hypertension was not maintained by a circulating pressor agent.

Another humoral factor proposed as a pressor agent contributing to hypertension is an inhibitor of Na+,K+-ATPase. Such a "ouabainlike factor" would depolarize the vascular smooth muscle cell by inactivating the electrogenic pump. Depolarization leads to activation of potential operated calcium channels in the cell membrane and thus to an increase in intracellular calcium concentration and vascular smooth muscle contraction. Additionally, inactivation of the pump would lead to an accumulation of intracellular sodium, which would further increase intracellular calcium concentration by activating the sodium-calcium exchanger. Hamlyn et al45 have recently made significant progress in the isolation of such a humoral factor that presumably inactivates the pump. Evidence has been presented indicating that this pump inhibitor is increased in the plasma of some forms of hypertension,46,47 yet in vitro observations indicate that this pump activity is increased instead of decreased in hypertension.48,49 We have compared pump activity in vivo in DOCA hypertensive and normotensive pigs with rubidium clearance from the plasma used as an index of pump activity levels (L. Vo and D.F. Bohr, unpublished observation). Rubidium was more rapidly cleared from the hypertensive than from the control pigs, indicating that the pump was more active rather than depressed. We have evidence that this hyperactivity results from an increase in sodium leak into the cell in hypertension,50 which in turn drives the pump to an extent that it masks any inhibitory action that might be exerted by a circulating ouabainlike factor.

Interesting pressor activities have recently been described in the red blood cells51 and plasma52 of SHR.

Central Nervous System

Mechanisms involving the brain have received strong support as playing an important role in the
development of experimental hypertension. Brody et al.\(^6\) observed that the placement of a small lesion in the anteroventral region of the third ventricle prevented the development of renal or mineralocorticoid hypertension. Gomez-Sánchez\(^4\) added supportive evidence for the involvement of this region in mineralocorticoid hypertension when she observed that chronic infusion of minute amounts of aldosterone into the lateral cerebral ventricle of the rat produced hypertension. This amount of the systemically infused steroid had no effect.

Recent observations made by Wyss et al.\(^5\) suggest that salt sensitivity in SHR is associated with a decrease in norepinephrine stores and turnover in the hypothalamus. This decrease in associated with a rise in blood pressure when the rat is placed on a high salt diet. Interestingly, the decrease in norepinephrine and rise in pressure do not occur when the rat is also given a high calcium diet.

### Vascular Reactivity

The increase in total peripheral vascular resistance that causes hypertension may reflect an intrinsic defect in the wall of the resistance vessels. Folkow et al.\(^6\) demonstrated that the greater arterial wall thickness in hypertension produces a mechanical advantage for vascular smooth muscle contraction and thus increases vascular reactivity. Lever\(^7\) suggested that the primary defect responsible for the wall thickness in hypertension could be a greater growth rate of the vascular smooth muscle cells. We\(^8\) have focused on the evidence that the vascular defect is an increase in vascular smooth muscle excitability.

### Plasma Membrane

Possible abnormalities in the plasma membrane are currently being evaluated to determine their role in the development of experimental hypertension. In the SHR, Postnov and Orlov\(^9\) have observed an increase in red blood cell membrane permeability to sodium associated with a decrease in calcium binding by the membrane. Devynck et al.\(^10\) have described a deficit in calcium binding by the plasma membrane of several tissues from SHR, including red blood cell, heart, liver, and brain. The increased excitability seems to result from the failure of calcium to produce a normal stabilization of the plasma membrane.\(^11\) Jones\(^12\) observed increased vascular smooth muscle permeability to potassium in SHR and mineralocorticoid hypertensive rats. Jones and Hart\(^13\) also described the stabilizing effect of extracellular calcium on this membrane, which is evidenced by the decrease in potassium permeability that occurred as calcium concentration of the physiological salt solution was increased over concentrations from 0.1 to 5.0 mM.

Furspan and Bohr\(^14\) have made entirely parallel observations on the passive fluxes of sodium and potassium through the lymphocyte membrane. Increasing calcium concentration decreased the fluxes of these monovalent cations. At any calcium concentra
tion, the flux was always greater through the lymphocyte membrane of the hypertensive than the normotensive rat.

Furspan et al.\(^15\) have recently implicated this membrane abnormality in the elevated arterial pressure of the SHRSP. A 10-week feeding of a high calcium diet to SHRSP, which caused only a 10% increase in plasma calcium level, resulted in a reduction in sodium and potassium flux in the lymphocytes. Blood pressure and intracellular free calcium concentration returned to normal levels. We have reported this paradoxical effect of chronic exposure to a small increase in plasma calcium concentration in an entirely different setting.\(^16\) The 20% elevation in plasma calcium concentration in primary hyperparathyroidism is accompanied by a lower-than-normal intracellular calcium concentration in platelets. We attributed these changes to a membrane-stabilizing effect of chronic exposure to a small increase in extracellular calcium concentration.

There is clearly a problem of too many different abnormalities that could be causing hypertension. The current quest in experimental hypertension is to understand the interrelation of the mosaic of hypertension abnormalities in renal, humoral, neural, vascular, and membrane functions.

### References


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