Review

Endocrine and Auto-Paracrine Factors in the Pathogenesis of Primary Hypertension

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Referring to the mosaic theory of Page, the authors present an overview of recent topics related to the participation of endocrine and auto-paracrine factors, such as steroids, ouabain-like substance, insulin, renin-angiotensin and endothelin, in the pathogenesis of primary hypertension. These factors promote the development of hypertension in either a direct or indirect manner; in addition, they promote, to some extent, the proliferation of vascular smooth muscle cells. Future research should attempt to elucidate interactions between these factors in cardiovascular tissues and to define how these factors interact with various vasodepressor substances to regulate blood pressure. (Hypertens Res 1995; 18: 171-179)

Key Words: steroids, ouabain-like substance, insulin, renin-angiotensin, endothelin, primary hypertension

Although primary hypertension accounts for 95% of all cases of hypertension, the multifaceted mosaic concept for the pathogenesis of this disease, proposed by Page (1) in 1963, still remains valid. One can only say that a number of new facets, which integrate closely into the mosaic theory, have appeared during the past few decades.

The blood pressure can be defined as the product of cardiac output (pumping action of the heart) and peripheral resistance (arterial tone). Moreover, complex mechanisms, involving interactions among genetic, hormonal and physical factors, affect each of the determinants. Recently, the potential roles of various hormones in modulating arterial tone, not only by the functional regulation of resistance vessels but also by their structural alterations (remodeling), have attracted considerable interest. The reason for revived interest in the hormonal aspects of the pathogenesis of primary hypertension seem to be due, in part, to the discovery of new vasoactive substances derived from the vascular tissue, acting as an auto-paracrine organ. Assuming a certain subgroup of primary hypertension can be identified as being caused by a state of vasopressor hormone excess or dilator deficiency, that subgroup then should be categorized as a form of secondary (endocrine) hypertension.

In the present review, the authors present an overview of recent advances in endocrinological aspects of primary hypertension and address questions that have emerged as a result of advances in the research of altered steroid metabolism, ouabain-like substances, the renin-angiotensin-aldosterone system, and endothelin.

Altered Steroid Metabolism

During the 1960s and 1970s, Kornel et al. (2-4) reported a series of articles suggesting that altered cortisol metabolism results in increased excretion of polar derivatives of cortisol (non-A-ring-reduced 20-hydroxysteroids and 6β-hydroxysteroids). They also provided some evidence for the predominant role of sulfoconjugation rather than glucurononconjugation in primary hypertension. However, the precise implications of these findings in the pathogenesis of primary hypertension are still unclear.

Recently, the pathophysiological importance of cortisol inactivation by 11β-hydroxysteroid dehydrogenase (11β-HSD) in various tissues has attracted considerable attention among steroidologists. The deficiency of this enzyme, whether congenital, as in the syndrome of apparent mineralocorticoid excess (AME) (5-7), or secondary to licorice (8) or carbadoxolone ingestion (9), results in a unique hypertension, in which cortisol acts as a potent mineralocorticoid to produce sodium retention, followed by volume-hypertension associated with a suppressed state of the renin-angiotension-aldosterone system and hypokalemia. Based on our current understanding, the pathophysiological mechanism of the above sequence of events can be explained by the dual actions of 11β-HSD. Under normal conditions, renal 11β-HSD appears to act to prevent contact between renal mineralocorticoids and glucocorticoids such as cortisol; consequently, the effect of cortisol as a mineralocorticoid is modest as 11β-HSD is present in target cells with mineralocorticoid receptors (10-}

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Received January 6, 1995.
Under conditions in which 11\beta-HSD activity is inhibited, cortisol binds easily to the mineralocorticoid receptor upon the loss or attenuation of protective action to shut out cortisol, and greater mineralocorticoid action is expressed. Thus, 11\beta-HSD may be defective in some patients with primary hypertension. Evidence for this theory was provided by Walker et al. (13-17) who reported that the half-life of 11 alpha-H cortisol was prolonged in a subgroup of hypertensives and that it correlated with blood pressure but not with indices of renal mineralocorticoid receptor activation. Moreover, Edwards et al. (18) demonstrated that attenuated 11\beta-HSD activity may link low birth weight and high placental weight with hypertension.

The authors (19) also assessed 11\beta-HSD activity based on the ratio of tetrahydrocortisone (THE) to tetrahydrocortisol (THF) plus allo-tetrahydrocortisol (alpha THF) in urine. In contrast to previous reports, no significant difference was found in 11\beta-HSD activity between primary hypertensives and normotensive controls, nor was any correlation found between enzyme activity and electrolytes in urine, measured as indices of renal mineralocorticoid activation. On the other hand, 5\beta-reductase activity, expressed as the ratio of THF to alpha-THF was significantly lower in primary hypertension than in normotensive controls (p<0.05) (20).

In addition to the liver and kidney, 11\beta-HSD is known to be present in almost all tissues, including vascular beds, though there exist multiple, tissue-specific isoforms of the enzyme. The authors (19) demonstrated the expression of 11\beta-HSD in the mesenteric artery by immunocytochemical staining methods, in situ hybridization, and by Northern blot analysis. Therefore, it is likely that 11\beta-HSD activity is diminished at the site of resistance vessels per se, as we demonstrated for the mesenteric artery by immunocytochemical staining (21). If this true in primary hypertension, the vasoconstrictor response to cortisol would be potentiated, even in minor stressful conditions, through the enhanced binding of cortisol to the mineralocorticoid receptor. It is noteworthy that in our study 11\beta-HSD activity positively correlated with 5\beta-reductase activity. This leads us to assume further that in primary hypertension an unknown mechanism relevant to either the cause or the effect of high blood pressure may function to inhibit the activities of both 11\beta-HSD and 5\beta-reductase.

Ouabain-Like Substance

An endogenous substance, which is associated with active volume expansion or increased sodium intake, has been found to exert an inhibitory action on sodium-potassium ATPase activity similar to ouabain. Considerable efforts have been expanded to characterize the chemical nature of such cytochemically detectable substances as Na-K ATPase inhibitor (22-32). Some investigators have suggested that the substance is a hypothalamic peptide while others have shown that it is a steroid (23) or steroid-sugar complex (24), most likely originating from the adrenal gland. Recently, a circulatory inhibitor of Na-K ATPase (ouabain-like substance; OLS) was identified (30-32), initially as ouabain, by gas chromatography-mass spectrometry of purified extracts from huge volumes of human plasma. Moreover, with the development of a suitable immunoassay for OLS (33), the substance was found to be secreted by adrenal glands (34-36) and to be present in increased concentrations in the plasma of patients with volume-expanded hypertension and congestive heart failure (37). It is interesting to note that in one patient with an aldosterone-producing adenoma associated with a high plasma OLA level, the level was normalized following surgical removal of the tumor (38). More recently, however, Gomez-Sanchez et al. (39) demonstrated that the Na-K ATPase inhibitor which circulates in plasma is not ouabain, but a structurally similar substance. This substance appears to a stereoisomer which cross-reacts with the some antibodies against ouabain.

Hyperinsulinemia-Hypertension Hypothesis

In recent years, a controversial debate has focused on whether hyperinsulinemia is a direct causal factor of high blood pressure or just an epiphenomenon arising as a result of obesity, which is often associated with primary hypertension. The hyperinsulinemia-hypertension hypothesis for the pathogenesis of primary hypertension originates from the report of Welborn et al. (40), who first noticed elevated insulin levels in essential hypertension and peripheral vascular disease. After almost twenty years, the observation of Welborn et al. was taken up again by Modan et al. (41), who suggested that hyperinsulinemia may play an etiological role in hypertensive subjects. This theory was revived later as the concept of syndrome X, proposed by Reaven (42, 43). According to this concept, multiple actions of insulin on the sympathetic nervous system, which is implicated in relation to epinephrine release (44), the renal handling of sodium, and vascular growth and remodeling, facilitate the development of hypertension. In addition, modified cation flux across cell membranes, promoted by the insulin-stimulated activation of Na^+H^+ antiporter, results in intracellular Ca^2+ accumulation, thereby potentiating the vasoactivity to vasoactive substances. Dyslipoproteinemia (high very-low-density lipoproteinemia: VLDL and low high-density lipoprotein-cholesterolemia: HDL-C) often associated with hyperinsulinemia could also accelerate the atherosclerosis and thereby reduce the elasticity of the vascular wall. In clinical experimental studies using the euglycemic insulin-clamp technique, angiotensin converting enzyme (ACE) inhibitors have been shown to ameliorate insulin resistance, and to reduce not only blood pressure but also hyperlipidemia (45, 46).

Does hyperinsulinemia really play a primary role in the development of hypertension and is it linked to the constellation of risk factors responsible for atherosclerosis? No clear-cut answer is yet available.
First of all, there have been considerable discrepancies among the hitherto reported data as to whether or not plasma insulin levels correlate with blood pressure and with morbidity and mortality attributed to ischemic heart diseases. Many studies support a strong positive correlation between insulinemia and blood pressure in patients with essential hypertension (47-53) and even in their young normotensive offspring (54), while others (55-62) have shown a modest or insignificant association. Such discrepancies are probably due to differences in the demographic composition of the study populations and to inaccurate assessment of body composition of the study participants, as pointed out in a recent review by Williams (63). Therefore, even though statistical analyses suggest a significant relation between insulin levels and blood pressure, the correlation becomes weaker or insignificant (64-68) after adjusting for the confounding factors of age, gender, body mass index, body fat distribution, and blood sugar levels. In particular, it has been emphasized that intra-abdominal (visceral) fat accumulation is the factor most strongly associated with insulinemia (69). The authors (68) noted a weak, but significant, positive correlation between fasting serum insulin values and either systolic or diastolic blood pressure in a study of 2,828 male bank employees, aged 30-65 years, who received a regular medical checkup. However, when the data for only the non-obese subjects with BMI below 25 were re-analyzed, there was no correlation between serum insulin values and blood pressure. The results indicate that insulin is somehow involved in the development of hypertension, and that its role is mediated by obesity. Muller et al. (61) investigated whether hyperinsulinemia is associated with hypertension in 421 men and 228 women ranging in age from 17 to 95 years. Either systolic or diastolic blood pressure was found to be statistically related to insulin levels. However, after adjusting for the confounding variables of age, body fat and fat distribution, correlations between blood pressure and insulin levels became entirely insignificant. Second, as pointed out by several groups of investigators, ethnic variability (69-71) in the relation between insulinemia and blood pressure might be responsible for the discrepancies among previous reports.

Hyperinsulinemia is generally considered to be a secondary phenomenon which compensates for the impaired action of insulin at target tissues, i.e., “insulin resistance,” as reflected by a decline in insulin-mediated glucose disposal. Many factors, ranging from biochemical to structural abnormalities in the insulin target tissues, may be involved in the mechanism(s) of insulin resistance. Here, the authors would like to mention several intriguing studies on the causal relationship between insulin resistance and hypertension. The first one is referred to as the “hemodynamic hypothesis”. This hypothesis is based primarily on the observations that: 1) microvessel density in skeletal muscle is reduced in SHR (72), in a one-clip, one-kidney model of renal hypertension (73), and in primary hypertension(74); 2) such vascular rarefaction possibly causes under-perfusion into the target tissue for insulin, and thus contributes to insulin resistance via reduced glucose uptake into peripheral tissues. The second hypothesis seems to be a variation of the above-mentioned hemodynamic hypothesis and is based on the concept that insulin resistance is a state resulting from attenuation of the originally present vasodilating effect of insulin. Laakso et al. (75) have recently shown that insulin-induced vasodilatation is blunted in obese subjects, and lack of vasodilatation in response to insulin release after nutrient intake may be contributory to the development of hypertension. However, several opposing arguments have also been proposed. Neither the structural rarefaction nor the functional abnormality of the vasculature in skeletal muscle causes reduced tissue perfusion, which can lead to insulin resistance and hyperinsulinemia. Increased peripheral resistance and vascular rarefaction of skeletal muscle, which are observed in hypertension, may be a consequence of the reduced metabolic requirements of muscle tissues. Presently, we have no confirmed evidence that hyperinsulinemia associated with insulin resistance can raise blood pressure or potentiate hypertension, except for a few animal experiments, exemplified by the report of Hall et al. (76) who demonstrated that long-term insulin infusion could elevate blood pressure in dogs when plasma insulin concentrations increased to levels similar to those in obese subjects. Subsequent reports have also argued against the insulin hypothesis, on the basis of the observations that chronic hyperinsulinemia in patients with insulinoma is not associated with a detectable elevation of blood pressure (77, 78) and that surgical removal of insulinoma does not decrease blood pressure.

**Extra-Renal Renin-Angiotensin and Aldosterone Systems**

1. **Tissue Renin-Angiotensin System**

The existence of the renin-angiotensin system (RAS) in the extrarenal tissue has been previously suggested on the basis of the biological activities and concentrations of each component of the RAS in the heart (79), blood vessels (80-83), adrenal gland (84), and reproductive organs (85). In the cardiovascular system, direct evidence for the presence of a RAS was recently obtained in mRNA expression studies. Renin mRNA was detected in cultured myocytes and fibroblasts from neonatal rat hearts, supporting the concept that angiotensin II is formed partly by renin located in the heart (86). Angiotensinogen mRNA has also been demonstrated in the vessel wall (87). ACE mRNA can also be detected in aorta subjected to pressure overload (88). Considered together, these findings suggest that each component of the RAS is produced in cardiovascular tissue and that these components may exert some physiological function independently of the circulating RAS. However, it is still controversial whether angiotensin formed in these tissues is dependent on renin and angiotensinogen synthesized locally or those taken up from plasma. A carefully designed experiment that measured cardiac renin and
angiotensins in the healthy pig heart suggested that angiotensin production depends on plasma-derived renin and angiotensigen (89). In that study, after the animals were nephrectomized, cardiac renin and angiotensins disappeared within 30 hours, in parallel with plasma levels, suggesting that most cardiac renin is of renal origin. From these results, the authors concluded that renin taken up by the heart from the circulation acts on angiotensigen to form angiotensin I, which is then converted to angiotensin II locally by cell-membrane-bound ACE. Interestingly, angiotensin II formation can be achieved by chymase, a chymotrypsin-like serine protease, which is not blocked by ACE inhibitors. Human chymase has a high substrate specificity (90) and does not form angiotensigen. Recently, Miyazaki's group has shown that chymase activity is increased along with its mRNA expression, particularly in the intimal thickening process of arterial restenosis (91). Differences in the distribution of ACE and chymase between and within tissues (92) may result in different levels of local angiotensin II during treatment with ACE inhibitors, leading to variable clinical effects. Angiotensin I present in increased levels in the circulation in association with ACE inhibition may be trapped by the cardiovascular system and converted to angiotensin II only in those tissues which contain chymase.

2. Cardiovascular Hypertrophic Effects of Angiotensin II
Proliferative effects of angiotensin II on both endothelial cells and vascular smooth muscle cells (SMC) have recently been demonstrated in conjunction with growth factors. In a rat aortic SMC culture model, angiotensin II stimulated DNA synthesis dose-dependently, as evidenced by increased labeled thymidine incorporation (93). Angiotensin II also stimulated SMC proliferation associated with increased expression of specific endogenous growth factors, such as transforming growth factor β1 (TGF β1) and platelet-derived growth factor (PDGF). In previous reports, angiotensin II induced hypertrophy, but not proliferation, in primary adult rat vascular SMC (94, 95). Increased cell size and cell number with angiotensin were reported in human SMC (96), whereas in the rat a hypertrophic response with no mitogenic effect has been shown in a similar setting (94). Different serum concentrations in the media may partly explain these discrepancies, as hyperplasia occurs in the presence of serum and hypertrophy occurs in its absence (97). Furthermore, these discrepancies may be related to phenotypic differences among the various vascular SMC lines used in the experiments (98). We have performed [3H]thymidine and [3H]leucine incorporation studies using endothelial and SMC cultured from human pulmonary artery (99). Our results indicated that angiotensin II stimulated [3H]leucine incorporation by SMC, suggesting that angiotensin II promotes hypertrophy in this cell line.

The extracellular matrix and mechanical strain have been shown to play a role in the potentiation of angiotensin-induced mitogenic activity in rat vascular SMC (100), which suggests that both increased pressure per se and humoral factors, act such as angiotensin and endogenous growth factors, synergistically and contribute to vascular remodeling.

The proliferative and mitogenic effects of angiotensin are mediated via the angiotensin receptor, since specific angiotensin receptor antagonists can prevent these effects of the peptide (101).

3. Vascular Aldosterone – A Link to Angiotensin II-induced Hypertrophy of Vascular Smooth Muscle Cells–
Recent studies conducted by Brilla et al. (102) and our group have demonstrated that vascular cells possess not only a renin-angiotensin system but also a mechanism for aldosterone production. Aldosterone is known to alter vascular smooth muscle cell (SMC) permeability to electrolyte ions, resulting in increased smooth muscle tone and responsiveness to various humoral and neurogenic vasoconstrictors. Studies (99) have provided direct evidence that vascular cells, including both endothelial cells and smooth muscle cells, cultivated from human pulmonary artery, express the CYP 11 B 2 mRNA encoding the key enzyme for the biosynthesis of aldosterone. In fact, we have identified by GC/MS at least two steroids (aldosterone and corticosterone) in perfusates from rat isolated mesenteric artery. Four other unknown steroids that cross-react with anti-sera for corticosterone and aldosterone were detected by HPLC, suggesting that the arterial vessels possess their own steroidogenic pathway (103). In addition, the amount of aldosterone released from the mesenteric artery in the perfusion circuit was significantly decreased when the artery was taken from rats given quinapril, an ACE inhibitor with high tissue penetration.

Aldosterone receptor gene was also found to be expressed in SMC and, to a lesser extent, in endothelial cells. Notably, the angiotensin II-induced increase in [3H]leucine incorporation in SMC was significantly enhanced by aldosterone but inhibited by ZK 91587, a type I mineralocorticoid receptor antagonist. These results indicate that up-regulated aldosterone may potentiate the hypertrophic and/or hyperplastic response to angiotensin II. It is therefore more likely that vascular aldosterone participates in modulating vascular tone in an “autocrine” or “paracrine” manner rather than via the systemic circulation. Considered with our recent findings that angiotensin II can induce vascular endothelin receptor expression, the vascular renin-angiotensin system may play a role in hypertensive vascular remodeling by interacting with endothelin.

4. Mineralocorticoid and Cardiovascular Fibrosis
Extraepithelial effects of mineralocorticoids have been reported to contribute to cardiovascular hypertrophy (104, 105). Aldosterone administration in uni-nephrectomized rats given 1% sodium chloride for 9 weeks markedly increased blood pressure and cardiac hypertrophy in association with increased left ventricular interstitial collagen (106). Increases
in α-1-I procollagen mRNA and in α-1-III collagen mRNA are not known.

In view of the recent finding that ET is synthesized in the left ventricle in a similar experiment (107). This cardiovascular effect is mediated through type 1 (mineralocorticoid) receptor in the heart, independently of hemodynamic changes. The heart is an organ that is not protected by 11β-HSD (108), an epithelial enzyme that inactivates glucocorticoid and thus prevents it from binding to type 1 receptor. Therefore, cardiac or perivascular fibrosis may occur both in mineralocorticoid- and glucocorticoid excess, as mentioned before. However, animal experiments suggest that cardiac fibrosis is a consequence of mineralo- and glucocorticoid excess in the presence of volume overload. In support of this, patients with Bartter's syndrome do not manifest clinical signs of diastolic dysfunction.

**Endothelin in the Cardiovascular System**

Regarding the potential roles of endothelin (ET) in the cardiovascular system and hypertension, we focus, in this section, on three short reports from our laboratory, since an extended and detailed review of ET was recently written by Masaki (109).

1. **Role of ET-1 in Blood Pressure Regulation**

   The potent vasoconstrictive property of ET led us to consider initially that ET may play a pivotal role in blood pressure regulation (110). Subsequent studies showed that the plasma concentration of ET was too low to maintain vascular tone; the concentrations, in fact, were similar in normal subjects and hypertensive patients (111), and the administration of a selective ET(A) antagonist failed to alter steady-state blood pressure (112). These results suggest that the ET in the systemic circulation may not play a role in steady-state blood pressure control. We tested whether ET in the circulation contributes to blood pressure in conscious rabbits (113). Porcine ET-1, at a concentration of 10^-12 to 10^-10 mol/kg, was given intravenously via an ear vein, and blood pressure was monitored. Renal, mesenteric and carotid blood flows were measured with a flow meter. The effect of ET-1 antibody on these hemodynamic variables was also examined. The results demonstrated that ET-1 administration dose-dependently increased blood pressure and decreased regional blood flow. ET-1 antibody alone in a dose that inhibited the exogenous ET-1 response failed to influence steady-state blood pressure. This finding also refutes the role of ET as a circulating vasopressor hormone.

   Current concepts of ET-1 in the circulatory system indicate that in some experimental models, such as DOCA treated SHR and Dahl salt-sensitive strain rats, ET contributes to the pathogenesis of hypertension indirectly (114, 115).

2. **ET-1 as a Pathogenic Factor and Marker for Diabetic Angiopathy**

   In view of the recent finding that ET is synthesized and released from the dysfunctional endothelium, we evaluated the effects of an ET receptor antagon-

   In diabetic patients with varying degrees of angiopathy, we measured plasma concentrations of ET-1. We found that plasma ET-1 levels correlated with the severity of vascular complications and proposed ET-1 as a marker for angiopathy in diabetic patients (117).

3. **A Possible Role of ET-1 in Cyclosporine-Induced Hypertension**

   Cyclosporine is an immunosuppressive agent that has improved long-term survival after organ transplantation and has proven to be beneficial in the treatment of various autoimmune disorders. Cyclosporine therapy, however, is associated with the development of hypertension, although the mechanism is poorly understood. We have shown that ET-1 release is increased during cyclosporine-induced hypertension (118). In another study we demonstrated that ET-1 mRNA is increased, confirming this mechanism (119).

   Interleukin (IL)-2, which has been used to treat malignant hematological disorders, is also known to cause increased vascular permeability, resulting in edema formation, weight gain and, occasionally, elevated blood pressure. We demonstrated elevated plasma levels of ET-1 in patients with these adverse reactions; measurement of ET-1 may therefore be useful as a marker for this adverse effect (120).

**Concluding Remarks**

In this paper, the authors provided an overview of endocrine and auto-paracrine hormones, such as steroids, renin-angiotensin, ouabain, insulin and endothelin, and discussed their roles in the pathogenesis of primary hypertension. The magnitude of their contribution to the regulation of blood pressure and to vascular remodeling remains unclear. It is unknown whether there is a subtype of primary hypertension where only one of the above hormones plays a dominant role. Another topic for future investigations is how vasodilating factors, such as prostaglandins, natriuretic peptides, adrenomedullin and nitric oxide, interact with these hypertensi-
nogenic hormones. The interaction between locally generated angiotensin II, aldosterone and ETs within the cardiovascular tissues may well become an important issue for future research on hypertension.

References

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