Role of oxidative stress in angiotensin-induced hypertension

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Reckelhoff, Jane F., and J. Carlos Romero. Role of oxidative stress in angiotensin-induced hypertension. Am J Physiol Regul Integr Comp Physiol 284: R893–R912, 2003; 10.1152/ajpregu.00491.2002.—Infusion of ANG II at a rate not sufficient to evoke an immediate vasoconstrictor response, produces a slow increase in blood pressure. Circulating levels of ANG II may be within ranges found in normotensive individuals, although inappropriately high with respect to sodium intake. When ANG II levels are dissociated from sodium levels, oxidative stress (OXST) occurs, which can increase blood pressure by several mechanisms. These include inadequate production or reduction of bioavailability of nitric oxide, alterations in metabolism of arachidonic acid, resulting in an increase in vasoconstrictors and decrease in vasodilators, and upregulation of endothelin. This cascade of events appears to be linked, because ANG II hypertension can be blocked by inhibition of any factor located distally, blockade of ANG II, OXST, or endothelin. Such characteristics are shared by other models of hypertension, such as essential hypertension, hypertension induced by reduction in renal mass, and renovascular hypertension. Thus these findings are clinically important because they reveal 1) uncoupling between ANG II and sodium, which can trigger pathological conditions; 2) the various OXST mechanisms that may be involved in hypertension; and 3) therapeutic interventions for hypertension developed with the knowledge of the cascade involving OXST.

isoprostanes; endothelin; spontaneous hypertension; renovascular hypertension; sodium balance

THREE DECADES AGO, the production of free radicals was thought to be the result of severe injuries or pathological insults such as those resulting from exposure to high-energy radiation or toxins such as carbon tetrachloride (127). However, in 1968, the discovery of superoxide dismutase (SOD) by McCord and Fridovich (96) strongly suggested that all aerobically metabolizing cells are capable of producing superoxide ions, which could play a role in normal metabolic processes. The concomitant discovery of the biology of nitric oxide (NO) strongly suggested that free radicals could constitute the basis of metabolic regulation (67, 90, 118). One of the first findings supporting such a notion was the demonstration that the inhibition of the synthesis of NO produced by the administration of an NO synthesis competitor, such as Nω-monomethyl-L-arginine or Nω-nitro-L-arginine methyl ester (L-NAME), produced a marked vasoconstriction (13, 136, 173), sodium retention (82, 83), and thereby a sustained increase in mean arterial pressure (MAP) (13, 82, 83, 136, 173). The identification of a specific pathological situation in humans, where a decrease of NO may end up in an elevation of blood pressure was thought to be linked to endothelial dysfunction (105), such as those involved in aging, arteriosclerosis, etc., but the specific metabolic alterations involved in this process were poorly understood. However, the relationship between a fall in NO with oxidative stress (OXST) became apparent when Beckman et al. (14) reported in 1990 that NO could combine with superoxide with a high degree of affinity to form peroxynitrite and that peroxynitrous acid was a constant source of active hydroxyl radical with potent oxidative properties (14). Furthermore, the study showed that superoxide and NO react together with a rate constant that is as large or larger than those for the reaction of superoxide with SOD or for NO with heme compounds (97, 170). The role of OXST in the regulation of the circulation was emphasized by the study of Rajagopalan et al. (129), who showed that the administration of large doses of ANG II to rats induced an increase in both blood pressure and the formation of superoxide from isolated aortic strips. This response could not be evoked by producing an equivalent elevation in blood pressure with other vasoconstrictors, such as norepinephrine. The specificity of ANG II to stimulate OXST, however, was questioned by an experimental finding showing
that the circulating levels of ANG II during sodium restriction can be much higher than those necessary to induce hypertension and other pathological effects (138).

All these findings raise a number of important questions with regard to the relationship of angiotensin, OXST, and NO in the regulation of renal blood flow (RBF), sodium excretion, and ultimately blood pressure. In this review we will try to answer these questions. Furthermore, we will examine first the effects of intravenous infusion of pressor and subpressor doses of ANG II and the extent to which this maneuver can be used as an experimental model to mimic different forms of essential hypertension in humans. The understanding of this effect of angiotensin necessitates an examination of the distribution of this peptide in the intravascular, renal interstitial, and intracellular compartment. We will also focus on the extent to which an imbalance between the levels of angiotensin with respect to sodium intake and the synthesis of NO will favor the production of OXST and also the component of OXST that appears to be involved in the development of hypertension (138). We will examine evidence showing that the renal dysfunction generated by the disequilibrium between angiotensin, NO, and OXST specifically affects RBF, the afferent and efferent glomerular arteriolar tone, tubular glomerular feedback (TGF), and medullary blood flow. We will also analyze evidence showing the participation of these dysfunctions in models of experimental hypertension such as in spontaneously hypertensive rats (SHR). For reasons of space, we will not examine the alterations in intracellular signaling by which the stimulation of AT1 angiotensin receptors lead to an exaggerated formation of oxygen by the mitochondria as well as the intracellular components involved in other pathological actions of angiotensin such as hypertrophy, arteriole remodeling, inflammatory components, etc.

CHARACTERISTICS OF THE VASOCONSTRICTOR RESPONSE EVOKED BY ANG II

The vasopressor response to ANG II has been classified as both fast and slow.

Fast pressor responses. It is well known that the intravenous administration of a relatively large dose of ANG II (>300 mg·kg⁻¹·min⁻¹) given as a bolus produces a rapid contraction of the smooth muscle through the phosphoinositide-Ca²⁺-protein kinase C (PKC) effector system (4, 53, 110, 158). The response achieves a maximal pressor effect in seconds and returns to normal levels in 2–3 min (60, 109). Comparative studies suggest that the fast responses to ANG II may only constitute a pharmacological phenomenon, because a sustained increase in the circulating levels of ANG II that is necessary to evoke a rapid elevation of blood pressure of this magnitude is higher than 2,500 pg/ml of plasma (23). Concentrations of this high level of circulating ANG II are never seen in a physiological (severe sodium deprivation) or in pathological (severe renovascular hypertension or hemorrhage) situations.

Slow pressor responses. Another type of vasopressor response induced by ANG II consists in the progressive elevation of MAP induced by the continuous administration of a subpressor dose of ANG II; that is to say, at doses of ANG II that do not evoke an immediate pressor response. For example, in rats, Hu et al. (62) showed that the continuous administration of a dose as low as 3.5 ng/min of ANG II produced hypertension almost 1 wk after commencing the infusion. This kind of “delayed or slow pressor response” was first demonstrated by Dickinson and Lawrence in 1963 (38). They observed that a continuous infusion of an amount of ANG II below the threshold of the direct vasoconstrictor effect, elicited a gradual increase in blood pressure. Two years later, McCubbin et al. (98) reported similar findings in dogs. Slow pressor responses have also been demonstrated in a studies conducted in different species, such as rabbits (38), dogs (98), rats (23, 62), swine (55), and humans (5). At present, the minimal amount of ANG II capable of producing a slow response (delayed elevation of blood pressure) in humans and animals has not been determined, although the slow pressor response to ANG II appears to be evoked at much lower doses in humans (30, 48, 49, 152) than in animals (5, 23, 38, 55, 62, 98), as discussed below.

A more detailed analysis of the characteristics of the slow pressor response is pertinent because this model of hypertension resembles most of the characteristics of essential hypertension found in humans: 1) the levels of circulating ANG II necessary to produce the development of delayed hypertension can be within the range of those found in normotensive individuals; 2) the hypertension evolves with an early increase in intrarenal resistance that coexists with sodium retention (this latter effect subsides after hypertension has been achieved); and 3) there is a significant elevation of peripheral resistance that plays an important role in the elevation of blood pressure since cardiac output remains normal.

PERIPHERAL LEVELS OF ANG II NEEDED TO PRODUCE HYPERTENSION

The levels of circulating ANG II in humans with essential hypertension remains undefined. In these patients, determination of plasma renin activity (PRA) has been routinely measured rather than circulating levels of ANG II (25). However, it is difficult to compare studies, because the levels of circulating ANG II cannot actually be derived from measurements of PRA, although under most conditions there is a correlation between the two determinations. However, when PRA is measured and plotted against urinary sodium excretion, 50% of essential hypertensive patients exhibit levels of PRA no different from that seen in normotensive individuals (25). Furthermore, a subset of the population of hypertensive individuals exists (25%) in whom the level of circulating PRA is significantly less than that measured in normotensive individuals (25). ANG II does contribute to the maintenance of hypertension in these essential hypertensive individuals because blood pressure is markedly reduced by the administration of either converting en-
zyme inhibitors (72a, 42) or ANG II receptor antagonists (44). Furthermore, this is particularly true in the diabetic low renin state (126) and in reduced renal mass hypertension (73) in which circulating levels of ANG II are not different from normal (33, 81, 190).

These observations raise the question of whether hypertension can be produced or even maintained with normal levels of ANG II. This issue can be better understood by examining the peripheral levels of ANG II that are actually needed to produce hypertension through slow pressor responses. In the above-mentioned study performed by Brown et al. (23), it was found that the continuous intravenous infusion of 20 ng·kg⁻¹·min⁻¹ of ANG II to conscious rats failed to elicit any detectable pressor response over the control values (103 ± 4 mmHg) during the first hour of the infusion. However, the following day, MAP increased by 15 mmHg. On day 7 of infusion, MAP reached 153 ± 6 mmHg. The level of circulating ANG II in these hypertensive animals on day 7 was 249 ± 25 pg/ml of plasma, which is threefold higher than the level recorded in normotensive controls infused with dextrose (80 ± 19 pg/ml). It was also found that the amount of ANG II needed to produce, during 1 h (i.e., a fast response), an elevation of MAP to levels comparable to that seen on the 7th day in the chronically infused hypertensive animals was 2,700 pg·kg⁻¹·min⁻¹. This finding proved that continuous infusion of subpressor doses of ANG II, which is accompanied by small increments in plasma ANG II (see later), is capable of producing the same increment in blood pressure as that induced by the fast infusion of a dose of ANG II that is ~10 times higher than that needed to produce the slow response. This observation led Dickinson and Lawrence (38) to state that “ANG II may bring into action some secondary mechanism which sustains arterial pressure by means other than general arterial vasoconstriction due directly to the hormone.”

One of the conceptual problems that one encounters when attempting to model the development of essential hypertension with the infusion of subpressor doses of ANG II is that a mild increase of ANG II presupposes the requirement of a concomitant increase in PRA. This implies an excessive release of renin without the modification of factors that control the velocity of the renin-angiotensinogen reaction (134), particularly a rapid depletion of renin substrate or even the absence of changes of renin binding by extrarenal renin receptors (28, 150). An important study to define this problem was undertaken by Hu et al. (62), who showed that slow responses can be developed in the rat by chronic administration of either ANG II (3.5 ng/min) or rat recombinant renin (0.6 ng/min), compared with vehicle control (Fig. 1). The results are somewhat similar to those reported by Brown et al. (23) in that the control levels of plasma ANG II were statistically elevated by 2.5-fold from ~4.5 ± 0.8 up to 10.7 ± 0.7 pg/ml (23). However, it differs in that on days 12–13, the concentrations of ANG II in plasma return to levels similar to those recorded during the control period (Fig. 1). The renin-infused animals experienced a slow and progressive increase in blood pressure very similar to that found in ANG II-infused animals. The studies of Hu et al. (62) and Brown et al. (23) clearly show that the administration of subpressor doses of ANG II or renin induced an increase in blood pressure that was sustained, despite the fact that the concentration of ANG II in plasma was never increased by more than a small amount (23) or was only transiently increased (62).

Highly relevant to the subject under discussion are the studies of Ames et al. (5), who were the first to investigate the chronic effects (6–11 days) of ANG II in humans. These investigators infused in normal volunteers an amount of ANG II (~25 ng·kg⁻¹·min⁻¹) that was capable of producing an initial increase in blood pressure of 20–30 mmHg (fast pressor response). However, as soon as sodium retention occurred, the subjects became increasingly sensitive to the pressor effect of ANG II in a manner comparable to that seen during the development of slow pressor responses (Fig. 2). Under these conditions blood pressure was maintained as ANG II doses were progressively diminished to one-fourth of the initial dose (Fig. 2). Similar observations were later made by Laragh et al. (85). In later studies Gandhi et al. (49), Cargill et al. (30), Shoback et al. (152), and Gaboury et al. (48) reported that ANG II infusions as low as 2–3 ng·kg⁻¹·min⁻¹ over 20–30 min caused acute increases in blood pressure in normotensive individuals on a normal salt diet. These studies show first that much lower doses of ANG II are typically required to increase blood pressure in humans than in experimental animals. The doses of ANG II that produced slow pressor effect in experimental animals were below 50 ng·kg⁻¹·min⁻¹ (23, 38, 55, 62, 98). These doses produce fast pressor responses in humans (30, 48, 49, 152). Second, as shown by Ames et al. (5) and Laragh et al. (85), a potentiating pressor effect similar to that seen in slow pressor responses devel-

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**Fig. 1.** Plasma ANG II levels during renin (solid bars) or ANG II (open bars) infusions. Each time point represents results from equal volume of plasma from a 3-day period. C, control days; E, experimental days; R, recovery days. Values of blood pressure (bottom) are average obtained from both groups during the 3-day period. *P < 0.05 vs. control period; †P < 0.05 vs. recovery period. (Modified from Ref. 62.)
oped on top of the fast pressor responses. The chronic pressor potentiation in response to ANG II is then an effect that occurs whenever ANG II is infused (or increased above normal) in a chronic fashion. The doses of ANG II that are within the subpressor range in humans have not yet been established, although Hollenberg et al. (60) reported that ANG II infusion of 1 ng·kg\(^{-1}\)·min\(^{-1}\) had no effect on MAP in humans but caused a marked reduction in RBF. Unfortunately, the infusion was not continued beyond the acute phase, so the development of slow pressor responses was not evaluated. In any case, the consistent delay of small subpressor doses of ANG II to produce a chronic increase in blood pressure is indicative of a time requirement necessary for the activation of additional vasoconstrictor processes, which can then trigger an autocatalytic reaction that accelerates or potentiates the vasoconstrictor effect of ANG II.

**CIRCULATING AND INTRARENAL LEVELS OF ANG II**

Before investigating the vasoconstrictor mechanism(s) that could potentiate the effect of ANG II, it will be important to understand the manner in which peripheral levels of ANG II relate to the concentrations in the interstitial fluid and in the intracellular compartment of some organs. It has been known for years that the kidney releases renin into the general circulation where it acts upon a microglobulin (103, 157) from which several active peptides are produced, ANG II being the one that exerts the most prominent pressor effect (21, 128). ANG II can also be generated by renin within the kidney. In fact, it has been emphasized in several studies that renin, angiotensinogen, ANG I, or ANG II are localized in secretory granules around the glomerular vascular pole and macula densa (65, 68, 70, 169). Hence, ANG I and II are probably cosecreted with renin by these cells (65, 68). The intrarenal renin-ANG II system appears to be important because high concentrations of ANG II have been detected in renal interstitial fluid (156) and in proximal tubular fluid (22, 151). Furthermore, measurement of renin in renal lymph indicated that renal interstitial fluid contains high renin concentrations (10, 88), and the interstitial fluid compartment is thought to be the site of intrarenal ANG II production. All these findings suggest that there should be a gradient of ANG II concentration that favors diffusion from the kidney to plasma and from there to other organs, their interstitial spaces, and intracellular compartments. This may not be the case, however. Navar et al. (113) found that there is a significant renal uptake of ANG II from plasma. This observation was confirmed by van Kats et al. (174), who observed that continuous infusion of a very small amount of \(^{125}\)I-labeled ANG II into the left ventricle of rats resulted in an accumulation of this peptide in the renal cortical tissue and was 4.1 ± 0.6 fold higher than in arterial plasma, whereas the medullary tissue/arterial plasma concentration ratio was 1.8 ± 0.2 (Fig. 3). The kidney was capable of clearing 88% of the ANG II that got into the organ through the renal artery. Furthermore, it was also found that during the infusion of \(^{125}\)I-labeled ANG II, the concentration of endogenous (naturally synthesized ANG II) in the renal cortex was 102 ± 30 times higher than in plasma, whereas the concentration in the renal medulla was 64.4 ± 14 times higher than in the arterial plasma (Fig. 3). The concentrations of endogenous (not labeled) ANG II in renal vein was 0.4 nmol. Thus the tissue-to-blood plasma concentration ratio was much higher for endogenous ANG II than it was for the infused \(^{125}\)I-ANG II. These results essentially show that although there is an uptake of ANG II by the kidney, ~85% of renal tissue ANG II originates from local production in the kidney.

**Fig. 3. Schematic representation of the relative concentration of infused \(^{125}\)I-labeled ANG II (solid line) and endogenously formed ANG II (dashed line) in renal cortex and medulla and in arterial and venous plasma.** For simplicity, the amounts of both forms of ANG II are given in arbitrary units (pg/ml) assuming that during the infusion of ANG II, the concentration achieved in plasma was 1 pg/ml.

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An additional finding of van Kats et al. (174) was that when infusion of ANG II was repeated in the presence of converting enzyme inhibitors, the tissue-to-plasma ratio of infused labeled ANG II or endogenous ANG II was not significantly altered. However, the administration of ANG II receptor antagonist significantly decreased the renal uptake of labeled ANG II, the tissue concentration of which reached levels below that recorded in plasma. ANG II receptor antagonists also resulted in a 75–80% decrease in the concentration ratio of endogenous ANG II, although endogenous ANG II remained higher in renal tissue than in plasma. This latter part of the study confirms previous observations by other investigators that cellular uptake of ANG II in the kidney depends on binding to cell membrane AT1 receptors. This process is then followed by receptor-mediated endocytosis. Cell-associated, blood-derived ANG II has a longer half-life than ANG II in the circulation (175), but the function of internalized ANG II is not completely known. The finding that ANG II receptor antagonist has a lowering effect on renal ANG II uptake and decreases endogenous concentrations of renal ANG II, effects that are not obtained by converting enzyme inhibitors, may have clinical repercussions that should be further investigated.

These results of van Kats (174) agree with those published by Nishiyama et al. (115), who conducted a study to determine the existing endogeneous levels of ANG I and ANG II in plasma and renal interstitium by means of microdialysis probes implanted in the renal cortex of rats. It was observed that the concentration of ANG I and ANG II in the renal interstitium were, respectively, 0.84 ± 0.04 and 3.07 ± 0.43 nmol/g (Fig. 4). These values were much higher than the respective plasma levels (ANG I 0.112 ± 0.014 nmol/l; ANG II 0.106 ± 0.008 nmol/l). This is to say that, in renal interstitium, the concentration of ANG II was 3.65-fold higher than ANG I, whereas interstitial levels of ANG II were 29-fold higher than in plasma. ANG I in renal interstitium was only 7.5-fold higher than in plasma (Fig. 4). These investigations also found that the administration of an angiotensin-converting enzyme inhibitor decreased the concentration of ANG II in plasma but failed to change the concentration of ANG II in the renal interstitium.

Thus from these studies it can be concluded that the intrarenal concentration of ANG II may not necessarily be related to the peripheral levels in plasma, because ANG II can accumulate in the renal interstitium and renal cells by continuous formation within the kidney and this concentration can be further enhanced by renal uptake from peripheral circulation. These findings have important physiological and pathological connotations, because they may indicate that the levels of ANG II that matter are not those found in plasma but those contained within the kidney itself. In fact, as we will see later, the kidney could be the most indicated place for the organism “to monitor” changes in the release of renin in relation to changes in extracellular fluid volumes.

**THE EFFECTS OF SODIUM INTAKE ON THE PRESSOR RESPONSE TO ANG II**

As mentioned above, the physiological levels of PRA correlate inversely with the amount of sodium intake. Brunner et al. (25) measured the levels of PRA at different levels of sodium intake in hypertensive and normotensive patients. This allowed the investigators to construct a nomogram in which low urinary excretion of sodium (from 0 to 30 meq/day) was associated with 12–13 ng·ml⁻¹·h⁻¹ of PRA, whereas urinary excretion of sodium in the range of 300 meq/day resulted in suppression of PRA to 0.5–1 ng·ml⁻¹·h⁻¹ (Fig. 5). This relationship indicates that for any given level of sodium intake, the existing levels of ANG II and NO are within the range needed to maintain sodium balance and normal arterial pressure. Therefore, an increase of ANG II to levels that are capable of producing
a slow pressor effect should necessarily be above those concentrations that are at that moment determined by the existing amount of sodium intake or volume repletion (ANG II-fluid volume coupling). This relationship can be monitored by the kidney, because there is an inverse relationship between sodium intake and renal synthesis of renin (52, 69) while renal interstitial pressure correlates well with volume expansion (27). One important corollary of this relationship between renin and sodium intake is that the levels of ANG II that may be excessive for the existing fluid volume when sodium intake is high (300 meq/day) and are capable of triggering slow pressor responses may be much lower than the physiological levels that are spontaneously produced when sodium intake is reduced to very low levels. Consequently, any plasma or renal level of PRA within the normal range can induce hypertension if they are inappropriately high with respect to fluid volume. Studies to determine the consequences of uncoupling body fluid volume from the level of ANG II were initially conducted by DeClue et al. (36). These investigators showed (Fig. 6) that in volume-depleted animals the extracellular fluid volume expansion induced by progressive increases of sodium intake from 3 meq·kg\(^{-1}\)·day\(^{-1}\) (a total of 63 meq/dog) to 25 meq·kg\(^{-1}\)·day\(^{-1}\) (a total of 525 meq/dog) produced a small 3% increase in MAP. Extracellular fluid volume was measured with radiolabeled \(^{22}\)Na. However, when a comparable sodium load was given in dogs in which ANG II was “clamped” by continuous infusion of small subpressor doses of ANG II (5 ng·kg\(^{-1}\)·min\(^{-1}\)), blood pressure was increased by 45 ± 3.8 mmHg or 46% over the control period (see Fig. 5). This finding was interpreted to indicate that although the infusions of ANG II were too small to produce any change in blood pressure in volume-depleted animals, it was sufficient to shift pressure natriuresis impairing sodium excretion during volume expansion inducing hypertension. Therefore, when ANG II was clamped, the progressive increase in body fluid volume and renal interstitial pressure became excessive (or inappropriate) to the existing plasma or renal levels of ANG II. The word inappropriate may be more pertinent because the hypertensinogenic effect of ANG II was produced in the presence of volume expansion that was not different from animals that did not receive ANG II and remained normotensive, although many other investigators have shown that increased sodium intake causes a marked potentiation of ANG II pressor effect (6, 35, 74, 108, 142, 154). DeClue et al. (36) was the first to demonstrate that a dissociation between extracellular fluid volume and the plasma or perhaps tissue levels of ANG II may be an important alteration underlying the development of high blood pressure. Such dissociation (or uncoupling) can be set by either inducing a progressive volume expansion in the presence of a fixed level of ANG II or, vice versa, by increasing the level of ANG II in the presence of a fixed volume. For example, a progressive infusion of small doses of ANG II that do not produce a pressor effect in dogs in normal conditions become hypertensive when infused into volume-expanded animals (36). This latter study was performed in dogs fed with 544 meq of sodium a day for 5 days, a sodium load similar to the highest intake given to the dogs in the experiment of DeClue et al. (36). In these dogs, progressively increasing infusions of subpressor doses of ANG II from 0.25 to 1, 2.5, and finally 5 ng·kg\(^{-1}\)·min\(^{-1}\) resulted in 10, 20, and 40% increments in MAP, respectively (36).

Finally, an additional observation that corroborates the importance of the dissociation between the levels of ANG II and body fluid volume required to produce hypertension is that in dogs made hypertensive by a large sodium load (500 meq/day) on top of ANG II infusions (5 ng·kg\(^{-1}\)·min\(^{-1}\)), the administration of a diuretic (furosemide) tends to normalize blood pressure by reducing fluid volumes to levels that are presumably “appropriate” to the concentration of ANG II (Fig. 7) (36). This observation may explain the therapeutic success in hypertensive patients of both ANG II inhibitors (because it renders the activity of ANG II appropriate to fluid volumes) or diuretics, which render fluid volume appropriate to the levels of ANG II (36). However, diuretics are less effective in normalizing blood pressure than an ANG II antagonist because volume contraction stimulates the release of renin, thus increasing the levels of renal and circulating ANG II. Hence, volume depletion should be driven to almost a maximum to drop MAP close to normal levels. As shown in Fig. 7 the dose of furosemide necessary to lower MAP was 85 mg/day. Relevant to the role of volume in ANG II-induced hypertension are the studies of Krieger and Cowley (78), who demonstrated that ANG II-induced hypertension can be prevented if volume expansion is servocontrolled and maintained within normal levels. The concept of excessive levels of ANG II with respect to the levels of sodium intake was introduced by Hollenberg et al. (60) when they observed that there was a human population of hypertensive individuals who were incapable of lowering the levels of PRA during fluid volume expansion. They called this group “nonmodulators.” This group of investigators also found that this alteration was corrected.
by converting enzyme inhibitors. It should be mentioned here that Dickinson and Yu (39) strongly emphasized that sodium retention and/or volume expansion is not an important condition to elicit slow responses to ANG II. These investigators showed that a progressive rise of blood pressure of 20–30 mmHg obtained by the infusion of 0.01 g/kg 1 of ANG II for 3 days was not accompanied by any significant change in urinary excretion of sodium, urine volume, or body weight. We concur with such a conclusion that slow responses to angiotensin are not dependent on a measurable volume expansion. However, the results of Dickinson and Yu (39) can be reinterpreted as indicating that ANG II exerted a strong antinatriuretic effect because the increase in blood pressure did not induce the habitual pressure natriuresis. Under these conditions, it can be safely assumed that the levels of sodium excretion and/or blood pressure were inappropriate to the levels of angiotensin in plasma or in the kidney.

SALT SENSITIVITY INDUCED BY ALTERATIONS IN THE CONCENTRATION OF INTRARENAL NO WITH RESPECT TO ANG II

As it is apparent, the study of DeClue et al. (36) constitutes the first salt-sensitive model obtained in a dog by interfering with the lowering effect that volume expansion has on the level of ANG II. Under these conditions, the level of blood pressure is essentially determined by the amount of sodium intake. A related model of salt sensitivity also reported long ago was caused by the partial inhibition of NO. Lahera et al. (83) first observed that the acute and progressive inhibition of NO in rats obtained by the successive infusions of 0.1, 1, and 10 mg of a NO synthesis inhibitor, L-NAME, produced significant decrements in urine volume, urine sodium excretion, RBF, and glomerular filtration rate (GFR) before the elevation of blood pressure was detected. With the passage of time MAP went up, bringing urine sodium excretion back to normal. This sequence of events was also produced in 180 min during the intravenous infusion of 10 μg·kg⁻¹·min⁻¹ (Fig. 8). This finding clearly suggests that the most sensitive parameter affected during partial systemic inhibition of NO is a decrease of sodium and water excretion and an elevation of intrarenal vascular resis-

![Figure 7: Decreasing effects of furosemide on blood pressure in dogs made hypertensive by the combination of a high intake of sodium chloride intake (500 meq/day) plus continuous angiotensin infusion (5 ng·kg⁻¹·min⁻¹).](image)

![Figure 8: Changes in MAP, glomerular filtration rate (GFR), and urine sodium excretion (UNaV) at 60, 120, and 180 min of infusion of N⁴-nitro-L-arginine methyl ester (L-NAME) 10 μg·kg⁻¹·min⁻¹.](image)
tance whose threshold of activation is higher than that of systemic blood pressure (136). This sequence of events resembles those obtained during the infusion of small doses of ANG II, which produces an increase in intrarenal resistance and a fall in water and sodium excretion before the overall elevation of peripheral resistance and blood pressure (56, 60, 62). This observation is consistent with the idea that the effects of NO are continuously counterbalanced by the vasoconstrictor effect of ANG II. Therefore, much of the effects seen during the progressive inhibition of NO are, in fact, produced by ANG II, whose antinatriuretic and vasoconstrictor effects are left unbalanced (136). A finding that supports this concept is the study of Salazar et al. (140), who showed that the continuous administration (for 5 days) of 0.1 g·kg⁻¹·min⁻¹ of L-NAME to dogs produces a marked decrease in water and sodium excretion, although this small dose was not sufficient to alter blood pressure. Under these conditions, the administration of a high sodium intake, which ranged from 80 to 300 meq/day, produced a very significant increase in blood pressure. This study shows that partial inhibition of NO synthesis produces salt sensitivity with similar characteristics as that obtained by clamping the levels of ANG II (36).

**INTRARENAL FUNCTIONS REGULATED BY ANG II AND NO**

It is pertinent to briefly comment here on those effects of intrarenal ANG II that are modulated by NO (Fig. 9) because an imbalance between NO and ANG II produced by OXST could explain a specific renal dysfunction that fosters slow pressor responses and thereby hypertension. The first of these processes involves the so-called TGF mechanism that operates at a single-nephron level to maintain a balance between the reabsorptive function of each nephron and the amount of solids and fluids filtered at this glomerulus (17, 111, 147, 184). The macula densa cells of the thick ascending limb of the loop of Henle detect changes in the composition of tubular fluid entering the terminal portion of the thick ascending limb of the loop of Henle and transmit signals that alter glomerular vascular resistance, glomerular capillary pressure, and thereby single-nephron GFR. Specifically, increases in the sodium chloride or total solid concentration of the tubular fluid flowing past the macula densa cells in response to increases in fluid flow into the ascending limb of the loop of Henle lead to increases in glomerular vascular resistance and decreases in single GFR (17, 111, 147, 184). Although the available evidence indicates that ANG II does not directly mediate TGF responses, it is clear that the prevailing ANG II levels do exert an important modulatory influence on the overall sensitivity of this mechanism (102, 112, 114, 146, 147). Several studies have shown that systemic administration of ANG II receptor antagonist or ACE inhibitors attenuates the TGF in response to increase in distal nephron perfusion rate (63, 122, 123). When these alterations are induced by converting enzyme inhibitors, the mechanism can be restored by the infusion of ANG II (146, 148). All together, these findings indicate that ANG II acts to enhance the sensitivity of the vascular element that mediates TGF, inducing alterations in single-nephron hemodynamic function (112, 114, 146, 147). Interestingly, the intensity of the response is modulated by the production of NO because the blockade of neuronal NO synthase produces an increase in tubuloglomerular response similar to that observed when angiotensin is infused (181). The physiological importance of this dualistic mechanism is that during volume repletion produced by excessive sodium intake there is a decrease of intrarenal synthesis of ANG II (181) with a relative enhancement of NO. This decreases the sensitivity of the TGF, which allows an increased natriuresis by permitting the passage of a bigger distal delivery of solutes through the distal nephrons. The opposite effect occurs during volume depletion where the increased synthesis of ANG II will increase the sensitivity of TGF, producing efferent arteriole vasoconstriction. This latter effect is important because it could explain the early renal vasoconstriction in essential hypertension that occurs when there is an excessive amount of ANG II.

A second important effect of ANG II is the interaction with NO in the regulation of glomerular resistance. Under normal conditions, the infusion of angiotensin into the kidney at low doses produces a more pronounced vasoconstriction in the efferent than in the afferent arteriole; however, after the blockade of NO with L-NAME, ANG II-induced vasoconstriction of the afferent arteriole is comparable to the vasoconstriction of the efferent. It is interpreted that the selective efferent vasoconstriction will protect GFR from excessive amounts of angiotensin produced within the kidney during volume depletion. These effects have been demonstrated by Ito et al. (72) in the isolated glomeruli with attached afferent and efferent arteriole and by Granger et al. (145) in the whole animal.

A third important effect of intrarenal angiotensin is the stimulation of proximal tubular sodium reabsorption. This action takes place at real minimum doses of ANG II that are not sufficient to alter MAP (60, 112) or aldosterone secretion. Under these conditions, ANG II

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Fig. 9. Schematic representation of the major renal functions (right) that are influenced by the equilibrium of ANG vs. nitric oxide (NO). BF, blood flow; GFR, glomerular filtration rate; interst, interstitial.
produces a marked sodium retention that leads to an increase in arterial pressure after a few days of initiating the infusion (112). This antinatriuretic effect can be largely explained by a direct tubular effect, which, in micropuncture studies, was shown to be exerted at doses of ANG II that range from $10^{-10}$ to $10^{-8}$ (58, 64, 121, 161). Such an effect, which has been discussed in other publications (135), appears important because it is quantitatively larger than those exerted by aldosterone (56).

The antinatriuretic effects of ANG II contrast with the natriuretic actions of NO, which appears to be exerted by stimulation of cGMP in distal nephrons, thus modifying amelioride NO-sensitive channels (71, 149, 162). Moreover, NO appears to exert a tonic vasodilation of the vasa recta in the renal medulla. Variations in NO synthesis will be followed by proportional changes in renomedullary blood flow and thereby in renal interstitial pressure and will produce an inversely related change in sodium reabsorption (132). We will examine this mechanism later on.

**ANG II AND NO EQUILIBRIUM IN THE CONTROL OF RENAL FUNCTION AND BLOOD PRESSURE**

The experiments discussed above suggest that a balance between the synthesis of NO and the formation of ANG II is necessary for the appropriate modulation of renal function and blood pressure (136, 138). This concept has been well demonstrated by studies of several investigators showing that most of the effect produced by NO synthesis inhibition are minimized or abolished by the simultaneous blockade of ANG II and, vice versa, that many of the consequences that result from the blockade of ANG II (vasodilatation, fall in blood pressure, natriuresis) are diminished by previous blockades of NO (100, 124, 153). This interaction between ANG II and NO indicates that the enhancement of the vasoconstrictor response of ANG II seen during the development of slow pressor responses could be produced by the activation of a process that impairs the activity of NO. A process that exhibits such characteristics is OXST, because it has the ability to quench NO and decrease its concentration in interstitial fluid. However, OXST also involves other pressor mechanisms, which are commented on below.

**THE MAJOR COMPONENTS OF OXST: STIMULATION BY ANG II**

Two of the major components of OXST are superoxide and hydrogen peroxide. Superoxide and hydrogen peroxide ($H_2O_2$) can be generated in many vascular cells and are derived from NADPH oxidase, cyclooxygenase, lipoxygenase, heme oxygenases, peroxidases, and hemoproteins, such as heme and hematin. The mechanism(s) by which ANG II causes production of superoxide has not been entirely elucidated. However, Mollnau and colleagues (104) found that chronic (7 days) ANG II infusion increased expression of nox 1, gp91(phox), and p22(phox) subunits of NADPH oxidase via a PKC mechanism (Fig. 10). This ANG II-mediated increase in the production of superoxide sets into motion a series of events that may play important roles in hypertension.

Under normal conditions, the superoxide that is produced in the cell combines with SOD that dismutates it to $H_2O_2$ (Fig. 10). $H_2O_2$ is further metabolized to $H_2O$ by the action of catalase and glutathione peroxidase, but under pathological conditions when an oxidative environment is present in cells, $H_2O_2$ can be a source of hydroxyl radical. Also under oxidative conditions, tetrahydrobiopterin ($BH_4$), a cofactor required for the NO synthase activity, can be oxidized to dihydrobiopterin ($BH_2$) (Fig. 11). In this case, NO synthase will produce superoxide rather than NO (163, 176, 187), making this another mechanism by which reactive oxygen spe-
cies (ROS) can be produced and NO concentrations can be reduced.

In 1990, Beckman et al. (14) reported that the product of the combination of superoxide and NO radicals is peroxynitrite, which is in equilibrium with peroxynitrous acid (Fig. 12A). Peroxynitrite/peroxynitrous acid are potent oxidants, and peroxynitrous acid can be a source of the biologically active hydroxyl radical as can H₂O₂ (14). Peroxynitrite has been shown to upregulate cyclooxygenase (COX)-mediated production of prostaglandin E₂ in macrophages from old mice (15). In addition to stimulation of COX activity, peroxynitrite can inhibit prostacyclin synthase activity as shown in an endothelial cell line (195), which could lead to a reduction in the vasodilator prostacyclin (PGI₂). In addition to the above-mentioned systems, ROS have also been shown in numerous studies to play a role in signal transduction in various cell types (32, 188). Therefore, in a situation where ROS, such as superoxide, H₂O₂, hydroxyl radical, are produced in concentrations that are not able to be controlled by the usual mechanisms employed by the cell, the increase in oxidation can produce a variety of negative effects on cellular function, including alteration of transcription factors, kinases, protein synthesis, and redox sensitive genes, which, in turn, could influence hypertrophy, migration, proliferation, endothelial dysfunction, and inflammation. In addition, the overproduction of ROS could set into motion mechanisms that could cause vasoconstriction and thereby influence blood pressure, as mediated by reduction in vasodilators NO and prostacyclin, or increases in vasoconstrictors, F₂-isoprostane, and endothelin (Fig. 12B). Most of the results of the studies discussed here suggest the importance of having appropriate methods to evaluate the magnitude of OXST to determine the correlation with hypertension and organ damage. This constitutes an extensive chapter that is beyond the scope of this review. Readers interested in this subject can consult Tarpey and Fridovich (168).

ROLE OF OXST IN ANG II HYPERTENSION

A number of studies have been conducted to elucidate the specific components of OXST that are involved in the development of ANG II-induced hypertension. Haas et al. (55), in pigs, and Reckelhoff et al. (130), in rats, were among the first to demonstrate that the slow hypertensive response to ANG II (10 ng·kg⁻¹·min⁻¹) was accompanied by a significant elevation of OXST as estimated indirectly by increases in plasma F₂-isoprostanes, an oxidative metabolite of arachidonic acid (106). Nishiyama et al. (115) also demonstrated that a prolonged infusion of ANG II in rats stimulates OXST. In this study, the administration of tempol, a SOD mimetic, reversed the vasoconstriction and produced vasodilation via an NO-dependent mechanism. Ortiz et al. (116) found in rats that the development of slow pressor responses to ANG II (10 ng·kg⁻¹·min⁻¹) could be inhibited by the administration by antioxidants such as tempol and vitamin E. As a result of antioxidant treatment, there was a fall in RBF and GFR, whereas the indexes of OXST, thiobarbituric acid reactive substances, and isoprostanes were found to be decreased in peripheral circulation as well as the renal vein.

Most of the studies commented on above indicate that the reduction in the concentration of NO (as a result of being scavenged by superoxide) constitutes a major component in the development of the observed vasoconstriction. Supporting the assumption that inhibition of NO synthesis enhances the vasoconstrictor effect of ANG II are the studies of Kitamoto et al. (77). These investigators found that the continuous administration of L-NAME to Sprague-Dawley rats for 7 days induced OXST, which was dependent on ANG II, because the effect was blocked by the administration of ANG II receptor blockers. Relevant to these findings are the studies of Usui et al. (172), who also found an increase in OXST produced by L-NAME, which was blocked by the administration of antioxidants. In this study, L-NAME blockade was associated with an increase in ACE activity in the aorta. The studies of Kitamoto et al. (77) and Usui et al. (172) reveal an interesting aspect of ANG II, NO, and OXST. They suggest that a simple decrease in NO synthesis leaves unbalanced ANG II, which induces OXST. This situation will be further stimulated by the increase in converting enzyme activity, which can accelerate the production of ANG II causing a positive feedback for OXST.

![Fig. 12. A: combination of NO and superoxide is peroxynitrite (ONOO), which is in equilibrium with peroxynitrous acid (ONOOH). Peroxynitrous acid is a source of hydroxyl radical (OH⁻). B: peroxynitrite, itself a vasodilator, produces vasoconstriction by converting arachidonic acid (aa) to F₂-isoprostanes and by increasing thromboxane A₂ (TxA₂). Peroxynitrite also causes vasoconstriction by inhibiting prostacyclin (PGI₂) synthase and thereby decreasing vasodilator prostacyclin (PGI₂).](http://ajpregu.physiology.org/Downloadedfrom)
The question of whether NO inhibition alone can cause OXST without the participation of ANG II should be further explored. As mentioned previously, NO can produce superoxide when BH4 is oxidized to BH3, leading to an increase in OXST and a reduction in NO (see Fig. 11). This could set up a vicious cycle that accentuates NO dysfunction and tends to perpetuate OXST (180, 187). These alterations may constitute an important mechanism of dysregulation that produces hypertension and renal dysfunction (see later). An example of this alteration is the SHR in which blood pressure can be normalized by the administration of BH4 (76, 99).

ASSOCIATION OF OXST TO ARACHIDONIC ACID METABOLITES

An additional area of interest about the hypertensigenic effects of OXST are the actions of ROS on the metabolism of arachidonic acid. As mentioned above, superoxide is likely to bind NO forming peroxynitrite, which is a component with the highest oxidative capacity known (14). This compound could oxidize arachidonic acid, releasing iso-PGF2α, a stereoisomer of PGF2α, simply called isoprostane (106). Isoprostane has been proposed as a sensitive marker of OXST (8, 127, 167). Isoprostanes can produce renal vasconstriction in a dose-dependent manner, reducing RBF and GFR (167) at low doses and producing hypertension at high doses, which are not likely to be seen even during pathologic situations. Hence it is more likely that the isoprostane-induced increase in systemic blood pressure is secondary to its renal effects (increase in renal vascular resistance and sodium retention). However, this assumption needs the support of more studies, particularly on the hemodynamic effects of isoprostanes on general circulation, which, at present, are missing. The role of isoprostane should be further explored considering that the binding of this compound to its receptor is nonspecifically inhibited by thromboxane (Tx) A2 receptor antagonists (107). Yamaguchi et al. (189) observed that Tx receptor antagonists block much of the pressor action of ANG II. Similar observations have been made by Keen et al. (75). It would be of interest to determine whether these effects are produced by the specific blockade of Tx from its receptor or by the nonspecific blockade of the Tx receptor to isoprostanes. This latter assumption implies an effect of ANG II on OXST that releases isoprostanes, which may be responsible for some of the constrictor effects of ANG II. In addition, peroxynitrite can stimulate PGI2 synthase (171) and produce the vasconstrictor Tx (9). At the same time, peroxynitrite inhibits prostacyclin synthase, inhibiting production of the vasodilator prostacyclin (34, 195). Another mechanism that may impact hypertension, depending on OXST, is the depletion of glutathione synthase, which results in decreases in GSH (177). An additional mechanism that may impact hypertension, depending on OXST, is the depletion of glutathione synthase, resulting in decreases in GSH (177). This alteration appears to be present during lead intoxication (40). The extent to which a reduction in antioxidant systems, such as GSH, SOD, and catalases, renders the organism more susceptible to the ANG II-induced OXST-mediated hypertension must be explored.

WHICH ALTERATIONS OF OXST COULD YIELD ANIMAL MODELS OF HYPERTENSION?

The evidence examined above invites one to consider if the characteristics of ANG II-induced hypertension are being shared by other models of hypertension. The inbred SHR, which is now considered a paradigm for OXST-induced hypertension (76), shares similar characteristics with those found in angiotensin-induced hypertension. In fact, in SHR, hypertension is corrected by the administration of renin inhibitors (183), converting enzyme inhibitor (7, 57), or ANG II receptor blockers (26, 51, 182). The plasma levels of ANG II in SHR are within the range exhibited by their normotensive Wistar-Kyoto counterparts, because PRAs are similar between these strains (26, 155). Because these animals do not exhibit an imbalance in water and electrolyte equilibrium, one can therefore argue that the existing normal levels of PRA (26, 155) are “inappropriate” to fluid volume, which is maintained within a normal range by a well-demonstrated shifting of pressure natriuresis (16, 54). This further suggests that the inappropriate amount of angiotensin is responsible for increasing OXST, because blood pressure in SHR is corrected by either short-term infusion of tempol (143) or when tempol is administered chronically in their drinking water (144). Under these conditions, the reduction of OXST and hypertension is accompanied by a comparable reduction of isoprostanes (144). Similar hypotensive effects have been obtained by the administration of vitamin C or E in this model (31).

MODULATION OF TGF BY OXST IN SPONTANEOUS HYPERTENSIVE RATS

In an attempt to pinpoint the specific defect involving OXST that could explain the pathogenesis of hypertension in SHR, Wilcox and Welch (181) looked at the regulation of TGF in this model of hypertension. This investigator found that in the SHR, a decrease in the synthesis of NO by neuronal NO synthase is responsible for increasing OXST, because blood pressure in SHR is corrected by either short-term infusion of tempol (143) or when tempol is administered chronically in their drinking water (144). Under these conditions, the reduction of OXST and hypertension is accompanied by a comparable reduction of isoprostanes (144). Similar hypotensive effects have been obtained by the administration of vitamin C or E in this model (31).
tensin AT1 receptor antagonists, but it remains the same when blood pressure is corrected by the administration of nonspecific therapy that consists of hydralazine, hydrochlorothiazide, and reserpine (179). It should be remembered here that the changes in pressure natriuresis in the SHR are corrected by the administration of ACE inhibitor or by angiotensin antagonists. All this evidence strongly suggests that in the SHR there is a subtle imbalance that favors the ANG II activity over NO that is manifested by an exaggerated TGF response that explains excessive afferent vasoconstriction and a simultaneous increase in tubular sodium reabsorption. Hence pressure natriuresis is shifted.

**ROLE OF OXST ON ENDOTHELIAL FUNCTION AND ON MEDULLARY CIRCULATION**

The consequences of altering the intrarenal equilibrium of ANG II and NO on RBF and afferent vs. efferent arteriolar tone were examined above. However, it should be mentioned that the intrarenal endothelial dysfunction appears to also influence renomedullary blood flow.

A group of investigators at the Medical College of Wisconsin has accumulated a significant amount of evidence showing that, in the rat, the integrity of the renal medullary circulation is very critical in maintaining pressure natriuresis (132). In fact, they found that increments in renal perfusion pressure above 78–85 mmHg produced proportional changes in renal papillary flow, whereas renal cortical flow does not change because it is effectively autoregulated (94). These characteristics are explained by the regional differences in the production or actions of NO and ANG II (93). Wu et al. (185) reported that NO synthase activity is 26 times the production or actions of NO and ANG II (93). Wu et al. (185) reported that NO synthase activity is 26 times higher in the inner medulla than in the renal cortex. Similarly, using a microdialysis tubing technique, Zou and Cowley (193) showed that medullary interstitial NO is much greater than in the cortex. This observation agrees with the study showing that the vasa recta in the renal medulla accounts for most of the NO synthesis in the kidney (95). The production of NO in the renal medulla is three times higher than in the afferent arterioles or the intralobular arteries (95).

The synthesis of NO in the renal medulla has also been suggested to be important to protect against increments in vasoconstrictor substances such as ANG II (166, 194), norepinephrine (192), and vasopressin (119). However, the ability of ANG II to increase the medullary NO was found to be significantly blunted in the Dahl salt-sensitive rat compared with the Brown Norway (166). Similarly, in DS, hypertension was induced with the infusion of 3 ng·kg−1·min−1 of angiotensin, which is a dose that failed to produce changes in blood pressure in the Brown Norway rat (166). Caution has to be exercised in attempting to extrapolate these observations from the rat to other mammals, because similar attempts in the dog have shown that medullary blood flow may be autoregulated as effectively as in the renal cortex (89). However, studies on the RBF distribution in a swine model performed with the highly sophisticated technique of computerized tomography have indicated that these animals’ papillary flow is not autoregulated due to different distribution of inner medullary RBF as in the rat (86).

These findings suggest two interesting perspectives on all the factors that participate in the pathophysiology of the spontaneous or salt-induced hypertension or angiotensin-induced hypertension. The first is that hypertension can be induced by a single defect, such as that produced by an inappropriate amount of angiotensin, alterations in BH4, deficient synthesis of NO, or deficiency in other antioxidants such as SOD, glutathione, catalases, etc., or enhanced vasoconstriction produced by some of the oxidant products such as isoprostane, etc. In this case, the normalization of the specific alteration will be the only way to correct hypertension. The second possibility is that, although a single alteration may constitute the initial event, it could promptly involve others that are directly or indirectly related to the same chain of events. For example, it is possible that the single defect consists of a deficient synthesis of BH4; however, this will produce less NO, which will not compensate for the vasoconstrictor effect of normal levels of ANG II and will then feedforward the stimulation of all the other interconnected events of OXST such as the enhancement of converting enzyme with more synthesis of angiotensin (see above). The experimental data favor this second possibility because in the SHR, DS, and angiotensin-induced hypertensive rat, hypertension can always be normalized by blocking the vasoconstrictor effect of angiotensin or OXST. Furthermore, most of the evidence also indicates that once concentrations of ANG II are rendered inappropriate, a sequence of events is triggered in a successive order, which fosters vasoconstriction and hypertension. That is why the specific blockade of OXST (with antioxidants) also normalize blood pressure. Most, if not all, models of hypertension, including human essential hypertension, are directly linked to the kidney. Thus the manner in which OXST produces renal dysfunction that is linked to hypertension is important (138).

**ROLE OF ENDOTHELIN IN ANG II HYPERTENSION**

In a study to determine the role of endothelin in slow pressor responses to ANG II, Ortiz and colleagues (116) observed first a significant increase in renal vein concentration of endothelin. This prompted a follow-up study (117) where they determined that the administration of a nonspecific endothelin receptor antagonist, bosentan, was capable of reducing blood pressure without altering OXST because the levels of isoprostane remained unchanged from ANG II-infused controls. These data suggest that that endothelin is distal to OXST and that endothelin may actually be stimulated by some component of OXST. Supporting this notion are the studies showing that F2-isoprostane, which is produced by OXST, can stimulate endothelin.
synthesis in endothelial cells (139). In other studies, Ballew and Fink (12) demonstrated that in high-salt diet-fed rats rendered hypertensive by the continuous infusion of 5 ng/min for 15 days, the administration of a specific ETA receptor antagonist (ABT-627) reduced MAP to normal levels without altering the blood pressure of normotensive controls fed a high-salt diet but not infused with angiotensin (12). In a subsequent study conducted by the same investigators (11), it was observed under similar experimental circumstances that the specific blockade of ETB receptors produced a further 18% increase in MAP over the hypertensive levels already induced by ANG II infusion. However, these hypertensive effects were not affected by sodium intake. Highly relevant to the results of Ortiz et al. (116, 117) and Ballew and Fink (11, 12) are the observations of Pollock and Pollock (125), who determined the effects of blocking selectively ETA or ETB receptors in rats submitted to high- and low-sodium diet without receiving ANG II. They observed that high sodium did increase the basal levels of MAP by 12 mmHg. The specific blockade of ETB receptors was followed in the high-salt diet-fed rats by a rapid (few hours later) increase in MAP of 55 mmHg (from 115 ± 2 to 170 ± 3 mmHg). In contrast, the elevation of MAP in low-sodium diet-fed animals treated with ETB receptor blockers was not significant. Furthermore, it was also shown that the hypertensive effect in high-salt diet-fed animals produced by the blockade of ETB receptors was due to the indirect activation of ETA receptors, because its blockade brought blood pressure down to normal levels (113 ± 3 mmHg). Collectively, all these results indicate that during high sodium load the activation of ETB receptors could play an important protective role against increments in blood pressure comparable to that obtained by the decrease in the synthesis in ANG II. The protective role of ETB receptors may be mainly manifested by the ability of this substance to maintain natriuresis and renal circulation (125). Hence endothelin may be compensating for the antinatriuretic effect of ANG II. In fact, Alexander et al. (3) showed that the intrarenal infusion of ANG II enhanced the renal expression of preproendothelin mRNA. However, more studies should be done to have a better understanding of the interaction between angiotensin and endothelin, particularly with respect to OXST. For example, the participation of endothelin in angiotensin-induced hypertension through OXST is supported by demonstrations such as the normalization of blood pressure obtained with either tempol or with nonspecific blockers of endothelin receptors (116, 117). Angiotensin could stimulate endothelin directly (3) or indirectly by the increased production of isoprostane (47, 139). Conversely, endothelin could directly stimulate superoxide production by activation of NADPH (43). All of these fragmentary observations make it difficult to offer a coherent explanation on the relationship that ANG II has with endothelin and OXST including salt metabolism. A recent review of Elijovich and Laffer (45) shows that most of the data in the literature strongly support the participation of endothelin in salt-sensitive hypertension. Furthermore, urinary endothelin excretion has also been shown to be increased by high sodium intake (141). In this manner, the vasoconstrictor effect of endothelin induced by high sodium along with OXST can exacerbate and potentiate this slow pressor response to ANG II.

It should be mentioned here that there are several studies demonstrating that deoxycorticosterone acetate and salt intake produce a significant elevation of OXST (19, 159, 186). However, these effects are not related to ANG II because they were not changed by the administration of ANG II receptor antagonists (186).

RENOVASCULAR HYPERTENSION

For a long time it has been known that the constriction of one renal artery is followed by two distinctive phases. In the first phase, there is a very rapid increase of renal renin (131) and PRA (20, 24, 137, 165), which generally correlates with the elevation of systemic blood pressure (Fig. 13) that lasts from 7 to 10 days (20, 91, 137). This first phase is also characterized by an increase in plasma aldosterone and certain volume retention, which may not be reflected in extracellular

Fig. 13. Correlations of PRA (ordinate) with increments in MAP in pigs with unilateral renal arterial stenosis at 1 (A) and 2 (B) mo after the arterial constriction. (Modified from Ref. 87.)
fluid volume expansion in a significant manner (20, 24, 165). After this phase, PRA comes down to near normal levels mainly due to the improvement of renal perfusion in the stenotic kidney because of hypertension. Consequently, aldosterone levels follow the same trend toward normalization (29, 165). At this point in time, hypertension is almost entirely due to an increase in total peripheral resistance (20, 29, 84, 137, 165) and coexists with normal levels of cardiac output, circulating renin, angiotensin, and aldosterone (20, 24, 29, 84, 91). This pattern resembles all the characteristics of hypertension induced by slow responses to ANG II, and is also comparable to spontaneous hypertension in genetically inbred rats such as the salt-sensitive hypertension in Dahl rats. In the past, there was a notion that these patients exhibited a blunted forearm blood flow response to the vasodilator effects of acetylcholine and also had an increased urinary excretion of 8-hydroxy-2’-deoxyguanosine and serum concentration of 8-methoxy-2-deoxyguanosine-modified LDL, which are well known markers of OXST. All these alterations were corrected after angioplasty. Interestingly, the response of forearm blood flow to acetylcholine was also significantly enhanced by the administration of an antioxidant, such as vitamin C. Krier et al. (79) found that the administration of isoprostane at the rate of 1 \( \mu \text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} \) induced a significant decrease in RBF, which affected mainly cortical perfusion and GFR. These effects were comparable to the effects produced by the intrarenal infusion of U46619, a thromboxane agonist, at the rate of 10 \( \text{ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} \). However, isoprostane was capable of reducing medullary blood flow only when administered to pigs with hypercholesterolemia and also induced a very significant increase in MAP. These effects were not observed in normal pigs. In this study, changes in the distribution of renal circulation were determined with an electron beam computed tomographer. The results were interpreted as indicating that in hypercholesterolemic pigs the renal functional responses to isoprostanes are largely similar to normal, but the systemic circulation exhibits an augmented sensitivity to pathophysiological levels of isoprostane. These findings suggest that isoprostanes may potentially play a role in the development of hypertension and vascular injury associated with dyslipidemias and OXST. Much of the participation of ANG II-stimulated OXST in the development of hypertension exists also in the Goldblatt model of unilateral renal stenosis after the removal of the contralateral kidney [1-clip, 1-kidney Goldblatt hypertension (1K,1C-GH)]. Dobrian et al. (41) showed that in this model, blood pressure was reduced by the administration of either an angiotensin receptor antagonist (losartan) or a superoxide mimetic (tempol). However, the

![Fig. 14. Correlations of plasma levels of isoprostanes with increments in MAP in pigs with unilateral renal arterial stenosis at 1 (A) and 2 (B) mo after the arterial constriction (Modified from Ref. 87.).](image-url)
effects of these drugs were additive, when administered together. Curiously in 1K,1C-GH, plasma isoprostane was not increased, despite the clear increase of oxygen production in the aortic rings. More studies should be performed to investigate if the characteristics of OXST in 1K,1C-GH differs from other forms of renovascular hypertension.

EFFECTS OF ANG II, OXST, AND NO ON THE REGULATION OF SYMPATHETIC OUTPUT AND BLOOD PRESSURE

It must be mentioned that the central nervous system was implicated in the slow pressor responses to angiotensin at a very early stage (1963). In fact, Dickinson and Lawrence (38) suggested that angiotensin-induced vasoconstriction of cerebral vascular vessels stimulate the intrinsic activity of the vasomotor centers in the hindbrain. Supporting this assumption was the demonstration that the slow responses were blunted by the administration of short-acting adrenergic blocking agent such as bethanidine. Furthermore, Dickinson and Yu (39) also show that the constant infusion of small doses of ANG II, being delivered alternatively into the vertebral arteries for 30 min or into the superior vena cava for the subsequent 30 min, produced higher levels of blood pressure when infused into the vertebral arteries. These results were interpreted as indicating that an effect of ANG II on the central nervous system was mainly responsible for the progressive pressor response. However, the role of the central nervous system in mediating the pressor effects of ANG II has been also discredited by a number of observations. For example, the slow pressor response to ANG II is not induced by norepinephrine or serotonin (1). In addition, the potentiation of vasoconstrictor responses to ANG II, which constitutes the important element underlying the slow responses, was also seen in isolated (pump perfused) mesenteric vascular bed (1). It will be impossible here to review the vast amount of data on the controversial aspects of the participation of the central nervous system in mediating the pressor effects of ANG II: the demonstration that the slow responses were independent of cyclooxygenase: use as clinical indicators of oxygen-derived reactive oxygen species convert flow-induced arteriolar dilation to constriction in hyperhomocysteinemia: possible role of peroxynitrite. Arterioscler Thromb Vasc Biol 22: 28–33, 2002.

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