Programming blood pressure in adult SHR by shifting perinatal balance of NO and reactive oxygen species toward NO: the inverted Barker phenomenon

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Racasan, Simona, Branko Braam, Hein A. Koomans, and Jaap A. Joles. Programming blood pressure in adult SHR by shifting perinatal balance of NO and reactive oxygen species toward NO: the inverted Barker phenomenon. Am J Physiol Renal Physiol 288: F626–F636, 2005. First published November 16, 2004; doi:10.1152/ajprenal.00314.2004.—The “programming hypothesis” proposes that an adverse perinatal milieu leads to adaptation that translates into cardiovascular disease in adulthood. The balance between nitric oxide (NO) and reactive oxygen species (ROS) is disturbed in cardiovascular diseases, including hypertension. Conceivably, this balance is also disturbed in pregnancy, altering the fetal environment; however, effects of perinatal manipulation of NO and ROS on adult blood pressure (BP) are unknown. In spontaneously hypertensive rats (SHR), NO availability is decreased and ROS are increased compared with normotensive Wistar-Kyoto rats, and, despite the genetic predisposition, the perinatal environment can modulate adult BP. Our hypothesis is that a disturbed NO-ROS balance in the SHR dam persistently affects BP in her offspring. Dietary supplements, which support NO formation and scavenge ROS, administered during pregnancy and lactation resulted in persistently lower BP for up to 48 wk in SHR offspring. The NO donor molsidomine and the superoxide dismutase mimic tempol-induced comparable effects. Specific inhibition of inducible nitric oxide synthase (NOS) reduces BP in adult SHR, suggesting that inducible NOS is predominantly a source of ROS in SHR. Indeed, inducible NOS inhibition in SHR dams persistently reduced BP in adult offspring. Persistent reductions in BP were accompanied by prevention of proteinuria in aged SHR. We propose that in SHR the known increase in ANG II type I receptor density during development leads to superoxide production, which enhances inducible NOS activity. The relative shortage of substrate and cofactors leads to uncoupling of inducible NOS, resulting in superoxide production, activating transcription factors that subsequently again increase inducible NOS expression. This vicious circle probably is perpetuated into adult life.

spontaneously hypertensive rat; nitric oxide; inducible nitric oxide synthase; molsidomine; tempol; proteinuria

There is now a large body of epidemiological data proving that environmental factors acting early in life, in particular in the intrauterine and postnatal period, correlate with chronic cardiovascular diseases. From these observations, Barker (10) developed the “programming hypothesis,” which proposes that fetal adaptation to adverse environmental factors in the perinatal period results in permanent structural and physiological changes that manifest as disease in adult life. As such, fetal programming is a form of phenotypic plasticity (10), which has conveyed an evolutionary advantage in the past but now has adverse effects because of relatively rapid changes in the environment in which we live (58). An example of this may be the explosion of hypertension in the African population (49). Experimental models that mimic these epidemiological findings have been successfully developed. Nevertheless, mechanisms responsible for this phenomenon are not fully understood.

Cardiovascular disease has been associated with a disturbed balance between nitric oxide (NO) and reactive oxygen species (ROS). Increased superoxide production and decreased NO bioavailability are present in human and experimental models of hypertension. Since the maternal disturbance is also present during the fetal and neonatal periods, this could afflict fetal development. Indeed, epidemiological and experimental data indicate a role for a disturbance in the NO-ROS balance in hypertension programming. Spontaneously hypertensive rats (SHR) are a widely used model for human essential hypertension. There is ample evidence that, in SHR, NO bioavailability is decreased and ROS is increased (80). Despite the genetic predisposition, the perinatal environment seems to play a role in the development of high blood pressure (BP) in SHR. Therefore, SHR can be used to explore the hypothesis that a maternal shift of the NO-ROS balance toward ROS contributes to the development of hypertension in the offspring.

In this paper, we propose that a perinatal shift in the NO-ROS balance permanently affects BP control. When this shift is toward more ROS and less NO, adult BP is increased. This could be the case in nutritional models associated with growth retardation and in genetically determined models such as SHR. In SHR, the shift in the NO-ROS balance seems to be critically linked to inducible NO synthase (iNOS); uncoupling of iNOS with superoxide production as a consequence could invoke a positive feedback loop, leading to a lifelong alteration in the redox state, with continuous changes in the redox-sensitive signaling to and transcription of genes involved in BP control. By breaking this feedback chain, one could permanently shift the NO-ROS balance toward NO and reduce BP (and potentially also prevent cardiovascular and renal injury): the inverted Barker phenomenon.

The BARKER HYPOTHESIS

Epidemiological studies have provided evidence that an inverse relationship exists between birth weight and cardiovascular diseases manifest only in adulthood, such as hypertension...
(11, 87), coronary heart disease (13), stroke (100), or type 2 diabetes (96). The initial proposal that chronic adult diseases are partly determined by events occurring in fetal and early postnatal life, put forward by Barker et al. (12), has since been confirmed by many studies (51, 63, 90, 136). Conversely, dietary manipulations in the perinatal period have beneficial effects on intrauterine growth restriction (IUGR) in humans. Caloric supplementation of pregnant women, as well as folate, vitamin B12, and iron supplements, reduced the incidence of low birth weight (22, 28, 30), whereas a diet rich in vegetables and fruits (important sources of folate, carotenoids, and antioxidants) was strongly associated with increased birth weight in rural India (122). Risk factors for cardiovascular disease, such as smoking, diet, a sedentary lifestyle, social status, and alcohol consumption, do not affect the relationship between low birth weight and cardiovascular disease (44, 63, 79, 123). The most frequently studied association is between low birth weight (or IUGR) and hypertension (68, 88). Importantly, IUGR refers to babies small for gestational age, rather than premature babies with extreme growth retardation. The early postnatal period is also important, since rapid postnatal catch-up growth (50, 89) increases the risk for high BP.

Traditionally, hypertension is thought to result from the interaction between genetic endowment and the adult environment. The programming hypothesis presumes that such interaction is also present in prenatal life and the concept of phenotypic plasticity, morphological or physiological variations of one genotype in response to different developmental conditions, adopted from other areas, seems applicable (10). This notion leads to a conceptual shift of therapeutic option from adult life to earlier stages of development, before hypertension (or overt cardiovascular disease) is manifest.

**EXPERIMENTAL EVIDENCE OF PROGRAMMING**

Experimental models developed to elucidate mechanisms that determine the programming phenomenon have found that limiting the perinatal supply of nutrition or of oxygen induces hypertension in the offspring later in life. Undernutrition in pregnancy results in IUGR and high BP in adult life in sheep (65), rats (86), and guinea pigs (40) and a reduced life span in rats (4, 149). Fetal hypoxia, induced by unilateral uterine artery ligation in guinea pigs (115), aortic ligation in rats (5), uterine artery ligation in rats (70), removal of endometrial caruncles in sheep (125), maternal hypobaric hypoxia in rats (151), and iron deficiency anemia in rats (32, 95) are also associated with IUGR and hypertension later in life. Together, these observations point at metabolism and oxygen supply as central factors. It has now become clear that maternal undernutrition or protein deprivation reduces vascular NO release, causing dysfunction both in the systemic circulation (17) and in the uterine arteries (148); hence the diverse models mentioned above seem to converge mechanistically. Recently, it was also documented in rats that induction of the metabolic syndrome during pregnancy by a supplemental feeding of lard induced hypertension and impaired endothelium-dependent relaxation in resistance arteries in the adult offspring (77).

**CANDIDATE GENES FOR HYPERTENSION IDENTIFIED IN SHR**

Classically, genetic hypertension implies that abnormal gene expression in a normal environment in early life results in hypertension. A number of specific candidate genes are implicated in the pathogenesis of hypertension in SHR. These include components of the renin-angiotensin system such as angiotensinogen (97, 135), renin (139, 162) (although transfer of the renin gene from the normotensive Brown Norway strain to SHR did not decrease BP) (134), and angiotensin-converting enzyme (ACE) (108, 169). Other hormonal systems such as kallikrein (117), neuropeptide Y (74), and atrial natriuretic factor (170) have also been identified. Finally, the Saa gene, expressed in renal proximal tubules (114, 129), cosegregates with high BP in SHR. However, there must be more genes involved, based on the number of quantitative trait loci (QTL) found to associate with BP regulation in this strain (37). Our idea is that the direct early result of this abnormal gene expression is an alteration of the perinatal environment and that this alteration in the perinatal environment negatively influences fetal development. This idea opens the possibility of normalization of the genetically hypertensive state by manipulating (i.e., correcting) the early environment.

**NONGENETIC FACTORS AFFECTING FETAL PROGRAMMING IN SHR**

Embryo transplantation from SHR to Wistar-Kyoto (WKY) rats has generally not affected adult BP in the offspring. However, indirect evidence for the hypothesis that nongenetic, perinatal factors affect fetal programming of BP in SHR can be found in experiments that modulate the postembryonic environment (171). Cross-fostering of SHR pups to normotensive mothers decreases BP (9, 29), but only if the cross-fostering takes place in the first 2 wk after birth (59, 60, 102). The immediate postnatal environment is characterized by differences in milk composition (higher sodium and lower calcium and protein content) (103) or lower milk intake (59) in SHR than WKY rats, modification of tubular responsiveness to ANG II (60), or by a modified behavioral pattern with increased frequency of milk delivery that elicits a higher heart rate and BP in the SHR pups (106). Renal 3A cytochrome P-450 (CYP3A) is upregulated in SHR, and inhibition of this rate and BP in the SHR pups (106). Renal 3A cytochrome P-450 (CYP3A) is upregulated in SHR, and inhibition of this metabolism, and oxygen supply as central factors. It has now become clear that maternal undernutrition or protein deficiency reduces vascular NO release, causing dysfunction both in the systemic circulation (17) and in the uterine arteries (148); hence the diverse models mentioned above seem to converge mechanistically. Recently, it was also documented in rats that induction of the metabolic syndrome during pregnancy by a supplemental feeding of lard induced hypertension and impaired endothelium-dependent relaxation in resistance arteries in the adult offspring (77).

**INAPPROPRIATE ACTIVATION OF THE RENIN-ANGIOTENSIN SYSTEM AND NO-ROS IMBALANCE IN CARDIOVASCULAR DISEASE**

A large body of evidence is now available for the pivotal role of ANG II in the development of cardiovascular disease. Inappropriate stimulation of ANG II, as present in several states predisposing a subject to atherosclerosis, such as hypertension (153), diabetes mellitus (36), heart failure (116), and
chronic renal failure (18), promotes the process of vascular wall damage through several mechanisms. ANG II can stimulate adhesion of monocytes (6), stimulate platelet adhesion (72), increase vascular wall oxidative stress via stimulation of NADPH-oxidase (62), stimulate vascular smooth muscle cell proliferation (33), and promote collagen formation (73). All these events promote plaque formation and overt atherosclerosis (137). Not surprisingly, the beneficial action of ACE inhibitors and AT1-receptor antagonists on target organ damage in the kidney, heart, macro- and microvascular circulation, as well as the brain, has been shown in a variety of clinical settings (43, 165).

Besides this inappropriate ANG II activity, cardiovascular risk factors are associated with an imbalance between NO availability and ROS production, resulting in endothelial dysfunction (20). Normal vascular structure and function are dependent on adequate production of NO. A decrease in NO bioavailability (52, 142) and an increase in oxidative stress (164) are present in human essential hypertension. Endothelial dysfunction has been demonstrated in essential (113) and renovascular (105) hypertension. Although the role of ROS is not completely elucidated in human hypertension, there are strong indications that ROS production, presumably driven by ANG II, contributes to hypertension (164). However, hypertension, in turn, may also induce ROS. This was clearly observed in aortic coarctation, where nitrotyrosine was increased in the aorta above, but not below, the suture (14). The NO-ROS imbalance does not seem to be specific for hypertension, since a shift toward ROS has also been described in other conditions predisposing a subject to cardiovascular disease. Diabetes mellitus (35, 143), hypercholesterolemia (39, 111, 152), a high-fat, refined carbohydrate (Westernized society) diet (124), and smoking (19, 91) are all associated with this imbalance, which is presumably a common intermediate between a variety of primary defects and endothelial dysfunction. From the perspective of fetal programming, these phenotypical (and not necessarily genetically determined) alterations that are also present in the perinatal situation will affect fetal development and create a nongenomic option of transmission of disease expression and severity to the next generation. Nevertheless, few attempts have been undertaken to correct the maternal NO-ROS balance. Supplementing a low-protein diet during pregnancy with L-arginine prevented hypertension in the offspring (69) by correcting vascular dysfunction in the mothers (17). Hypertension induced by a 50% reduction of food intake during pregnancy was completely corrected by oral supplementation of L-arginine (7). In a recent study from our laboratory, adult BP was permanently reduced in SHR and the aged-related increase in WKY was prevented by perinatal supplementation with L-arginine and antioxidants (120).

**INAPPROPRIATE ACTIVATION OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM AND NO-ROS IMBALANCE IN FETAL PROGRAMMING AND POTENTIAL CENTRAL EFFECTS**

The renin-angiotensin system has been implicated in the pathophysiology of IUGR-induced hypertension. Plasma renin activity is elevated at birth in growth-retarded humans and inversely correlates to birth weight in adults (78, 101). The female offspring of rabbits with secondary renin-dependent hypertension (2-kidney, 1-wrapped model) had increased BP (34). A low-protein diet in pregnant rats produces elevated BP in the offspring, associated with increased pulmonary and plasma ACE activity (85), and administration of an ACE inhibitor from 2–4 wk of age prevents the increase in BP in this model of IUGR (132, 133) up to 12 wk of age; however, long-term effects are unknown. Cerebral endothelial dysfunction persisted in hypertensive adult rats after maternal protein deprivation, despite normalization of BP by ACE inhibition for 1 wk before the measurements were made (82). This suggests that ANG II-mediated superoxide formation (62) is not the only factor leading to a disturbed NO-ROS balance in the fetal programming of hypertension.

Changes in the renin-angiotensin system induced by IUGR may be partly driven by increased maternal glucocorticoid activity (83). Postnatal glucocorticoid administration has been found to lead to hypertension in children at 14 yr of age (38). Mineralocorticoid activity has also been implicated in fetal programming. Indeed, some of the reported glucocorticoid effects may in fact be mineralocorticoid effects because of the inhibition of placental 11β-hydroxysteroid dehydrogenase type 2, the fetoplacental enzymatic barrier to maternal glucocorticoids (131). In fact, the offspring of rats exposed to carbon monoxide, an inhibitor of 11β-hydroxysteroid dehydrogenase, were hypertensive (84).

Evidence to support a disruption of the NO-ROS balance as a cause of altered programming is also available. IUGR induced by a 50% reduction of food intake during pregnancy decreased aortic and mesenteric endothelial relaxation in the adult offspring (54, 55), which was corrected by application of superoxide dismutase (SOD) and SOD mimetics ex vivo, indicating an important role for superoxide. This was confirmed in a follow-up study using intravital microscopy to visualize ROS generation in the mesenteric arterial wall, in which the source of ROS after intrauterine malnutrition was found to be NADPH oxidase, the activity of which was ANG II dependent (53). A recent study from this group demonstrated disruption of the NO-ROS balance due to uncoupling of NOS in this model, because superfusion of the mesenteric artery with tetrahydrobiopterin reduced ROS and increased NO production (56).

The NO-ROS balance also modulates sympathetic activity. Infusion of an NO donor or NOS blocker into the rostral ventrolateral medulla (RVLM), respectively, inhibits and stimulates sympathetic output (166). However, this issue is controversial. Downregulation of neuronal NOS (nNOS) accompanies sympathetic activity induced by intracerebroventricular administration of ANG II (21). Both nNOS and iNOS are expressed in the RVLM, and local inhibition of iNOS increased BP and sympathetic activity, whereas selective inhibition of nNOS in the RVLM reduced BP and sympathetic activity, suggesting a balance between iNOS and nNOS in the RVLM (26). Whether the increased sympathetic activity associated with UGR (70) is driven by an increase in cerebral activity of iNOS or a decrease in cerebral nNOS activity has not been studied.

**INAPPROPRIATE ACTIVATION OF THE RENIN-ANGIOTENSIN SYSTEM AND NO-ROS IMBALANCE IN SHR**

During normal renal development, the abundance of the different ANG receptor subtypes shifts; there is an increase in
the AT₁ receptor, whereas the AT₂ receptor tends to decrease (155). In SHR this shift appears to be accelerated, resulting in overexpression of the AT₁ receptor and deficiency of the AT₂ receptor at an early age. In 1-day-old SHR, the ratio of AT₁ to AT₂ receptor binding sites in the kidney is higher than in WKY rats (159), and this relative deficiency of AT₂ receptors persists at 7 days (159) and 4–5 wk (27, 42). In contrast, in conscious adult (19 wk old) SHR, the AT₂-receptor antagonist PD-123319 increases renal vascular resistance more than in WKY rats (93). Collectively, these findings suggest a relative deficiency of the renal AT₂ receptor in SHR during perinatal life. This could be crucial for development of the full-blown hypertensive phenotype. In cross-fostering studies, SHR pups reared by a normotensive WKY mother, besides having lower BP, had lower AT₁ receptor expression, and the increased renal sensitivity of SHR to ANG II was reversed (60, 61). ANG II strongly stimulates NAD(P)H oxidase, one of the important sources of superoxide in SHR (62, 163). Indeed, in young SHR renal expression of NADPH oxidase subunits is already increased from 4 wk of age (23), and treatment of young SHR with ACE inhibitors and AT₁ antagonists from 4 to 10 wk of age consistently blunts the development of hypertension (171).

Interventions in the prenatal or early postnatal period aimed at decreasing BP have been attempted in SHR; captopril administered to dams and pups reduced BP and extrarenal vascular hypertrophy up to 6 mo of age (158). In a similar study, we observed that perinatal captopril and losartan, despite the initial BP-lowering effect, caused gross malformations in intrarenal arteries at an early age, which eventually resulted in malignant hypertension and death more than 6 mo after cessation of treatment (121). Inhibition of the renin-angiotensin system in young SHR also induced a defect in the capacity to concentrate urine, confirming previous observations in normotensive rats (144) and piglets (64). However, this defect was much milder than the lack of concentrating ability observed in ACE knockout mice (47), and treated SHR also did not show any obvious abnormality of the renal papilla, as is common in knockout mice (121) and in human neonates exposed to ACE inhibitors (141).

A disturbed balance between NO and superoxide, as in other preatherosclerotic conditions, is characteristic of SHR. The L-arginine/NOS pathway is upregulated in SHR, with higher vascular endothelial NOS (eNOS) and renal iNOS protein mass than in WKY rats and increased nitrate plus nitrite (NOₓ) excretion directly after weaning at 3–4 wk, the so-called prehypertensive stage (146). In SHR, renal cortical and macula densa nNOS levels are increased (154), and NO production by vascular smooth muscle iNOS is enhanced (160). In the brain in SHR, the NOS system is programmed differently than in other tissues. In the cerebral cortex and brain stem, Ca-dependent NOS activity was decreased at 3–4 wk, although by 13–14 wk this was increased in the hypothalamus and brain stem compared with WKY rats (118). However, at 8–10 wk functional expression and synthesis of iNOS (which is Ca independent) were reduced in the brain stem (RLVM) in SHR (25), which was associated with sympathetic vasomotor hyperactivity (24).

Despite the upregulation of eNOS and basal NO release (99), this does not provide adequate compensation for increased ROS, because eNOS gene delivery in SHR results in BP reduction (94) and adult SHR are more sensitive to the increase in BP and kidney damage produced by chronic NOS inhibition (150). NO bioavailability seems to be determined by increased production of superoxide (1) from vascular NAD(P)H oxidase (163) and xanthine oxidase (81, 140) as well as from NOS itself. NOS can become a source of superoxide rather than NO when the substrate, L-arginine, or the cofactor, tetrahydrobiopterin (BH₄), are deficient (75). Endothelial NOS uncoupling has been demonstrated in SHR both at a prehypertensive age (31) and in adult SHR with established hypertension (76). However, all isoforms can become uncoupled, and iNOS seems to be particularly sensitive (112).

Information about antioxidant systems in SHR is limited. In the aorta, CuZn and Mn SOD protein levels and activity are increased at 28 wk of age (145). Extracellular CuZn SOD is decreased in SHR kidney at 11 wk of age (2), but CuZn SOD (extra- plus intracellular), catalase, and glutathione peroxidase activities were all normal in SHR renal cortex and medulla at 24 wk of age (167). Administration of modified human SOD or the SOD mimic tempol always decreases BP in SHR (107), as does supplementation from prenatal life up to 24 wk of age with an antioxidant-rich diet (vitamins C and E, zinc, and selenium) (167). Our findings in SHR that interventions aimed at shifting the NO-ROS balance in the perinatal situation toward NO result in a permanently lower BP in the offspring of SHR (120) support the concept that the balance between NO and ROS is already disturbed in the programming phase.

PLACENTAL DYSFUNCTION AND INTRAUTERINE ENVIRONMENT IN SHR

Three potential aberrations in the fetal environment can be discerned in SHR: 1) alterations in uteroplacental blood flow, 2) changes in uteroplacental (amino acid and mineral) transport and amniotic fluid volume and composition, and 3) alterations in placental and fetal redox state. A decrease in uteroplacental flow in SHR was inversely related to BP in the offspring (3). Note that this is in line with the experimental models of the fetal programming of hypertension as mentioned before. Amniotic fluid volume is lower in SHR at fetal day 15 and then higher at fetal day 20 with lower potassium concentration and significantly higher osmolality and sodium concentrations (45, 46). The role of such changes in amniotic electrolyte concentrations is unclear. Recently, it has been shown in preeclampsia that the umbilical blood and villous tissue contain less L-arginine, due to increased expression of the enzyme arginase II, which degrades arginine (110); as a consequence, uncoupling of eNOS occurs. This might explain, at least in part, the increased peroxynitrite formation in the placenta and vasculature in preeclampsia (127). In this respect, it is of interest that in SHR a deficit in placental amino acid transport exists (92) and arginine levels are reduced at an early age (71); hence a similar uncoupling of NOS as described in preeclampsia may occur. The amino acid supplementation we have employed in a recent study in SHR (see below) might have compensated for such a deficit. Despite the above-mentioned evidence of enhanced ROS formation in the young and adult SHR, no detailed studies have been undertaken to elucidate whether the prooxidant status affects the placenta (flow and growth) and fetal development (by affecting redox-sensitive transcription).
PERINATAL MANIPULATION OF NO AND ROS IN SHR

From the available data, it seems reasonable to propose that the disturbed ROS-NO balance in the pregnant dam is transmitted to and affects the development of the fetus. We do not argue against genetic background as an important factor for the development of hypertension in SHR. However, it is conceivable that besides these genetic factors, perinatal NO-ROS balance leaves a permanent footprint on the structure or function of the developing kidney or cardiovascular system of the fetus. Various antioxidants reduce BP in adult SHR with established hypertension (107, 109, 130, 147); some groups have started antioxidant treatment in the prenatal phase (126, 168) and continued treatment for the entire study period. Remarkably, before our recent studies the effect of the NO-ROS balance on fetal BP programming in SHR was not addressed. We have now studied the effects of different interventions aimed at modifying the oxidative status applied for a limited period of time in pregnant and lactating SHR on long-term BP and kidney damage in the offspring. The various interventions applied were intended to modify different links in the oxidative chain (Fig. 1).

Initially, we addressed whether a nonspecific dietary supplement that supported NO formation and scavenged ROS affected programming of BP in SHR, by use of a combination of L-arginine plus vitamins C and E and taurine. The treatment period we used encompasses nephrogenesis in the rat, in which glomeruli are formed until day 7 or day 10 and tubules continue to develop until 28 days in the outer medulla (104), and covered the last 2 wk of gestation until weaning at 4 wk or until 8 wk. Administration of the supplement mix resulted in persistently lower BP for at least 48 wk in SHR offspring. Interestingly, the same supplements also prevented the BP increase in aging WKY (Fig. 2) (120). The perinatally supplemented SHR offspring transiently had lower urinary thiobarbituric acid-reactive substance excretion, suggesting that supplemental antioxidants and excess NOS substrate in the perinatal period in SHR result in less ROS production, with a temporary improvement of the NO-ROS balance that has long-term implications for BP control in later life. Interestingly (Fig. 2) is that while some interventions resulted in lower BP starting from 8 wk of age, others mitigated the steep increase in BP seen in control SHR between 8 and 20 wk of age. The different patterns of reduced BP show that different periods of BP increase in SHR seem to be programmed separately. There was no difference in glomerular number between SHR and WKY offspring.

![Fig. 1. Production of reactive nitrogen and oxygen species (ROS) and interventions that increase nitric oxide (NO) and decrease ROS. L-NIL, L-N^6-(1-iminoethyl)lysine; iNOS, inducible NO synthase.](http://ajprenal.physiology.org/)

![Fig. 2. Systolic blood pressure (SBP) in control female Wistar-Kyoto (WKY; ○) rats and spontaneously hypertensive rats (SHR; ●) and perinatal vitamin C-, vitamin E-, taurine-, and L-arginine-supplemented SHR [until 4 (●) and 8 wk (●)] and similarly supplemented WKY rats [until 4 (○) and 8 wk (○)]. Values are means ± SE. *P < 0.05 vs. SHR control. †P < 0.05 vs. WKY control. Adapted from Ref. 120.](http://ajprenal.physiology.org/)

**Lower BP in SHR by perinatal shift of NO-ROS balance**

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rats, confirming recent findings using state-of-the-art counting technology (16), nor were there consistent increases in glomerular number in supplemented rats compared with controls, so this cannot explain the persistently lower BP. This is different from the IUGR models where marked decreases in glomerular number are a consistent finding in rats (157) and sheep (156).

To dissect further the roles of NO and ROS, we treated SHR dams for the same brief perinatal period with a combination of vitamin C, vitamin E, and the NO donor molsidomine (8, 48), as well as with the membrane-permeable SOD mimic tempol (130) and obtained a comparable life-long BP reduction (Fig. 3) and prevention of proteinuria (Fig. 4). Similar treatment with a combination of vitamin C and vitamin E (without molsidomine) had no effect (not shown). These findings suggest that manipulation of the NO-ROS balance in the perinatal period in favor of NO and away from ROS can be achieved using different compounds and combinations, with similar effects on BP and renal protection in the offspring (119, 120).

Fig. 3. SBP in control female WKY (○; n = 7) rats, control SHR (●; n = 6), SHR perinatally treated with vitamin C (594 mg/l drinking water), vitamin E (9 g/kg food), and molsidomine (120 mg/l drinking water) administered during the last 2 wk of gestation and until 8 wk of age (●; n = 8), and SHR perinatally treated with tempol administered during the last 2 wk of gestation and until 4 wk of age (172 mg/l drinking water; ■; n = 7). SBP was measured in prewarmed, nonanesthetized rats by tail cuff. *P < 0.05 vs. SHR control.

Fig. 4. Proteinuria in 48-wk-old female WKY rats (n = 7), control SHR (n = 6), SHR perinatally treated with vitamin C, vitamin E, and molsidomine (n = 8), and SHR perinatally treated with tempol (n = 7). Urine (24 h) was collected, and protein was measured with Coomassie blue. *P < 0.05 vs. SHR control. †P < 0.05 vs. WKY rats.

Fig. 5. Proposed mechanism by which hypertension is perinatally programmed in SHR, comprising increased oxidative stress through excessive superoxide production that results in a positive feedback loop. Note the pivotal role of iNOS overexpression and uncoupling. BH₄, tetrahydrobiopterin; AP-1, adapter protein-1; STATs, signal transducers and activators of transcription; AT₁, ANG II type 1.
IS UNCOUPLING OF NOS IN SHR INVOLVED IN FETAL PROGRAMMING OF BP SYSTEMS?

When insufficient substrate or cofactors are present, NOS can become uncoupled and become a source of superoxide (31, 75). Aminoguanidine, a specific inhibitor of iNOS, reduced BP in adult SHR (67), whereas a specific NOS inhibition with \(^{N^\omega}\)-nitro-L-arginine increased BP in SHR (150). Together, these findings suggest that iNOS could be a source of ROS rather than NO in SHR. Indeed, we found that treatment with the specific iNOS inhibitor L-\(^{N^\omega}\)-(1-iminoethyl)lysine during pregnancy and lactation persistently reduced BP in SHR offspring (119). The level of BH4 is low in young SHR. Sepiapterin, a BH4 precursor, corrects this and reduces systolic BP and ROS form a positive feedback loop. This creates the potential mechanism, which translates into altered pro-inflammatory phase in SHR the increase in AT1 receptor density, via dox-sensitive transcription factors, including NF-κB, adapter protein-1, and signal transducers and activators of transcription (98). Indeed, it has been proposed that a low-grade inflammatory reaction may underlie the pathogenesis of hypertension in SHR (138). Remarkably, despite the increased ROS activity in young and adult SHR, no information is available on the expression and activity of transcription factors in SHR kidney and vasculature, but it is clear that uncoupling due to incorrect matching of iNOS activity and BH4 availability may well result in positive feedback: excess ROS production activates NF-κB and adapter protein-1, which in turn stimulate iNOS gene expression (41). Taken together, iNOS uncoupling and increased oxidative stress are likely to result in alterations of redox-sensitive transcription factors during development and which could well persist for further development.

PROPOSED MODEL OF EARLY UNCOUPLING OF NOS IN SHR

The data described above suggest that in a crucial developmental phase in SHR the increase in AT1 receptor density, via excess superoxide production by NAD(P)H oxidase, enhances iNOS expression. Uncoupling of the increased iNOS activity due to a relative shortage of substrate and cofactors results in excess superoxide production that activates transcription factors, thus modulating gene expression. On the one hand, this affects development of cardiovascular and renal systems and on the other exacerbates the increased expression of iNOS. This potential mechanism, which translates into altered programming of BP control, is depicted in Fig. 5.

Central to this hypothesis is that iNOS, transcription factors, and ROS form a positive feedback loop. This creates the possibility of permanent correction. Either scavenging excess superoxide by antioxidants, providing NO directly, providing the enzyme with excess substrate, or inhibiting iNOS in the perinatal period interrupts this pathological mechanism. The consequence is transient improvement of the NO-ROS balance as shown by NOx and thiobarbituric acid-reactive substance excretion and the long-term persistent blunting of hypertension and renal injury.

CONCLUDING REMARKS

Experimental studies are required to unravel the mechanisms resulting in the programming phenomenon. Such knowledge could lead to simple maternal dietary interventions during pregnancy and lactation and thus spare the next generation(s) from the burden of a complex of related diseases and life-long antihypertensive treatment.

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