Intrauterine undernutrition: expression and activity of the endothelial nitric oxide synthase in male and female adult offspring

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Received 10 October 2001; accepted 30 May 2002

Abstract

Objective: Epidemiological studies suggest that intrauterine undernutrition plays an important role in the development of arterial hypertension in adulthood. In an attempt to define the mechanisms whereby blood pressure may be raised, we have hypothesized that arteries from offspring of nutritionally restricted dams exhibit abnormalities in the endothelial function and in nitric oxide synthesis. In order to investigate the existence of potential gender differences on the effects of intrauterine undernutrition, both male and female offspring of pregnant Wistar rats on normal and restricted diets were studied in adulthood.

Methods: Female pregnant Wistar rats were fed either normal or 50% of the normal intake diets, during the whole gestational period. At 14 weeks of age, the rats were used for the study of vascular reactivity, eNOS and iNOS gene expression, eNOS activity and, in the case of females, estrogen levels.

Results: Intrauterine undernutrition induced hypertension in both male and female offspring, but hypertension was more severe in male rats. Endothelium-intact aortic rings from male and female rats in the restricted diet group exhibited increased responses to norepinephrine, decreased vasodilation to acetylcholine and unaltered responses to sodium nitroprusside in comparison to aortic rings from control rats. No gender-related differences were observed in the vascular reactivity studies. Intrauterine undernutrition promoted decreased gene expression for eNOS in aorta isolated from male, but not female, offspring, reduction in eNOS activity in both male and female offspring and impairment in synthesis of estrogen in female offspring.

Conclusion: Our data show that intrauterine undernutrition: (1) induces hypertension both in the male and female offspring, hypertension being more severe in male than in female rats; (2) alters endothelium-dependent responses in aortas from the resulting offspring. The endothelial dysfunction is associated with a decrease in activity/expression of eNOS in aortas from male offspring. The mechanism involved in altered response to ACh in female offspring might be a consequence of reduction in estrogen levels leading to reduced eNOS activity.

Keywords: Endothelial function; Gender; Hypertension; Nitric oxide

1. Introduction

Maternal undernutrition during critical periods of organ development is known to impair fetal growth and it has been suggested that endocrine or physiological changes involved in the fetal adaptation to undernutrition persist and predispose to the development of adulthood diseases [1]. Hypertension [2], coronary heart disease [3], type II diabetes [4] and renal disease [5] are some of the disorders which have been linked to low birth weight. Whilst most of these associations are based on retrospective population studies, prospective investigations in animals are now providing substantial experimental evidence to support the
hypothesis of ‘fetal programming’. Recent animal studies demonstrated that a severe, but balanced, limitation of fetal substrate supply retards fetal growth and induces hypertension in rats and guinea pigs [6,7]. Furthermore, restriction of specific nutrients in the maternal diet also retards fetal rat growth and produces marked elevation of blood pressure in the adult offspring. In particular, the feeding of low protein diets in rat pregnancy results in permanently elevated blood pressure in the offspring from the age of weaning [8,9]. A number of potential mechanisms through which hypertension may be initiated by fetal undernutrition has been investigated and a possible role for the renin–angiotensin system [10], the kidney [11] and stress-induced stimulation of the hypothalamic–pituitary–adrenal axis [12] in the maintenance of raised blood pressure has been identified. In this study, we have investigated if severe restriction of all dietary constituents during pregnancy in the normotensive rat will induce hypertension in the offspring. In an attempt to define the mechanisms whereby blood pressure may be raised, we have hypothesized that arteries from offspring of nutritionally restricted dams exhibit abnormalities in the endothelial function and in nitric oxide synthesis. In order to investigate the existence of potential gender differences on the effects of intrauterine undernutrition, both male and female offspring of pregnant Wistar rats on normal and restricted diets were studied in adulthood.

2. Methods

2.1. Animals

All procedures used in this study were approved and performed in accordance with guidelines of the Ethics Committee of the Institute of Biomedical Science, University of São Paulo. Wistar rats from our colony (Laboratory of Hypertension, Institute of Biomedical Science, University of São Paulo) were maintained in a room at 22±1 °C with a 12-h light cycle and 60% humidity.

2.2. Feeding protocol

Timed matings were carried out in female Wistar rats (age range 9–11 weeks). To assess the stage of oestrus of the females, vaginal smear was checked prior to introducing the males. Day 1 of pregnancy was determined by the presence of spermatozoa in the vaginal smear. Following confirmation that mating had occurred, the animals were housed individually in standard rat cages. Female rats were randomly divided into two groups: control (C, \( n = 4 \)), fed standard chow ad libitum (diet: protein 22%; carbohydrates 43.5%; fat 4.2%; cellulose 8%; minerals 10%; water 12.5%; plus salt and vitamin mixtures) and a nutritionally restricted group (R, \( n = 4 \)), fed 50% of the ad libitum intake, determined by the amount of food consumed by the C group from day 1 of pregnancy until parturition (23 days). Following parturition, both offspring received food ad libitum. In order to prevent any variation in neonatal growth through availability of milk intake during suckling, litter size was standardized to eight pups at day 1. The pups were weighed and blood pressure levels determined at 4 and 14 weeks. At 14 weeks of age, the rats were used for the study of vascular reactivity, endothelial nitric oxide synthase (eNOS) activity, inducible nitric oxide synthase (iNOS) and eNOS mRNA expression and, in the case of females, estrogen levels. To avoid variations due to the estrous cycle, all the experiments were performed in females in the stage of estrous (EF).

2.3. Measurement of arterial blood pressure

Systolic blood pressure was determined in conscious rats by an indirect tail-cuff method, using a programmed E&M Instrument Co. electrosphygmomanometer (Narco Bio System, TX, USA). Rats were preheated at 40 °C for 5 min, and then three stable consecutive measurements of blood pressure were averaged. The cuff pressure was controlled automatically and the systolic pulses detected by the pulse transducer were monitored with the audio signal. Care was taken in selecting an appropriate cuff size for each animal.

2.4. Estrogen assay

The animals were killed, a 4-ml blood sample was removed from the abdominal aortic for serum estrogen levels by radioimmunoassay with a commercially available kit (Coat-A-Count Estradiol: Diagnostic Products) with a sensitivity of 8 pg/ml. Rats were staged for estrus cycle at the time of sample collection. As mentioned before, to avoid variations in the results due to the estrous cycle, only females in the state of estrous (EF) were used.

2.5. Vascular reactivity in isolated aortic rings

The rats were anesthetized with i.p. injections of chloral hydrate (300 mg/kg body weight), the thorax was opened and the descending aorta was immediately excised. After removal of loose connective tissue, two transverse rings of the same artery (about 4 mm in length) were cut and the descending aorta was immediately excised. After removal of loose connective tissue, two transverse rings of the same artery (about 4 mm in length) were cut and mounted at optimal length for isometric tension recording in an organ chamber (15 ml), as described by Furchgott and Zawadzki [13]. One ring served as the control while the other had the endothelium mechanically removed by gentle rubbing of the luminal surface with a small cylindrical piece of sponge attached to a thread to permit its insertion through the lumen. Each ring was mounted on two L-shaped stainless steel hooks with the short, straight
portion of each hook passing through the lumen of the ring. The lower hook was attached to the base of the organ chamber and the upper one to a strain gauge. The preparations were mounted in an organ bath containing Krebs–Henseleit solution with the following composition (in mmol/l): NaCl 113, KCl 4.7, CaCl₂ 2.5, NaHCO₃ 25, MgSO₄ 1.1, K₂PO₄ 1.1, EDTA 0.03 and glucose 11. The bathing solution was kept at 37 °C, and was gasified with a mixture of 95% O₂ and 5% CO₂. The preparations were allowed to equilibrate for at least 1 h under a resting tension of 1.5 g, which was maintained throughout the experiment. This procedure was found to produce optimal conditions for reproducible isometric force development and chosen based on previous experiments in which contractions to norepinephrine were studied under different preloads [14]. The tension developed was detected using an F-60 microdisplacement transducer and the response recorded on a polygraph (Narco-Bio-System, TX, USA). Cumulative concentration–effect curves to norepinephrine, acetylcholine and sodium nitroprusside were obtained in different aortic rings. In the case of the two latter agents, the preparations were pre-contracted with 10⁻⁷ mol/l norepinephrine (concentration that induces 60–80% of the maximum effect).

2.6. Endothelial nitric oxide synthase (eNOS) activity assay

The eNOS assay is based on the biochemical conversion of L-arginine to L-citrulline by NOS. This reaction involves a five-electron oxidation of a guanidinonitrogen of L-arginine to nitric oxide, together with the stoichiometric production of L-citrulline. Radioactive arginine is added to homogenate tissue. After incubation, the reactions are stopped with a buffer containing EDTA, which chelates the calcium required by eNOS and, consequently, inactivates the eNOS. Equilibrated resin, which binds to arginine, is added to the samples and they are then applied to spin cups. The citrulline, being ionically neutral at pH 5.5, passes through the column completely. The eNOS activity is then determined by quantitating the radioactivity in the eluate. Concentrations are expressed as final molar (mol per l) units.

2.7. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Rat aortas were dissected, frozen in liquid nitrogen, and stored at −70 °C. Total cellular RNA was isolated from the aortas using TRizol Reagent (GIBCO BRL, Life Technologies, Rockville, MD, USA). After DNA digestion (RQ1 DNase RNAse-free, Promega, Madison, WI, USA), 1 µg of total RNA from each preparation was reverse transcribed, in the presence of RNAase inhibitor (RnasH⁻, Promega, Madison, WI, USA), in a reaction volume of 20 µl containing 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3.0 mM MgCl₂, 10 mM dithiothreitol (DTT), 2.0 mM deoxyribonucleotidetriphosphates (dNTP), 200 U of Moloney murine leukemia virus reverse transcriptase (M-MLV RT; GIBCO BRL) and 1 µg of oligo (dT)₁₂-₁₈ primer. The reaction was carried out at room temperature for 10 min and at 37 °C for 60 min and terminated by heating at 100 °C for 5 min. The reverse-transcribed cDNA (100 ng) was amplified in a final volume of 50 µl by PCR under standard conditions (1.5 mM MgCl₂, 450 µM dNTP, 2.5 U Taq polymerase) with specific primers for rat eNOS (CCAGCTAGCCAAAGTCACCAT.sense / GTCTCGGAGCCCATACAGGATT.antisense), iNOS (GAGGAGTGGGCGAGGAGATG.sense / GTAGTAGAAAGGG-GACAGGAC.antisense) and GAPDH (GTGAAGGTCGGGTGTGAACCGGATT.sense / CACTGCTTCTCTGAGTG- GCAGTGAT antisense). GAPDH was used as an internal control for the coamplification. In order to identify the optimal amplification conditions, a series of pilot studies was performed using a thermal cycler with temperature gradient (Eppendorf Mastercycler gradient, Eppendorf-Netheler-Hinz, Hamburg, Germany) at the annealing step, various amounts of RT products from 2 to 200 ng RNA, and 26–40 cycles of PCR amplification. The amplification was carried out using an initial denaturing cycle at 94 °C for 5 min and the subsequent cycles as follows: denaturation, 30 s at 94 °C; annealing; and extension, 45 s at 72 °C. PCR products (10 µl per lane) were electrophoresed using 1% agarose gel containing ethidium bromide 0.5 µg/ml. The gel was subjected to ultraviolet light and photographed. The band intensities were measured using a software package (Kodak Digital Science, Eastman Kodak Company, New Haven, CT, USA) and the signals were expressed relatively to the intensity of the GAPDH amplicon in each co-amplified sample.

2.8. Drugs

Chemicals (Sigma, St Louis, MO, USA) were prepared daily and dissolved in Krebs–Henseleit solution and the concentrations are expressed as final molar (mol per l) concentrations in the organ chamber.

2.9. Statistical analysis

The results are shown as mean±S.E.M. for maximal responses or mean and 95% confidence intervals (95% CI) for EC₅₀. Statistical analysis was carried out using: two-way ANOVA with Bonferroni’s correction for multiple comparisons and unpaired Student’s t-test for comparison of a single observation between restricted and control groups (SigmaStat, version 2.0, Jandel Scientific Software). Values were considered statistically significant when P<0.05.
3. Results

3.1. Characteristics of the pregnant rats and their offspring

During pregnancy, maternal weight gain was monitored weekly. The nutritional restriction during pregnancy resulted in a marked reduction in maternal body weight from conception until day 15 of gestation. From day 15 of gestation until parturition, at day 23, the undernourished dams gained weight returning to their premating weights (Fig. 1A). Maternal undernutrition resulted in fetal growth retardation, which was reflected in a clear decrease in birth weight in the offspring exposed to intrauterine undernutrition (Fig. 1B). However, no differences were observed in mean litter size at birth in control (10.10±0.41 pups/litter, n=4) and nutritionally restricted (9.50±0.40 pups/litter, n=4) groups, indicating that the reproductive ability was unaffected.

3.2. Effect of intrauterine undernutrition on blood pressure levels in offspring

Wistar offspring exposed to intrauterine undernutrition had higher blood pressure levels than C offspring. At 4 and 14 weeks of age, both male and EF female R groups showed clear elevation of blood pressure compared to C of the same sex and age (Fig. 2). Gender differences in blood pressure levels were noted only in the 14-week-old R groups (Fig. 2); with the EF female rats exhibiting lower blood pressure values than male rats. No gender differences were observed in the C offspring (Fig. 2).

3.3. Serum estrogen levels

Serum estrogen levels were lower in R EF females (17.16±2.77 pg/ml, n=4) than in C EF females (39.22±6.26 pg/ml, n=5) at 14 weeks of age.

3.4. Effect of intrauterine undernutrition on vascular reactivity in the adult offspring

All experiments were performed in aortic rings with (E+) or without endothelium (E−), obtained from the same vessel from male and EF female C and R rats. Mean optimal resting tension was the same for aortic rings from all groups (i.e. between genders and between R and C groups). Pre-constrictor tension development in response to norepinephrine was not significantly different between the C and R groups.

3.4.1. Endothelium-dependent relaxation in response to acetylcholine (ACh)

The cumulative concentration–effect curves to ACh in aortas from both male and EF female from R offspring exhibited a significant rightward shift (changes in the EC_{50}) in comparison to those in the respective C offspring (Fig. 3). The maximal relaxation to ACh was decreased in arteries from both male and EF female from the R offspring when compared with respective control arteries (Fig. 3). The relaxing action of ACh in R and C groups did not differ significantly.

Fig. 1. (A) Maternal body weight of nourished dams (●) and undernourished dams (■) during gestation. Each point represents the mean±S.E.M. of four animals. *P<0.05 compared with nourished dams. (B) Bar graphs show neonatal body weight in offspring from control (C, open bars) and nutritionally restricted groups (R, solid bars). Values are expressed as mean±S.E.M. of 32 animals. *P<0.05 compared with control group.
Fig. 2. Blood pressure (mmHg) in male and female rats from the control (C, open bars) and nutritionally restricted (R, solid bars) groups at 4 and 14 weeks of age. Values are expressed as mean±S.E.M. of seven animals. *P<0.05 for R versus C of same sex; **P<0.05 for male versus female of same group.

not exhibit gender differences since similar responses were observed in both males and EF females from both groups (Fig. 3). Loss of relaxant response of the preparations from all groups of animals occurred after removal of the endothelial cells (data not shown).

3.4.2. Smooth muscle responsiveness to sodium nitroprusside (SNP)

Similar responses (maximal relaxation and EC\textsubscript{50} values) to the endothelium-independent agent, SNP, were observed in aortas, with and without endothelium, from C and R offspring (Table 1).

3.4.3. Vasoconstriction to norepinephrine (NE)

Aortic rings E\textsuperscript{+} isolated from R offspring were more responsive to NE than the respective preparations from the C group (Fig. 4A). On the other hand, no difference was observed in NE responses in aortic rings E\textsuperscript{−} from the R group when compared to those from the C group, in both sexes (Fig. 4B). In addition, the concentration–effect curves to NE in aortic rings E\textsuperscript{+} from both male (1.4×10\textsuperscript{-7} M; 95% CI: 1.2–4.3, P<0.05) and EF female (2.4×10\textsuperscript{-7} M; 95% CI: 1.1–2.7, P<0.05) rats from R offspring showed a significant leftward shift (changes in the EC\textsubscript{50}) when compared with C males (12.3×10\textsuperscript{-7} M; 95% CI: 1.4–6.4) and C EF females (13.1×10\textsuperscript{-7} M; 95% CI: 1.4–26.6), respectively (Fig. 4A). No gender differences were observed in the R and C groups.

3.5. Endothelial nitric oxide synthase activity measurement

As illustrated in Fig. 5, endothelial nitric oxide activity was markedly reduced in aorta from R in comparison to
Table 1
Effective concentration 50% (EC₅₀) and maximal response (MR) values for sodium nitroprusside (SNP) tested in aorta isolated from control (C) and nutritionally restricted (R) rats

<table>
<thead>
<tr>
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<th>C</th>
<th>R</th>
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<tr>
<td></td>
<td>MR² ± S.E.M.</td>
<td>MR² ± S.E.M.</td>
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<tr>
<td>Male</td>
<td>EC₅₀ (×10⁻⁶ M) ± S.E.M.</td>
<td>EC₅₀ (×10⁻⁶ M) ± S.E.M.</td>
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<tr>
<td>SNP (with endothelium)</td>
<td>98.3±1.1</td>
<td>99.2±0.8</td>
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<tr>
<td>SNP (without endothelium)</td>
<td>98.3±0.9</td>
<td>97.5±1.4</td>
</tr>
<tr>
<td>Female</td>
<td>SNP (with endothelium)</td>
<td>98.3±0.9</td>
</tr>
<tr>
<td>SNP (without endothelium)</td>
<td>98.4±0.7</td>
<td>97.8±1.4</td>
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*Results are the mean ± S.E.M.
Results are the mean with 95% confidence intervals in parentheses.

Fig. 4. (A) Concentration–effect curves for norepinephrine (NE) in aortic rings with endothelium isolated from control (C) male (●, n=6) and female (○, n=6) or from nutritionally restricted (R) male (□, n=7) and female (■, n=7) rats. Values are expressed as mean ± S.E.M. *P<0.05 for R versus C of same sex. (B) Concentration–effect curves for norepinephrine (NE) in aortic rings without endothelium isolated from control (C) male (●, n=6) and female (○, n=6) or from nutritionally restricted (R) male (□, n=7) and female (■, n=7) rats. Values are expressed as mean ± S.E.M. *P<0.05 for R versus C of same sex.

3.6. Effect of intrauterine undernutrition on mRNA expression for eNOS and iNOS in the adult offspring

mRNA expression for eNOS was lower in R males than in C males at 14 weeks of age, whereas no statistical differences were observed between the R and C EF females groups (Fig. 6). There were no differences in the mRNA expression for iNOS between the R and C offspring (Fig. 6). Gender differences in the mRNA expression for eNOS were noted within both R and C groups (Fig. 6), with expression of mRNA for eNOS being higher in EF females than in males of the same age and group (Fig. 6).
adaptation to undernutrition persist and predispose to the adult hypertension-programming of development [15]. However, criticism on this hypothesis has recently been presented. Epidemiological data demonstrated that high blood pressure was not linked to prenatal exposure to a balanced reduction of macro-nutrients in the maternal diet [16]. In addition, experimental studies do not support the concept of an inverse relationship between birth weight and blood pressure in adulthood [17]. The discrepancies found may be related to differences in the feeding protocol used since the consequences of malnutrition depend on several factors, including the severity and duration of nutritional deficiencies.

In the present work, we studied male and female offspring from nutritionally restricted dams and detected differences in the magnitude of the increase in systolic blood pressure levels in these animals. Since we detected higher blood pressure in both male and female offspring from undernourished dams than in their respective controls, we suggest that the effects of severe nutrient restriction during pregnancy in Wistar rats are gender-independent. In addition, the severity of the hypertension is greater in males than in females similar to that found in other models of hypertension. This gender dichotomy in the manifestation of the severity of the hypertension would seem to be unrelated to the initiating causative mechanism of the hypertensive disorder.

There are conflicting findings in the literature about the effect of the intrauterine undernutrition on the development of hypertension in males and females. Kwong et al. [15] demonstrated increased blood pressure levels only in male offspring of rats subjected to intrauterine undernutrition. These authors suggested that male preimplantation embryos have higher capacity to respond to the maternal environment and may, as a consequence, exhibit heightened sensitivity to specific programming influences. Woodall et al. [18] considered male and female offspring together as no gender differences were apparent in offspring of control or restricted diet groups. On the other hand, Ozaki et al. [19] demonstrated that maternal under-nutrition during pregnancy causes gender-related hypertension in the resulting offspring. Methodological differences could explain these contradictory results. In the various studies, the composition of the diets varied not only in the protein content but also in the fat, carbohydrate and salt contents. Thus, changes in dietary composition may contribute to the differences in the consequences of the fetal undernutrition on the developing cardiovascular system in male and female rats.

In order to investigate alterations of vascular reactivity in the offspring from nutritionally restricted dams, responses to acetylcholine, an endothelium-dependent vasodilator, to sodium nitroprusside, an endothelium-independent vasodilator and to norepinephrine, a vasoconstrictor whose effects are modulated by the endothelium, were investigated. Whereas the responses induced by acetyl-

4. Discussion

In the present study, we demonstrated that the maternal nutritional restriction throughout gestation in Wistar rats resulted in severe maternal body weight loss during gestation and in intrauterine growth retardation, characterized by a significant decrease in birth weight of the offspring. In addition, we demonstrated that severe nutrient restriction in utero elevated blood pressure to hypertensive levels in both male and female Wistar rats.

These observations suggest that intrauterine nutrition may be important in the cardiovascular system regulation. Endocrine and physiological changes involved in the fetal
choline were decreased in aorta isolated from offspring of nutritionally restricted dams, no differences could be detected to sodium nitroprusside. Therefore, we may suggest that it is not the smooth muscle vasodilating capability that is reduced in aorta isolated from offspring of nutritionally restricted dams, but some function related to the endothelium. In fact, Goodfellow et al. [20] and Leeson et al. [21] demonstrated that growth restriction in human pregnancy is associated with endothelial dysfunction. The authors used a non-invasive method to evaluate endothelium-dependent dilatation in the brachial artery of children and reported significant, graded and positive association of low birth weight with impaired endothelial function in childhood.

The present study provides for the first time evidence that the intrauterine undernutrition promoted: (1) decreased gene expression for eNOS in aorta isolated from male, but not female, offspring; (2) reduction in eNOS activity in both male and female offspring.

There are few mechanisms that could explain how the endothelium dysfunction leads to the decreased response to acetylcholine observed in offspring of nutritionally restricted dams. Considering that nitric oxide (NO) is the main agonist responsible for the endothelium-dependent relaxation induced by acetylcholine, intrauterine undernutrition may cause a reduction in NO synthesis and/or bioavailability, decreasing the endothelium-dependent vasodilation. Therefore, the reduced NO synthesis by impairment of activity/expression of eNOS could be responsible for the decreased ACh-mediated vasodilatation observed in male offspring from nutritionally restricted dams.

Differently from that observed in male offspring, gene expression for eNOS was not altered in estrous female offspring of nutritionally restricted dams. However, eNOS activity was reduced in these animals and could explain, at least partially, the impairment response to ACh observed in female offspring. It has been proposed that estrogen has a vasoprotective effect on endothelium via a NO mediated mechanism. It was shown that long-term treatment of cultured human and bovine endothelial cell with estrogen up-regulates eNOS activity in a receptor mediated system dependent on calcium [22,23]. In addition, deficiency of estrogen (by ovariectomy) in female SHR promotes reduction in eNOS activity [24]. Borwick et al. [25] demonstrated that fetal undernutrition impairs ovarian development in ewes’ offspring, presumably by reduction in estrogen synthesis. Since females from dams submitted to undernutrition in pregnancy exhibited reduced estrogen levels, we suggested that the vasoprotective role of the estrogen on the vascular responses has been lost in females submitted to intrauterine undernutrition and this led to decreased eNOS activity.

It is well known that the endothelium modulates the vasoconstrictor response to norepinephrine, probably by releasing NO. Reduced release/synthesis/availability of NO may lead to increased responses to norepinephrine. The fact that the aortic rings with endothelium from offspring of nutritionally restricted dams were more responsive to norepinephrine, led us to suggest that the modulatory role of the endothelium on the vascular responses has been lost in Wistar rats submitted to intrauterine undernutrition. In males and females, this may be due to the reduction in the activity of eNOS and consequently, reduction in NO synthesis. On the other hand, vascular smooth muscle responses to norepinephrine have been preserved either in male or female, reinforcing the hypothesis that food restriction in utero affects mainly the endothelium.

Other parameters may also be involved in the alterations of vascular reactivity observed in offspring from nutritionally restricted dams. Since the endothelium releases contracting factors (EDCFs), we cannot exclude a role for these factors in the endothelial dysfunction induced by intrauterine undernutrition. Ozaki et al. [19] demonstrated that, in Wistar rats, maternal undernutrition during pregnancy caused abnormal response to thromboxane (TXA2) in the resulting offspring. Recently, an association between overexpression of the PGH2/TXA2 receptor and intrauterine growth restriction has been observed in mice [26]. Another EDCF that could be involved in alteration of endothelium-dependent responses is superoxide anion. In fact, recent studies from our laboratory have shown a marked increase in the concentration of superoxide anion in the mesenteric arteriolar bed from male Wistar offspring exposed to severe food restriction in utero [27]. In addition, reduction in estrogen levels (by ovariectomy) in female SHR has been linked to increased superoxide anion generation in mesenteric arterioles [28]. Therefore, EDCF's and particularly superoxide anion, that inactivates NO, might contribute to the alterations in blood pressure levels and vascular reactivity found in offspring of nutritionally restricted dams. Studies are in progress in our laboratory to investigate the role of EDCF in these animals.

In summary, our data show that intrauterine undernutrition: (1) induces hypertension both in the male and female Wistar offspring, hypertension being more severe in male than in female rats; (2) alters endothelium-dependent responses in aortas from the resulting offspring. The endothelial dysfunction is associated with a decrease in activity/expression of eNOS in aortas from male offspring. The mechanism involved in altered response to ACh in female offspring might be a consequence of reduction in estrogen levels leading to reduced eNOS activity.

Acknowledgements

The present study was supported by grants from FAPESP. The authors thank Marta Rodrigues da Silva and Sonia Maria Rodrigues Leite for excellent technical support.
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