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Prenatal stress changes rat arterial adrenergic reactivity in a regionally selective manner

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Abstract

A suboptimal fetal environment has been linked to increased risk of cardiovascular disease in adulthood. We investigated whether intrauterine stress (IUS) alters the development of adrenergic reactivity in different types of rat arteries. Intrauterine stress was induced by ligation of the uterine arteries at day 13 of pregnancy in Wistar rats. First-order mesenteric, renal, femoral and saphenous arteries of the 21-day-old male offspring were studied in a myograph. IUS in the rat changes arterial adrenergic reactivity in a regionally selective manner. Adrenoceptor-mediated responses are altered in the renal artery. Maximal contractile responses to phenylephrine were increased, while sensitivity to the α_1-adrenoceptor agonist was decreased. Intrauterine stress significantly reduced contractile responses to norepinephrine and enhanced relaxing responses to isoproterenol in the renal artery. Adrenergic responses were not modified in mesenteric, femoral and saphenous arteries. In the kidneys the densities of [^{3}H]prazosin binding sites, periarterial adrenergic nerves and of the glomeruli were not altered after intrauterine stress at day 13 of gestation. The observed regionally selective alterations in arterial reactivity might link a suboptimal fetal environment to the development of cardiovascular disease in the adult.

Keywords: Intrauterine stress; α-Adrenoceptor; β-Adrenoceptor; Sympathetic innervation; Renal artery; (Rat)

1. Introduction

Cardiovascular disease is associated with genetic predisposition, smoking, obesity, lack of exercise and diabetes mellitus. In addition, there is now substantial evidence that adverse influences during fetal life increase the susceptibility to cardiovascular, endocrine and metabolic diseases later in life (Barker, 2000). A suboptimal supply of oxygen (Lueder et al., 1995; Ruijtenbeek et al., 2000) and nutrients caused by maternal undernutrition (Hoet and Hanson, 1999) or by disturbed placental function (Pardi et al., 2002) alter the fetal development of cardiovascular control mechanisms. The exact mechanisms that link a suboptimal fetal environment to the programming of adult disease are unknown. A number of neuro-humoral systems and organs, like the renin–angiotensin system, the hypothalamic–pituitary–adrenal axis (Green, 2001), vascular endothelial function and sympathetic innervation (Holemans et al., 1999; Jansson and Lambert, 1999; Ruijtenbeek et al., 2000), and the kidneys (Marchand and Langley-Evans, 2001) have been proposed to be involved in what is increasingly recognized as signs of the epigenetics of chronic disease.

Sympathetic nerves innervate the vascular tree, but there are considerable differences in the density and regulatory actions of the sympathetic nervous fibres between different vascular beds. Adrenoceptors mediate the vascular actions of the sympathetic nervous system. The most important neurotransmitter, norepinephrine, can bind to α_1-, α_2- and β-adrenoceptors which cause vessels to contract or relax (Satoh et al., 1999; Tsuru et al., 2002). Hyperinnervation of the cardiovascular system precedes the structural and functional changes of the vasculature in spontaneously hypertensive rats (Head, 1989; Lee, 1985) and eventually leads to

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an increased peripheral resistance, cardiovascular hypertrophy and high blood pressure.

Studies in our own laboratory have demonstrated that a suboptimal fetal environment can cause sympathetic hyperinnervation of the peripheral arterial system (Ruijtenbeek et al., 2000). Since sympathetic nerves, neurotransmitter release and the subsequent stimulation of different receptor subtypes are important in maintaining normal cardiovascular dynamics and vascular structure, we hypothesize that a suboptimal fetal environment can change normal development of arterial adrenergic function.

In this study, we induced an experimental intrauterine stress (IUS) by bilateral ligation of the uterine arteries at day 13 of pregnancy in Wistar rats and investigated the consequences of intrauterine stress for adrenergic vascular responses in the offspring. To evaluate whether intrauterine stress affects regionally different vascular beds in a different manner, mesenteric resistance, renal, femoral and saphenous arteries were taken from the 21-day-old offspring and studied with pharmacological methods. Changes in pattern and density of cathecholamine-containing nerves were also assessed using fluorescence immunohistochemistry and α_1-adrenoceptors were approached by radioligand binding.

2. Materials and methods

2.1. Animals

Experiments were approved by the local ethical committee for animal research of the University of Maastricht (registration number 2000-68). Animals had free access to pelleted food and tap water and were maintained on a 12-h light/dark cycle at 21 °C.

2.2. Induction of intrauterine stress

Female Wistar rats (Charles River, Maastricht, The Netherlands) weighing 200–250 g were mated. Day 1 of pregnancy was defined as the day immediately following the night during which males were present and conception was confirmed by the vaginal smear method. To induce intrauterine stress, on day 13 and day 17 of pregnancy, animals were subjected to bilateral ligation of the uterine arteries (near the iliac arteries), according to the method of Wigglesworth (1964). A sham procedure was performed as described, except for the actual occlusion of the blood vessels. The offspring of mothers, which underwent bilateral ligation of the uterine arteries during pregnancy, are referred to as intrauterine stress (IUS) group and the offspring of mothers, which underwent a sham operation, are referred to as control group. Rats delivered spontaneously at 22 days and litter size and birth weight of the offspring were determined within 1 h after birth.

Because the number of life-born pups was reduced after intrauterine stress, litter sizes of the control and intrauterine stress group were matched within 1 h after birth. At 21 days after delivery, animals were killed by cervical dislocation.

2.3. Arterial reactivity

Arterial segments were isolated from the 21-day-old male offspring of both groups. Two stainless steel wires (diameter 40 μm) were inserted in the lumen of the arterial segments, which were then mounted in organ chambers between an isometric force transducer and a displacement device (Danish Myotechnology by J.P. Trading, Denmark). The organ chambers were filled with Krebs–Ringer bicarbonate solution which was maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2. Before the actual experiments started, arterial segments were stretched to their individual optimal lumen diameter for mechanical performance, i.e. the diameter at which maximal contractile responses K^+ (40 mM) were obtained (De Mey and Brutsaert, 1984). In most experiments, a first-order mesenteric resistance, right renal, right femoral and right saphenous arterial segment from one animal were mounted in an organ chamber and studied in parallel. At the start of the experiments, all arterial preparations were exposed to 1 μM capsaicin during 20 min to stimulate and permanently desensitise sensory–motor nerves, while it does not affect efferent fibres of the autonomic nervous system (Holzer, 1991; Szallasi and Blumberg, 1999).

Concentration-dependent (0.01–10 μM) contractile responses to phenylephrine, norepinephrine, 5-hydroxytryptamine and electrical field stimulation were expressed as active wall tension (N/m) or as percentage of the contraction, induced by a mixture of 125 mM K^+ and 10 μM phenylephrine. This latter response which we refer to as maximal tissue contraction (Tmax), results from strong activation of electro- and pharmacomechanical coupling by K^+ and phenylephrine, respectively. Sympathetic neuroeffector mechanisms were studied using electrical field stimulation (EFS; 0.25–32 Hz, 2 ms, 85 mA) via platinum electrodes, which were placed in the axial direction over the vessel segments, and a stimulator designed by the local technical department.

Concentration-dependent (0.01–10 μM) relaxing responses to isoproterenol, calcitonin gene-related peptide (CGRP) and forskolin were investigated during contraction induced by 40 mM K^+ to blunt effects of the tested compounds on sarcolemmal K^+ channels (Cohen and Vanhoutte, 1995), in order to concentrate on ultimate stimulatory influences on adenylyl cyclase. Relaxing responses were expressed as percentage change of the pre-existing contractile tension. Effects of isoproterenol on α_1-adrenoceptors were prevented by 1 μM prazosin. After experimentation, arteries were fixed overnight at their optimal diameter in phosphate-buffered (pH 7.4) formaldehyde (4%).

Table 1
Birth weight and litter size of the offspring at birth

	Ligation at day 13		Ligation at day 17	
	CON	IUS	CON	IUS
Birth weight	5.44 ± 0.08 (n = 84)	6.04 ± 0.10[a] (n = 38)	5.35 ± 0.04 (n = 204)	5.03 ± 0.05[a] (n = 137)
Litter size	8 ± 1 (n = 14)	4 ± 1[a] (n = 13)	11 ± 1 (n = 16)	5 ± 1[a] (n = 22)

IUS: intrauterine stress. Values are mean ± S.E.M.
[a] $P < 0.001$.

2.4. Morphometric analysis of the arteries and their adrenergic nerve fibres

Structural properties of first-order mesenteric resistance, renal, femoral and saphenous arteries were determined according to the morphometric methods described by Stassen et al. (1997). Glyoxylic acid was used to visualize and quantify periarterial adrenergic nerve fibres in whole mount preparations (Ruijtenbeek et al., 2000).

2.5. Morphometric analysis of the kidneys

Left kidneys were dissected, fixed overnight in phosphate-buffered (pH 7.4) formaldehyde (4%) and embedded in paraffin. Parallel transversal sections were stained with Jones methenamine silver. Number, area and circumference of the glomeruli were measured using video images generated by a Zeiss Axioscope (Zeiss, Germany), a standard CCD camera (Sony) and commercially available software (Sigmascan Pro 2.0, Jandel Scientific, Erkrath, Germany). The number of glomeruli within a known volume was calculated according to the formula: $n = G/FA(D + T)$ (Lucas et al., 1996), where n is the density of glomeruli (/mm^3); G is the number of glomeruli counted in 10 fields of the kidney; F is the number of fields counted; A is the area (1,200,356 μm^2); D is the average diameter of the glomeruli (62 μm); and T is the section thickness (4 μm). The glomeruli counted were localized in the cortical area immediately under the capsule.

2.6. α_1-Adrenoceptor binding assay

Radioligand binding was used to determine the characteristics of α_1-adrenoceptors in right kidney microsomes. Analysis of [^{3}H]prazosin binding was performed essentially as described by Michel et al. (1993).

2.7. Drugs and solutions

Krebs–Ringer bicarbonate buffer contained (in mM): NaCl 118.5, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, KCl 4.7, $CaCl_2$ 2.5 and glucose 5.5. A 40 mM K^+ solution was prepared by replacing part of the NaCl by an equimolar amount of KCl. Phosphate-buffered solution consisted of 0.1 M, $NaH_2PO_4 \cdot H_2O$ and 0.1 M $Na_2HPO_4 \cdot 2H_2O$. Calcitonin gene-related peptide (CGRP) was obtained from Bachem (Bubendorf, Switzerland). Capsaicin, phenylephrine, 5-hydroxytryptamine, norepinephrine and prazosin were obtained from Sigma (St. Louis, MO, USA), isoproterenol and forskolin from ICN Pharmaceuticals (Costa Mesa, CA, USA) and Lawson solution from Boom (Meppel, The Netherlands). Phenylephrine, 5-hydroxytryptamine, norepinephrine, isoproterenol and CGRP were dissolved in distilled water and capsaicin, prazosin and forskolin in ethanol (100%).

2.8. Data analysis

Concentration response curves were analysed in terms of sensitivity ($pD_2 = -\log EC_{50}$) and maximal response (E_{max}) by fitting individual concentration–response data to a sigmoid regression curve and interpolation (Graphpad Prism version 2.01, Graphpad Software). Differences between findings in arteries from both groups of rats were tested with Student t-test or Mann–Whitney U-test when normality test (Kolmogorov–Smirnov) failed. A value of $P < 0.05$ was considered statistically significant. Data are presented as mean ± S.E.M.

3. Results

After bilateral ligation of the uterine arteries at day 13 of pregnancy, (1) fetal survival was reduced, as indicated by the small litter size, (2) birth weights were increased (Table 1) and (3) absolute and relative organ weights were unchanged at 21 days of age (data not shown). The induction of intrauterine stress at a later time point of pregnancy (day 17) resulted in significantly decreased birth weights (Table 1).

Optimal diameter and cross-sectional area of the media were larger in the renal and femoral arteries than in the mesenteric resistance and saphenous arteries. These arterial structural properties at 21 days of age were not modified by intrauterine stress (Table 2).

Table 2
Structural properties of first-order mesenteric resistance, renal, femoral and saphenous arteries of 21-day-old rats

	Control		Intrauterine stress	
	Diameter (μm)	MCSA ($\times$ 1000 μm^2)	Diameter (μm)	MCSA ($\times$ 1000 μm^2)
Mesenteric artery	194 ± 6	5.2 ± 0.4 (n = 8)	197 ± 10	5.1 ± 0.6 (n = 7)
Renal artery	373 ± 13	21.2 ± 1.8 (n = 16)	375 ± 26	21.9 ± 2.2 (n = 11)
Femoral artery	440 ± 10	33.9 ± 2.1 (n = 9)	475 ± 14	36.1 ± 1.7 (n = 8)
Saphenous artery	304 ± 6	16.2 ± 1.1 (n = 9)	320 ± 10	16.6 ± 0.7 (n = 9)

MCSA: media cross-sectional area. Values are mean ± S.E.M.

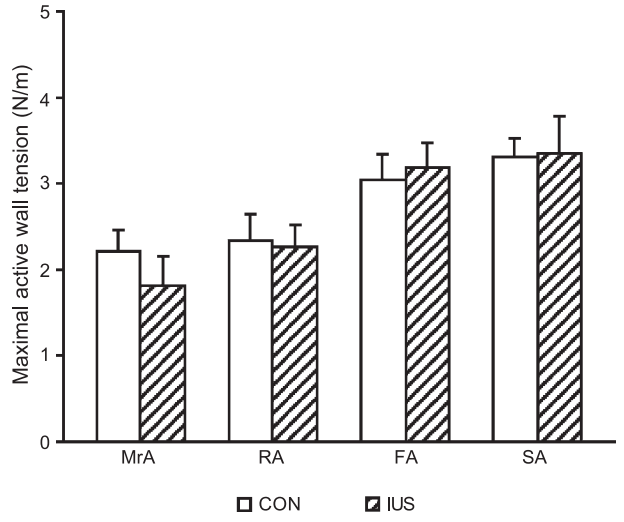

Fig. 1. Maximal contractile responses (T_{max}) to 125 mM K^+ plus 10 μM phenylephrine in first-order mesenteric resistance (MrA), renal (RA), femoral (FA) and saphenous arteries (SA) of control rats (□, CON) and rats that survived intrauterine stress (▨, IUS). Data are mean ± S.E.M.

3.1. Arterial reactivity

Maximal contractile responses (Tmax) to a mixture of 125 mM K^+ and 10 μM phenylephrine were comparable for the four types of arteries, despite differences in diameter and media mass. Tmax was unchanged after intrauterine stress (Fig. 1).

All arterial preparations contracted in response to the α_1-adrenoceptor agonist phenylephrine. Responses of the mesenteric resistance and saphenous arteries were considerably larger than those of renal and femoral arteries of control animals (Fig. 2). Following intrauterine stress, maximal contractile responses to phenylephrine were significantly increased in the renal artery and reached a comparable reactivity as the mesenteric resistance and saphenous arteries (Fig. 2). In addition to an increased maximal response, sensitivity to phenylephrine was significantly decreased in the renal artery after intrauterine stress (Table 3). Intrauterine stress did not modify sensitivity and maximal responsiveness to the α_1-adrenoceptor agonist in the mesenteric resistance, femoral and saphenous arteries (Fig. 2).

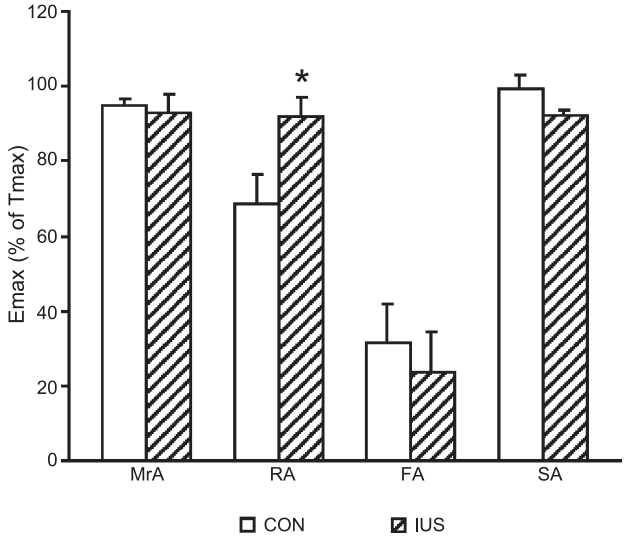

Fig. 2. Maximal contractile responses (% of T_{max}) to phenylephrine in first-order mesenteric resistance (MrA), renal (RA), femoral (FA) and saphenous arteries (SA) of control rats (□, CON) and rats that survived intrauterine stress (▨, IUS). Data are mean ± S.E.M., *$P<0.05$ vs. control.

To investigate whether intrauterine stress changes contractile responses to other agonists, we exposed renal arteries of control animals and of animals that survived intrauterine stress to increasing concentrations of 5-hydroxytriptamine and vasopressin. Maximal responses to 5-hydroxytriptamine were significantly increased after intrauterine stress and maximal responses to vasopressin seemed to be increased as well, but the differences were not statistically significant (Table 3). Sensitivities to 5-hydroxytriptamine and vasopressin were not altered after intrauterine stress (Table 3).

Fig. 3 summarizes maximal contractile responses to exogenous norepinephrine. Mesenteric resistance, renal and saphenous arteries contracted to the same extent, but the femoral artery barely responded to stimulation with norepinephrine. Intrauterine stress significantly decreased maximal contractile responses to norepinephrine in the renal artery, while sensitivity was not modified (Table 3). Intrauterine stress did not change the responses to norepinephrine in the other types of artery (Fig. 3).

Table 3
Maximal contractile responses and sensitivity to vasoconstrictor and vasodilator stimuli in renal arteries at 21 days of age

		CON	IUS
5-Hydroxytryptamine	E_{max} (% T_{max})	85.81 ± 4.04	99.82 ± 4.52[a]
	pD_2	6.09 ± 0.15	6.01 ± 0.10
Vasopressin	E_{max} (% T_{max})	76.92 ± 8.13	92.10 ± 7.87
	pD_2	6.69 ± 0.10	6.59 ± 0.19
Phenylephrine	E_{max} (% T_{max})	68.56 ± 7.84	91.96 ± 5.11[a]
	pD_2	6.24 ± 0.15	5.61 ± 0.11[b]
Norepinephrine	E_{max} (% T_{max})	97.15 ± 3.34	70.75 ± 4.35[a]
	pD_2	6.10 ± 0.31	6.00 ± 0.36
Electrical field stimulation	E_{max} (% T_{max})	23.02 ± 5.89	16.78 ± 4.92
	EF_{50} (Hz)	16.13 ± 0.32	16.96 ± 0.46
Isoproterenol	E_{max} (% K^+ 40 mM)	− 26.67 ± 10.78	− 57.55 ± 7.31[a]
	pD_2	6.89 ± 0.17	7.03 ± 0.07

E_{max}, maximal response. T_{max}, maximal tissue contraction, induced by a mixture of 125 mM K^+ and 10 μM phenylephrine. EF_{50}, frequency required for 50% of maximal response. Values are mean ± S.E.M.

[a] $P<0.05$.

[b] $P<0.001$.

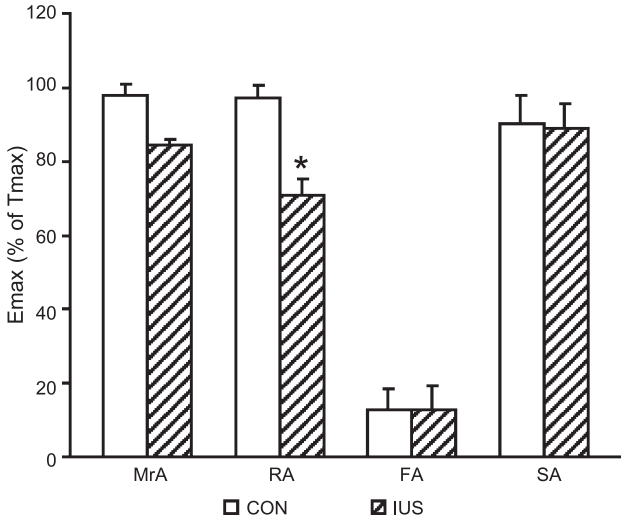

Fig. 3. Maximal contractile responses (% of T_{max}) to norepinephrine in first-order mesenteric resistance (MrA), renal (RA), femoral (FA) and saphenous arteries (SA) of control rats (□, CON) and rats that survived intrauterine stress (▨, IUS). Data are mean ± S.E.M., *$P<0.05$ vs. control.

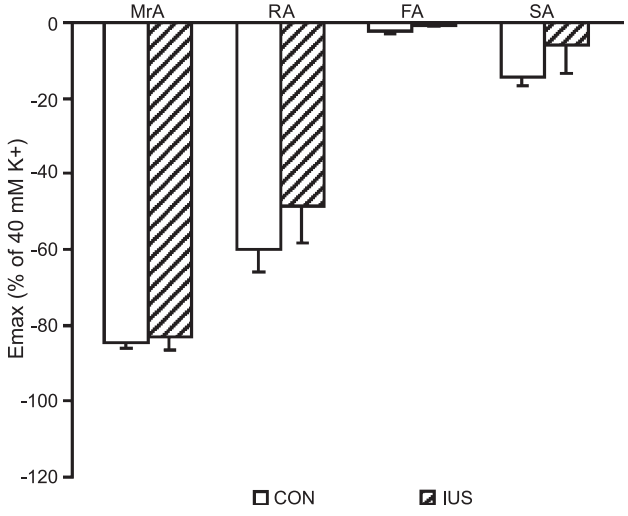

Fig. 5. Maximal relaxing responses (% of 40 mM K^+) to CGRP in first-order mesenteric resistance (MrA), renal (RA), femoral (FA) and saphenous arteries (SA) of control rats (□, CON) and rats that survived intrauterine stress (▨, IUS). Data are mean ± S.E.M.

Stimulation of β-adrenoceptors with isoproterenol, during precontraction with 40 mM K^+, caused all vessels to relax. The maximal relaxing responses were similar in the mesenteric resistance, femoral and saphenous arteries, while the renal artery displayed smaller relaxing responses to isoproterenol. When the animals had suffered intrauterine stress, renal arterial relaxing responses to β-adrenoceptor stimulation were significantly increased compared to the control animals (Fig. 4). Sensitivity to isoproterenol was unchanged in the renal artery after intrauterine stress (Table 3). Intrauterine stress did not modify β-adrenergic relaxation in mesenteric resistance, femoral and saphenous arteries (Fig. 4). Also when intrauterine stress was induced at day 17 of pregnancy, which decreased birth weight (Table 1), renal arterial relaxing responsiveness to isoproterenol was enhanced (− 12.04 ± 5.59% vs. − 51.64 ± 11.30%, $P=0.016$).

Isoproterenol causes relaxation via stimulation of adenylyl cyclase. Consequently, we evaluated responses to direct activation of adenylyl cyclase by forskolin (0.01–10 μM). All vessels fully relaxed during contraction with 40 mM K^+. Intrauterine stress did not alter maximal relaxing responses to forskolin, nor did it change sensitivity to the diterpene in either type of artery that we investigated (data not shown).

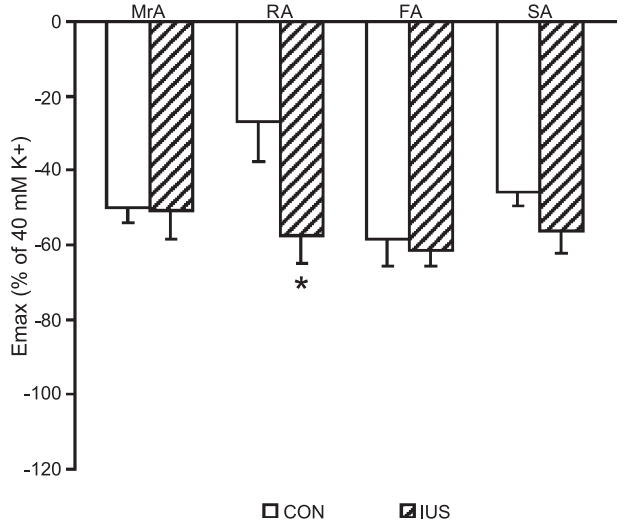

Fig. 4. Maximal relaxing responses (% of 40 mM K^+) to isoproterenol in first-order mesenteric resistance (MrA), renal (RA), femoral (FA) and saphenous arteries (SA) of control rats (□, CON) and rats that survived intrauterine stress (▨, IUS). Data are mean ± S.E.M., *$P<0.05$ vs. control.

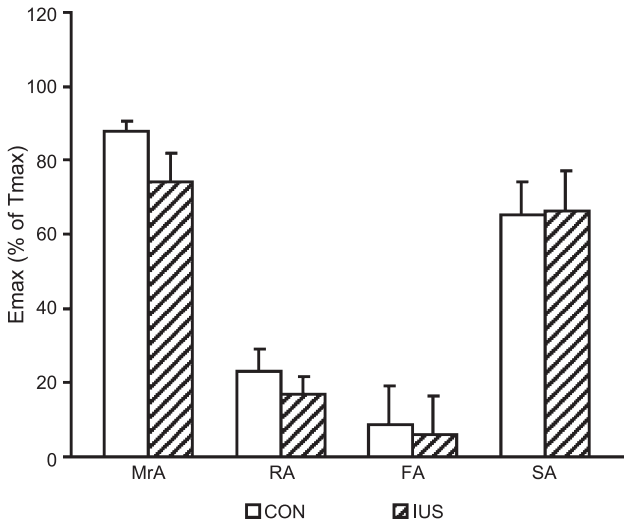

Fig. 6. Maximal contractile responses (% of T_{max}) to electrical field stimulation (EFS) in first-order mesenteric resistance (MrA), renal (RA), femoral (FA) and saphenous arteries (SA) of control rats (□, CON) and rats that survived intrauterine stress (▨, IUS). Data are mean ± S.E.M.

Another agonist that acts via adenylyl cyclase, CGRP (0.1 μM), induced a considerable relaxation in the mesenteric resistance and renal artery, but hardly in the femoral and saphenous artery. Intrauterine stress did not modify relaxing responses to CGRP in either type of artery (Fig. 5).

Maximal contractile responses to sympathetic nerve stimulation (electrical field stimulation after exposure to capsaicin) of the renal and femoral artery were smaller than in the mesenteric resistance and saphenous artery (Fig. 6). Despite altered responses to α_1- and β-adrenoceptor agonists in the renal artery after intrauterine stress, contractile responses to electrical field stimulation were unchanged after surviving a suboptimal fetal environment (Fig. 6). Neither did intrauterine stress alter neurogenic vasoconstriction in mesenteric resistance, femoral and saphenous arteries (Fig. 6).

3.2. Ligand binding in kidney

To gain information about α_1-adrenoceptors that might be indirectly relevant for renal arteries, we determined the affinity (K_d) and density (B_{max}) of [^{3}H]prazosin binding sites in kidney microsomes of control animals and of animals that suffered intrauterine stress. Although the affinity for α_1-adrenoceptors seems lower in the kidneys of intrauterine stress animals, these differences did not reach statistical significance (K_d, 0.07 ± 0.02 vs. 0.09 ± 0.01 nM). The B_{max} values calculated from saturation binding curves were comparable in kidneys of control and intrauterine stress animals (B_{max}, 67.1 ± 6.9 vs. 58.0 ± 7.4 fmol/mg protein).

3.3. Morphometry of the kidneys

Our results in Table 4 demonstrate that the density of glomeruli is not significantly modified in 21-day-old rats that have suffered intrauterine stress. Moreover, the area and the circumference of the glomeruli are not significantly altered after intrauterine stress.

3.4. Nerve density

Adrenergic nerve fibre density differs markedly between vascular beds of the rat. Mesenteric resistance and saphenous arteries display a dense innervation, while renal and femoral arteries display only few nerve fibres. Staining of catecholamine-containing nerves with glyoxylic acid indicated that adrenergic fibre density was not modified in either type of artery of 21-day-old rats that survived intrauterine stress induced at 13 days of gestation (data not shown).

Table 4
Morphology of kidneys of 21-day-old animals

	CON	IUS
Kidney weight (g)	0.28 ± 0.02	0.30 ± 0.01
Density of glomeruli (/mm^3)	373 ± 36	429 ± 37
Area of glomeruli (μm^2)	2699 ± 202	2495 ± 167
Circumference of glomeruli (μm)	210 ± 18	190 ± 6

Values are mean ± S.E.M.

4. Discussion

Epidemiological findings suggest that an unfavourable fetal environment, resulting in low birth weight, predisposes individuals to develop chronic cardiovascular and metabolic diseases in adult life (Adabag, 2001; Barker, 2000, 2001). In the present study, we investigated whether experimental intrauterine stress in the rat alters the development of adrenergic reactivity in arteries from regionally different parts of the vascular tree, which play a major role in the control of total peripheral resistance and blood pressure.

The main finding of our study is that intrauterine stress resulted in a regionally selective alteration of arterial adrenergic function at 3 weeks after birth. While mesenteric resistance, femoral and saphenous arteries were not affected, renal arteries displayed selective pharmacological alterations.

Intrauterine stress induced at day 13 of pregnancy resulted in a marked reduction of the number of fetuses and an increased birth weight. Intrauterine stress induced at a later stage of gestation (day 17) reduced birth weight, as previously reported (Engelbregt et al., 2002; Jansson and Lambert, 1999). Thus, birth weight alone seems not to be an ideal index of the exposure of the fetus to an environment that leads to persistent cardiovascular alterations (Hoet and Hanson, 1999; Pardi et al., 2002). We recently reached a similar conclusion during a comparison of the effects of protein malnutrition and chronic moderate hypoxia on arterial endothelium-dependent reactivity in the chicken embryo (Ruijtenbeek et al., 2003b).

In the four types of arteries that we studied, maximal contractile responses were comparable, despite a larger optimal diameter and cross-sectional area of the media in renal and femoral arteries than in mesenteric resistance and saphenous arteries. This is likely due to a more pronounced contractile phenotype of smooth muscle cells in small resistance than in large elastic arteries (Van Der Loop et al., 1997).

All arteries contracted in response to α_1-adrenergic stimulation, but as in the rabbit (Satoh et al., 1999), there were regional differences in responses to phenylephrine between vascular beds. However, after intrauterine stress, sensitivity to phenylephrine was decreased and maximal contractile responses to phenylephrine were increased in the renal artery, while responses of mesenteric resistance, femoral and saphenous arteries were unaffected by intrauterine stress. These changes are probably not due to an increased number of α_1-adrenoceptors, as the density and affinity of α_1-adrenoceptors in the kidneys were not affected after intrauterine stress. This suggests that the increased responsiveness to α_1-adrenoceptor stimulation

is probably due to alterations in the signal transduction pathway that is engaged.

In the rat renal vasculature α_1-adrenoceptors mediate the action of sympathetic stimulation (Wolff et al., 1987) and an increased renal vascular α_1-adrenergic responsiveness is associated with hypertension in adult spontaneously hypertensive rats (Uchino et al., 1991). Therefore, the increased renal arterial response to stimulation with phenylephrine in rats that suffered intrauterine stress, might lead to the development of hypertension later in life. It is also possible that the increased renal α_1-adrenergic responsiveness causes an imbalance between afferent and efferent arteriolar tone (Azar et al., 1979), which can lead to an altered glomerular filtration and renal damage on the long run.

Hyperresponsiveness of blood vessels from hypertensive subjects to contractile agonists has long been known. One of them, 5-hydroxytryptamine, has numerous vascular effects, mediated by at least $5HT_2$ receptors and possibly α_1-adrenoceptors (Vanhoutte et al., 1988). Boston and Hodgson (1997) reported that the reactivity of the renal vasculature to 5-hydroxytryptamine is enhanced in spontaneously hypertensive rats. In our experiments, stimulation of the renal arteries with 5-hydroxytryptamine showed an increased contractile response after intrauterine stress, without altered sensitivity to the agonist.

Maximal contractile responses to exogenously applied norepinephrine were significantly reduced in renal arteries after intrauterine stress, even though intrauterine stress increased α_1-adrenoceptor-mediated responses. Mesenteric resistance, femoral and saphenous arteries were not changed after intrauterine stress and, as demonstrated before in 20-day-old normal rats (Ozaki et al., 2001), femoral arteries showed only weak responses to norepinephrine. Ozaki et al. studied the effect of dietary restriction in pregnant rats on vascular function in the offspring at different ages and found blunted contractile responses to phenylephrine and norepinephrine in the femoral artery of 20-day-old rats after moderate maternal undernutrition. In contrast to this study and experiments of our own group in the chicken embryo after chronic hypoxia (Ruijtenbeek et al., 2000), we did not observe any changes of responses to norepinephrine in the femoral artery. The model of placental insufficiency used in the present study involves reduced fetal supply of both oxygen and nutrients. The combination of both stimuli and the acute nature of the insult might have different effects on vascular function than the separate interventions.

The reduced renal arterial response to norepinephrine, despite an increased renal contractile response to α_1-adrenoceptor stimulation, might be explained by an enhanced renal dilator response to β-adrenoceptor stimulation. After intrauterine stress, maximal renal arterial relaxing responses to isoproterenol were increased, while responses of mesenteric resistance, femoral and saphenous arteries to isoproterenol were not modified. The same results were observed after intrauterine stress induced at day 17 of pregnancy. Thus, intrauterine growth acceleration (after intrauterine stress on day 13) as well as intrauterine growth restriction (after intrauterine stress on day 17) are accompanied by the same persistent effects on renal arterial β-adrenergic reactivity. The increased β-adrenergic response seems to be in contrast with findings demonstrating decreased β-adrenergic vasodilatation in hypertension and in ageing (Werstiuk and Lee, 2000). The β-adrenoceptor requires GTP-binding proteins (G-proteins) to link receptor activation to adenylyl cyclase. Activated β-adrenoceptors can stimulate both G_s (stimulatory G-protein), and the inhibitory G-protein, G_i (Kilts et al., 2000). After a period of intrauterine stress, released catecholamines might activate β-adrenoceptor-mediated responses and redirect the cardiac output to tissues that have an increased oxygen demand. Repeated administration of β-adrenoceptor agonists decreased neonatal G_i expression and enhanced G_s function (Zeiders et al., 1999a,b). Increased release of norepinephrine during intrauterine stress throughout late gestation (Simonetta et al., 1997) may continuously activate β-adrenoceptors and alter postnatal development of β-adrenoceptor regulation. Why this leads to increased β-adrenergic reactivity in renal arteries but not in other peripheral arteries and how this can persist for at least 3–4 weeks after the initial insult, remains to be explored.

To analyse the selectivity of the enhanced β-adrenoceptor-mediated relaxing responses, we used forskolin to directly activate adenylyl cyclase (Suzuki et al., 1988). All vessels fully relaxed in response to this diterpene and intrauterine stress did not alter maximal responses or sensitivity to this compound. Also, for calcitonin gene-related peptide, which acts primarily via the activation of adenylyl cyclase under our experimental conditions (Bell and McDermott, 1996), intrauterine stress did not alter arterial responses. The observed selectivity might imply that intrauterine stress modifies the density of β-adrenoceptors or their coupling to adenylyl cyclase. Future experiments are required to strengthen this hypothesis.

Nerve densities differ markedly between vascular beds. Renal arteries, which were hardly innervated with catecholamine-containing nerves, showed small responses to electrical field stimulation. It has been demonstrated that neonatal sympathetic denervation reduces maximal contractile activity and thickness of the media in the adult (Bevan, 1989). However, intrauterine stress did not change these arterial properties in any of the tested vessels nor did a negative fetal environment change sympathetic nerve densities and contractile responses to sympathetic nerve stimulation with electrical field stimulation. Studies from our own group in the chicken embryo demonstrated an increased sympathetic innervation after chronic moderate hypoxia (Ruijtenbeek et al., 2000). Yet, these were found not to persist at 21 days of age (Ruijtenbeek et al., 2003a). A balance between the increased contractile response to α_1-adrenoceptor stimulation and the increased relaxing response to β-adrenoceptor activation might result in the maintenance of normal renal arterial responses to nerve

stimulation after intrauterine stress. Alternatively, cotransmitters, such as neuropeptide Y and ATP, may help maintain renal arterial neurogenic vasoconstrictor responses.

Several studies in animals (Bauer et al., 2002; Merlet-Benichou et al., 1994), as well as in humans (Manalich et al., 2000; Paixao et al., 2001), have demonstrated that negative events during pregnancy have major consequences for renal hemodynamics and glomerular density and size, which might be linked to the development of cardiovascular disease. Here, glomerular density and size were not modified in 21-day-old rats. In the rat, nephrogenesis begins around day 12 of gestation and is not complete until 8 days after birth (Moritz and Wintour, 1999). Nephrogenesis involves the rapid remodelling of structures, which requires massive apoptosis (Coles et al., 1993; Koseki et al., 1992) and renal apoptosis in the rat peaks late in gestation (Malik et al., 2000). Thus, the induction of intrauterine stress, at day 13 of gestation, might be a too early time point to dramatically change normal nephrogenesis. This does not suggest that renal function cannot be affected by the changed arterial adrenergic reactivity. The kidneys play an important role in the regulation of blood pressure and renal nerves already influence renal function during development. A variety of functions, including renin secretion, salt and water balance, gluconeogenesis and vasoconstriction can be regulated by renal α- and β-adrenoceptors (McPherson and Summers, 1982). Therefore, a normal development of renal adrenoceptor function seems to be important for normal kidney function in the adult.

Our results demonstrated that a suboptimal fetal environment alters renal vascular adrenergic and serotonergic function, at least until 4 weeks after the insult, while other areas of the arterial tree are not noticeably affected. Whether these changes are persistent, regressive or progressive throughout adulthood and whether they have major consequences for the development of hypertension or renal dysfunction remains to be established.

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