British Journal of Pharmacology (1996) 118, 1012-1018

The vasoconstrictor effects of L-NAME, a nitric oxide synthase inhibitor, in pregnant rabbits

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1 We have used anaesthetized, acutely instrumented non-pregnant (NP) and late pregnant (P) New Zealand white rabbits to examine the possible role of nitric oxide (NO) in the pregnancy-induced fall of vascular tone and arterial pressure. Systemic, renal and pulmonary vascular resistance, as well as plasma concentrations of cyclic GMP (PcGMP) were compared before and after the inhibition of NO synthesis by N^G-nitro-L-arginine methyl ester (L-NAME).

2 P rabbits had lower baseline total peripheral resistance (TPR), mean arterial pressure (MAP) and higher PcGMP than NP controls (all $P<0.05$ or less). L-NAME (1, 10, 50 mg kg^{-1}, i.v.) resulted in dose-dependent elevation of TPR in both groups. However, the absolute, as well as percentage increases in TPR were greater ($P<0.05$) in NP than in P rabbits..

3 Cardiac output (CO) was reduced more ($P<0.01$) by NO inhibition in NP than P rabbits. Therefore, despite the smaller increase in TPR, the elevation of MAP was greater ($P<0.001$) in P than NP rabbits. After L-NAME, NP rabbits developed more severe bradycardia and a greater increase of pulmonary vascular resistance which might have contributed to the more pronounced reduction of CO.

4 PcGMP increased in both groups following L-NAME, but more ($P<0.01$) in NP than P rabbits.

5 Infusion of acetylcholine (ACh, 0.02 μmol l^{-1} kg^{-1}) reduced MAP and TPR more (both $P<0.05$) in NP than P rabbits and L-NAME reduced the ACh-induced depressor response only in NP rabbits.

6 These results suggest that the low vascular tone and arterial pressure in pregnant rabbits is not mediated by NO.

Keywords: Pregnancy; hypotension; vascular tone; cardiac output; pulmonary resistance; nitric oxide; cyclic GMP; L-NAME; acetylcholine

Introduction

Normal pregnancy is associated with systemic vasodilatation, hypotension, and vascular refractoriness to various pressor hormones (DeSwiet, 1995). The elucidation of the mechanisms of these vascular changes is important, since they fail to develop or disappear in pregnancies complicated with hypertension (Gant *et al.*, 1973). Recently, attention has been focused on the possible involvement of the vasodilator L-arginine-nitric oxide (NO) - guanosine 3':5'-cyclic monophosphate (cyclic GMP) system. Thus, Goetz *et al.* (1994) observed an enhanced vasodilator response to acetylcholine in isolated aortic rings of pregnant rats and hypothesized that endothelial NO release by acetylcholine is increased during pregnancy in the rat. Weiner *et al.* (1994) using uterine arteries, the heart, kidney and other organs of guinea-pigs showed the induction of a calcium-dependent NO synthase during pregnancy. Conrad and Vernier (1989) in studies on pregnant rats and earlier, Kopp *et al.* (1977) in studies on pregnant women measured higher plasma levels and urinary excretion of cyclic GMP, the intracellular messenger of NO (Katsuki *et al.*, 1977). Finally, Conrad *et al.* (1993a) recorded enhanced nitrite and nitrate concentrations (metabolites of NO) in body fluids during pregnancy of the rat.

Thus, it seems that synthesis of NO is enhanced during pregnancy, but its significance in the lowering of systemic vascular resistance and blood pressure is still in doubt. A greater elevation of arterial pressure in pregnant animals than in non-pregnant animals following administration of various drugs that are known to inhibit NO synthesis has been considered as an indication of greater NO synthesis-induced hypotension in pregnant animals. Similarly, since such agents might lead to a larger potentiation of the blood pressure-elevating effect of various pressor hormones in pregnant than non-pregnant animals, this raises the possibility that NO may be responsible for the blunting of responsiveness to pressor agents in pregnancy. In experiments performed on whole animals Umans *et al.* (1990) studied conscious, chronically implanted rats and found that N-methyl-arginine, a NO synthase inhibitor produced similar elevations of mean arterial pressure (MAP) and falls in heart rate in pregnant and virgin rats. On the other hand, Molnár & Hertelendy (1992) who also used conscious rats, demonstrated that inhibition of NO synthesis produced a greater elevation of MAP in pregnant rats, but the absolute value of MAP of non-pregnant rats remained higher than that of the pregnant rats just as before NO synthesis inhibition. Similarly, when Chu and Beilin (1993) inhibited NO synthesis in anaesthetized rats, both non-pregnant and pregnant animals showed a 40 mmHg increase of MAP and the pregnant rats remained relatively hypotensive. Moreover, the pressor hyposensitivity of pregnant rats to noradrenaline, but not to angiotensin II was reversed by NO blockade. In contrast, Nathan *et al.* (1995), who used anaesthetized, ganglion-blocked rats obtained a larger elevation of MAP in pregnant than in non-pregnant rats upon NO synthase inhibition, but found that this manoeuvre restored normal pressor responsiveness to angiotensin II, but not to phenylephrine.

The interpretation of these data is difficult, since the baseline vascular tone and its change during the infusion of pressor agents was evaluated indirectly, from the induced changes in arterial pressure. However, inhibition of NO synthesis induces vasoconstriction not only in the systemic, but also in the pulmonary (Persson *et al.*, 1990; Perrella *et al.*, 1991) and cor-

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onary (Amezcua *et al.*, 1989) vasculature and the latter will tend to decrease cardiac output (CO) and blunt the elevation of arterial pressure induced by systemic vasoconstriction. In other words, conclusions based on systemic blood pressure measurements alone, can be misleading.

Therefore, in the present study, we have used anaesthetized rabbits, in which we have recorded changes of MAP, CO, total peripheral resistance (TPR), heart rate (HR), stroke volume (SV), mean pulmonary arterial pressure (MPAP), pulmonary vascular resistance (PVR), renal blood flow (RBF), renal vascular resistance (RVR), and plasma cyclic GMP concentration (PcGMP). We have compared the changes induced in these variables by intravenous administration of N^G-nitro-L-arginine methyl ester (L-NAME), a potent NO synthase inhibitor, in late pregnant and age-matched non-pregnant New Zealand white rabbits.

Methods

Preparations

A total of 22 female non-pregnant [NP, body weight 3.6 ± 0.1 kg (mean $\pm$ s.e.)] and 23 late pregnant (P, body weight 3.9 ± 0.1 kg, 25th–30th days of the 30–32 days gestation) New Zealand white rabbits were individually housed and given standard rabbit chow and tap water *ad libitum*.

Experiments were performed under general anaesthesia induced by intravenous (i.v.) pentobarbitone [slow bolus of 60 mg kg^{-1} in 4 ml kg^{-1} sterile physiological saline (NaCl) + 10 mg kg^{-1}, given in 1 ml kg^{-1} as a constant infusion] into the right marginal ear vein. Rabbits of Series 1 and 3 breathed via a tracheotomy tube. Mean arterial pressure [diastolic pressure + 1/3 pulse pressure (MAP, mmHg)] and heart rate (HR, 1 min^{-1}) were monitored through a cannula containing 50 u heparin ml^{-1} NaCl which was inserted into the right femoral artery. The cannula was connected to a pressure transducer (BRP-01, Experimetria, Budapest, Hungary) and a blood pressure coupler (HG-02, Experimetria). The right femoral vein was catheterized for administration of L-NAME (Sigma, St. Louis) and acetylcholine (Sigma). A heparin-NaCl filled polyethylene cannula was introduced into the right jugular vein and advanced 5 cm deep from the thyroid cartilage so that its tip lay in the central venous space. The right carotid artery was cannulated so that a thermistor (PTH-01, Experimetria) could be inserted with its sensor tip in the aortic arch (7.0–7.5 cm from the thyroid cartilage), as described earlier (Losonczy *et al.*, 1993; 1995). Then, through a left paralumbar incision the left renal artery was exposed retroperitoneally and a 1RB Transonic Flow probe was placed around it, as described previously (Brown & Venuto, 1991). Renal blood flow (RBF) was measured by Transonic Flowmeter 101 (Transonic Systems, Ithaca, New York).

In rabbits of Series 2 the tracheotomy tube was connected to a Harvard dual-phase control respirator pump and the animal was ventilated at 35–45 breaths min^{-1} with a tidal volume of 10–14 ml kg^{-1} body weight. Arterial P_{O_2} was set between 65–110 mmHg and P_{CO} $SCP9_2$ between 17–32 mmHg (Radelkis, Type OP-925/2, Budapest). Before the chest was opened a dextran (average mol. weight 40 000 D, Rheomacrodex, Human, Godollo) infusion, 5 ml kg^{-1} 40 min^{-1} (into the left marginal ear vein) was started. Thoracotomy was performed by the removal of a 2 cm portion of the sternum between the 1st and 3rd intercostal spaces. After completion of surgery the infusion of dextran was stopped. Haematocrit of arterial blood was $42 \pm 1\%$ before and $39 \pm 1\%$ after thoracotomy. The anterior wall of the right ventricle was punctured by a 20G needle and a polyethylene cannula (PE-10) containing heparin-NaCl was slipped through the needle and advanced 3 cm into the main pulmonary artery. Placement was considered correct if diastolic pressure was $\geqslant 8$ mmHg. After the animal had been killed at the end of the experiment the position of the pulmonary cannula was always verified. These thoracotomized rabbits were also instrumented for the measurement of MAP and CO as described above.

In Series 1, 2 and 3, CO was measured by thermodilution by using a CO-100 Cardiac Output Computer (Experimetria). MAP, HR, SV and TPR were also calculated by the CO-100. RVR was calculated as MAP divided by RBF. PVR was calculated as MPAP divided by CO.

The blood temperature of all rabbits was maintained between 38–40°C with a heating pad. The surgical procedures were followed by a 60 min stabilization period.

Protocols

In Series 1, the systemic and renal haemodynamic effects of NO blockade were tested. Haemodynamic parameters were sampled 3 times, 5 min apart and their mean was considered as baseline. Then L-NAME in 50 mg ml^{-1} of NaCl was injected as an i.v. bolus, as successive doses of 1, 10 and 50 mg kg^{-1} given 17 min apart. The effects of each of these doses were tested at 5, 10 and 15 min after the bolus injection. Typically, the L-NAME induced changes were fully developed by 5 min and plateaued between 10 and 15 min. The mean of the 10 and 15 min readings was therefore considered as representative of maximum change evoked. In the open-chest animals of Series 2 a similar protocol was followed except that only the effects of 10 mg kg^{-1} L-NAME were studied.

In Series 3 on closed-chest animals, the actions of acetylcholine (ACh, 0.02 μmol min^{-1} kg^{-1}, in 0.1 ml NaCl min^{-1} kg^{-1} for 10 min) were tested. ACh infusion induced a steady-state fall of MAP and TPR between the 2nd and 10th min of infusion, and therefore the average of the 4th, 6th, 8th and 10th min readings was considered as representative of the maximum effect. After the ACh infusion was stopped, the rabbits were allowed to recover and 60 min later L-NAME, in a dose of 10 mg kg^{-1}, was administered i.v. When the elevation of MAP and TPR plateaued, the effect of infusion of ACh was re-tested.

Cyclic GMP assay

Plasma and urinary concentrations of cyclic GMP were measured by radioimmunoassay (RIA). Urine was collected from Series 1 rabbits placed in metabolic cages for 48 h before experiments. During the acute experiments blood (3 ml) was collected from the same animals into chilled tubes containing Na_2-EDTA, before the first CO measurements and 15 min after the 10 mg kg^{-1} dose of L-NAME. Plasma was separated by centrifugation at 3000 *g* for 15 min at 4°C. Plasma and urine samples were stored at −20°C. After being thawed, plasma samples were diluted fourfold with ethanol, allowed to stand for 1 h at 4°C, and centrifuged at 2000 *g* for 10 min. The supernatant was evaporated to dryness under reduced pressure, redissolved in phosphate buffer (50 mM, pH 7.3) to give an appropriate dilution of 1:10–1:100. Urine samples were centrifuged and assayed directly after dilution (1:10 000–1:30 000) by buffer. The cyclic GMP RIA was developed by I. Mucha. In this assay the [^{125}I]-succinyl-tyrosine-methyl ester derivative of cyclic GMP (Brooker *et al.*, 1979) competes with acetyl-cyclic GMP prepared *in situ* (Steiner *et al.*, 1972), for a specific antibody raised in rabbits against 2′-0-succinyl-cyclic GMP bound to human serum albumin (Harper *et al.*, 1975). Magnetizable particles coated with anti-rabbit secondary IgG were used to separate the bound, from free fraction. This assay is characterized by an average sensitivity of 1 fmol, and negligible cross-reaction with closely related compounds.

The urinary excretion of cyclic GMP ($U_{cGMP}V$) was expressed relative to creatinine excretion in order to reduce the influence of changes in renal function. The creatinine concentration was measured by colorimetry.

Results are presented as means $\pm$ s.e. Baseline data of NP and P rabbits were compared by paired *t* test. The effects of

treatments in the two groups were compared by ANOVA of two-factor experiments, followed by the least significance difference test. $P<0.05$ was considered significant.

Results

Table 1 summarizes the baseline haemodynamic values in the closed-chest non-pregnant (NP) and pregnant (P) rabbits of Series 1. In P rabbits, MAP and TPRI were lower, and plasma cyclic GMP concentrations higher, than in NP controls. The tendency for the increase in $U_{cGMP}V$ to be greater in P rabbits did not reach statistical significance. CO index (COI), HR, SV index (SVI) and RVR index (RVRI) did not differ between P and NP animals, but RBF was lower in P than NP rabbits. The administration of 1, 10 and 50 mg kg^{-1} L-NAME to Series 1 animals led to a dose-dependent elevation of MAP in P, but not in NP rabbits (Figure 1a). The blood pressure elevation of NP rabbits reached its maximum after the 1 mg kg^{-1} dose. After the injection of 50 mg kg^{-1}, the MAP of NP rabbits often fell below the original baseline, so that the average MAP was around baseline. In contrast, P animals showed a maximum change of $+22\pm4$ mmHg ($P<0.001$ vs NP) after the 50 mg kg^{-1} dose. On the other hand, L-NAME caused a dose-dependent reduction of COI in NP, but not in P rabbits (Figure 1b). After the 50 mg kg^{-1} dose COI was more than 40% below baseline in NP, but only 12% below baseline in P rabbits. TPRI increased dose-dependently in both groups, the change being steeper ($P<0.025$) in NP than in P rabbits (Figure 1c). Four additional P rabbits received 250 mg kg^{-1} L-NAME, but the resultant elevations of MAP and TPRI were identical to those observed after the 10 mg kg^{-1} dose. The reduction of COI was associated with bradycardia and reduced SVI (Figure 2a, b), the bradycardia being more pronounced in NP rabbits. The change induced by L-NAME in RVRI was moderate and not different between P and NP animals (Figure 3a, b). The concentration of cyclic GMP increased significantly when assessed after the 10 mg kg^{-1} (1 + 10 mg kg^{-1} cumulative) dose of L-NAME in both groups (Figure 4), but the elevation was greater in NP than P rabbits.

Table 2 summarizes the baseline pulmonary haemodynamic parameters in Series 2 (thoracotomized) animals. MPAP was similar, COI tended to be higher and PVRI somewhat lower in P than NP rabbits but these differences did not reach significance. Following the administration of 10 mg kg^{-1} L-NAME, the elevation of PVRI was 160% in NP and only 70% in P rabbits ($P<0.01$, Figure 5a, b, c).

In Series 3, before the infusion of acetylcholine (ACh), NP and P rabbits had an average MAP of 109 ± 6 and 71 ± 6 mmHg ($P<0.001$), COI of 144 ± 17 and 145 ± 10 ml min^{-1} kg^{-1} (NS), and TPRI of 0.82 ± 0.12 and 0.52 ± 0.05 mmHg. kg ml^{-1} min^{-1} ($P<0.001$), respectively. Figure 6 shows that the infusion of ACh caused a larger fall of MAP and TPRI in NP than in P rabbits. COI remained unchanged in both groups. Administration of L-NAME reduced the difference in ACh-induced depressor responses, because the NP, but not the P animals showed a smaller vasodilatation to ACh after L-NAME.

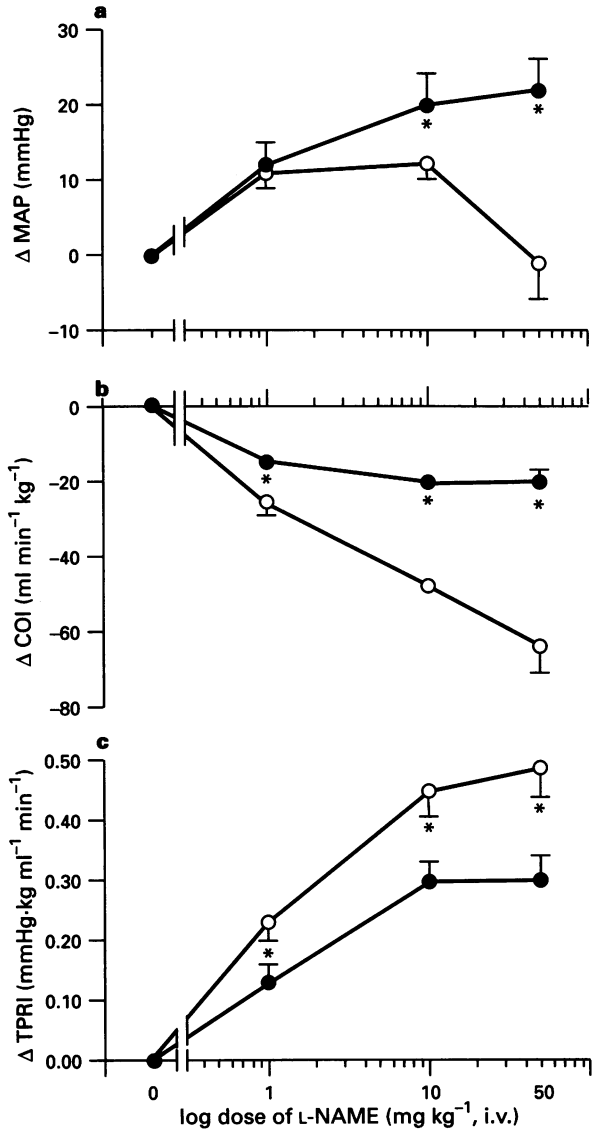

Figure 1 Series 1: changes (mean ± s.e) of (a) mean arterial pressure (ΔMAP), (b) cardiac output index (ΔCOI) and (c) total peripheral resistance index (ΔTPRI) following the intravenous injection of increasing doses of L-NAME, an inhibitor of NO synthesis. (○) Non-pregnant ($n=9$), (●) pregnant ($n=7$) rabbits; *$P<0.05$.

Table 1 Systemic haemodynamic and humoral variables of Series 1 rabbits at baseline

	Non-pregnant (n = 9)	*Pregnant* (n = 7)	P
MAP (mmHg)	105 ± 2	88 ± 3	<0.01
HR (min^{-1})	293 ± 8	275 ± 6	NS
COI (ml min^{-1} kg^{-1})	152 ± 6	150 ± 10	NS
SVI (ml kg^{-1})	0.52 ± 0.02	0.56 ± 0.04	NS
TPRI (mmHg. kg ml^{-1} min^{-1})	0.70 ± 0.03	0.56 ± 0.04	<0.05
RBF (ml min^{-1} kg^{-1})	17.6 ± 1.5	12.6 ± 1.3	<0.05
RVRI (mmHg. kg ml^{-1} min^{-1})	6.3 ± 0.5	7.3 ± 0.8	NS
PcGMP (fmol ml^{-1})	144 ± 23	261 ± 21	<0.01
$U_{cGMP}V$ (μmol μmol $creat^{-1}$)	237 ± 47	330 ± 42	NS

Results shown are means ± s.e. Abbreviations: MAP-mean arterial pressure, HR-heart rate, COI-cardiac output index, SVI-stroke volume index, TPRI-total perpherial resistance index, RBF-renal blood flow, RVRI-renal vascular resistance index, PcGMP-plasma cyclic GMP concentration, $U_{cGMP}V$-urinary excretion of cyclic GMP/urinary excretion of creatinine.

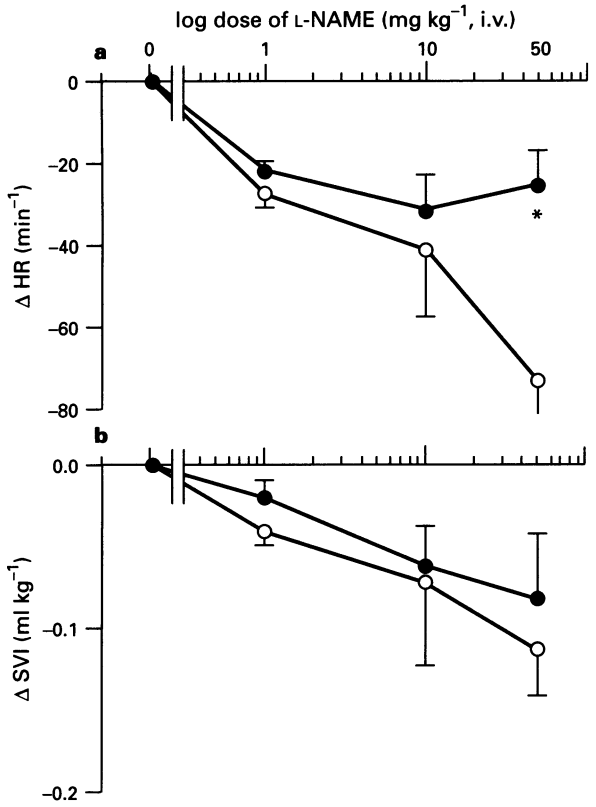

Figure 2 Series 1: changes (mean±s.e) of (a) heart rate (ΔHR) and (b) stroke volume index (ΔSVI) following the injection of L-NAME. (○) Non-pregnant ($n=9$), (●) pregnant ($n=7$) rabbits; *$P<0.05$.

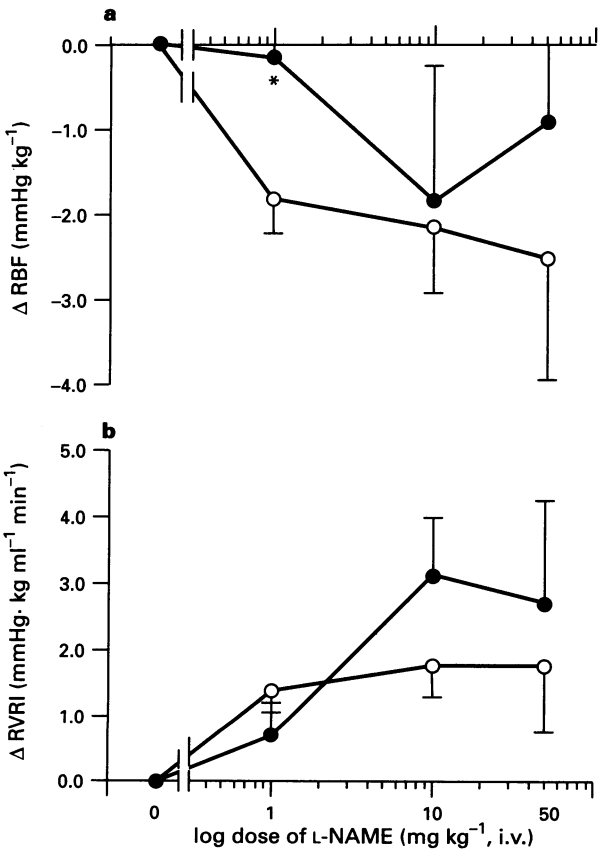

Figure 3 Series 1: changes (mean±s.e.) of (a) renal blood flow (ΔRBF) and (b) renal vascular resistance index (ΔRVRI) following the injection of L-NAME. (○) Non-pregnant ($n=9$), (●) pregnant ($n=7$) rabbits, *$P<0.05$.

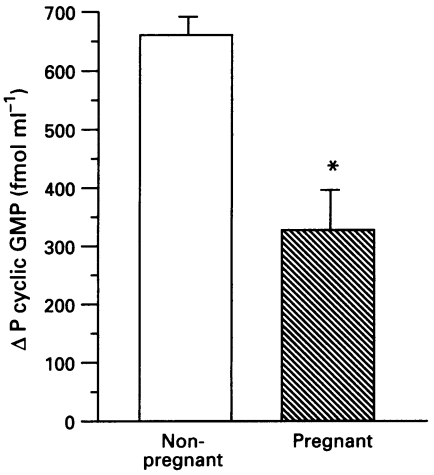

Figure 4 Series 1: changes (mean±s.e.) of plasma cyclic GMP concentration (ΔP cyclic GMP) in non-pregnant ($n=9$) and pregnant ($n=7$) rabbits after the administration of L-NAME ($P<0.001$ vs pre-L-NAME in both groups). $P<0.005$ vs non-pregnant rabbits.

Table 2 Pulmonary haemodynamics of Series 2 rabbits at baseline

	Non-pregnant (n=7)	*Pregnant (n=6)*	P
MPAP (mmHg)	15±1	15±1	NS
COI (ml min^{-1} kg^{-1})	113±5	135±16	NS
PVRI (mmHg. kg. ml^{-1} min^{-1})	0.13±0.0	0.12±0.1	NS

Data shown are means±s.e. Abbreviations: MPAP-mean pulmonary arterial pressure, COI-cardiac output index, PVRI-pulmonary vascular resistance index.

Discussion

The main finding of these experiments is that the elevation of MAP after the inhibition of NO synthesis was greater in P than NP rabbits only because of the relative maintenance of CO in P and not because of more severe peripheral vasoconstriction. The tonic synthesis of NO affects CO through the regulation of cardiac function (Katsuki *et al.*, 1977; Perrella *et al.*, 1991; Grocott-Mason *et al.*, 1994) and pulmonary vascular resistance in NP animals (Persson *et al.*, 1990; Perrella *et al.*, 1991). In the present study the blockade of NO synthesis induced greater bradycardia and pulmonary hypertension in NP than P rabbits. Thus, normal cardiac and pulmonary function may depend on normal NO synthesis more in NP than P rabbits. Cardiac depression and pulmonary hypertension reduced CO so significantly that the otherwise greater systemic vasoconstriction induced by L-NAME in NP rabbits resulted in less and not greater elevation of systemic arterial pressure in NP than P animals. These findings are partially consistent with earlier published data on the greater blood pressure elevating effect of NO inhibition in pregnancy (Ahokas *et al.*, 1991; Molnár & Hertelendy, 1992; Nathan *et al.*, 1995). However, our present data provide a different interpretation, namely, that the greater systemic blood pressure elevating effect of NO inhibition during pregnancy reflects a lower, not a greater dependence of systemic haemodynamics on tonic NO synthesis during pregnancy.

Cyclic GMP is the intracellular messenger synthesized by smooth muscle cells when they are triggered by NO (Katsuki *et al.*, 1977) and pregnancy has been shown to increase plasma and urinary cyclic GMP content in rats (Conrad & Vernier, 1989) and women (Kopp *et al.*, 1977). The present study showed that plasma and urinary cyclic GMP are also elevated

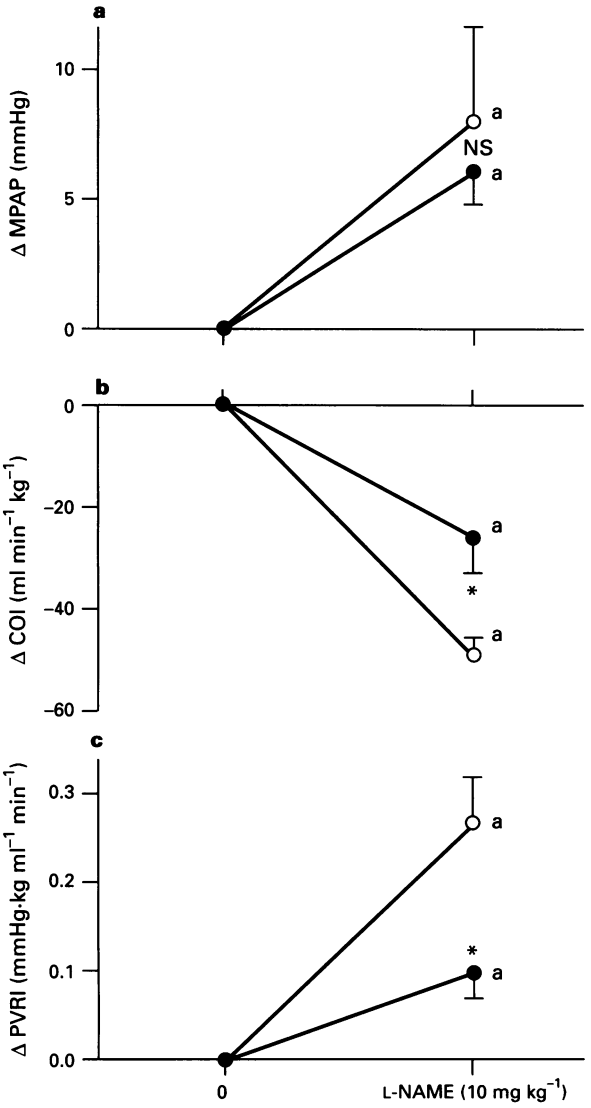

Figure 5 Series 2: changes (mean±s.e.) of (a) mean pulmonary arterial pressure (ΔMPAP), (b) cardiac output index (COI) and (c) pulmonary vascular resistance index (ΔPVRI) after the administration of L-NAME. (○) Non-pregnant ($n=7$), (●) pregnant ($n=6$) rabbits; ($^{a}P<0.05$ vs pre-L-NAME), $*P<0.05$, and NS: not significant, vs non-pregnant rabbits.

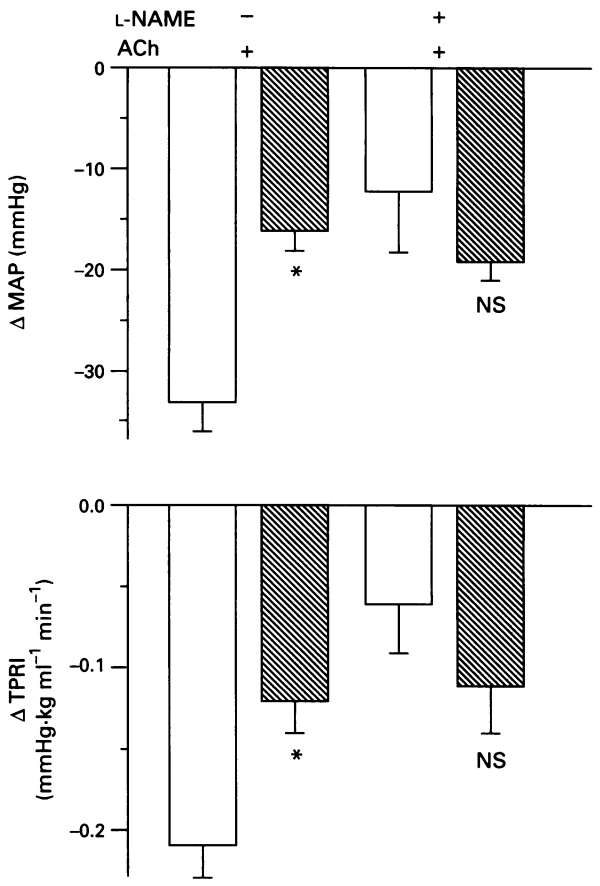

Figure 6 Series 3: changes (mean±s.e.) of mean arterial pressure (ΔMAP) and total peripheral resistance index (ΔTPRI) during the infusion of acetylcholine (ACh) in unpretreated and L-NAME pretreated non-pregnant (open columns, $n=6$) and pregnant (hatched columns, $n=6$) rabbits. $*P<0.05$, NS: not significant, vs non-pregnant rabbits.

in the rabbit during pregnancy. However, it seems questionable, whether this can be taken as a sign of enhanced NO synthesis. Cyclic GMP synthesized by soluble guanylate cyclase probably does not readily leave the cells (Arnal *et al.*, 1992) and a poor correlation was found between the urinary excretion of nitrite+nitrate and that of cyclic GMP (Sütö *et al.*, 1995). Our finding of increased and not decreased plasma cyclic GMP concentration in both NP and P rabbits during severe inhibition of NO synthesis (as evidenced by generalized vasoconstriction) further documents the independence of extracellular cyclic GMP level and NO synthesis. It may be that elevation of plasma and urinary cyclic GMP concentrations are more powerful indices of atrial natriuretic peptide (ANP, Hirata *et al.*, 1987). Indeed, ANP has been shown to be released in the rat and rabbit during myocardial ischaemia (Baertschi *et al.*, 1986) and we speculate that the higher cyclic GMP concentrations in our rabbits after L-NAME administration may reflect myocardial ischaemia-induced release of ANP. Since the fall of CO, SV and HR, as well as the elevation of cyclic GMP were more pronounced in NP than P rabbits, such an interpretation seems plausible. It may be noted that Arnal *et al.* (1992) have also demonstrated elevated plasma cyclic GMP concentration in rats during the administration of L-NAME.

Acetylcholine (ACh) induces vasodilatation through the release of NO, prostacyclin and an endothelium-derived hyperpolarizing factor (Bauersachs *et al.*, 1994; Umans & Levi, 1995). Kim *et al.* (1994) reported that mesenteric, but not renal arteries of P guinea-pigs showed larger vasodilatation to ACh than those of NP controls and that this pregnancy-induced enhancement of ACh-induced mesenteric vasodilatation disappeared after prior blockade of the synthesis of NO. In the present study no evidence was found for a more reactive systemic vasodilator response to ACh in P rabbits. Rather, the fall of MAP and TPR induced by ACh was less in P than in NP rabbits. In addition, the difference between the ACh responses in the two groups was abolished when they were treated with the NO synthase inhibitor L-NAME. This may indicate that NP rabbits responded with a larger release of, or response to, NO than P rabbits and not *vice versa*. This suggestion accords with the findings of St. Louis & Massicotte (1992) that the pregnancy-induced blunting of pressor responses of rat blood vessels was not mediated by endothelium.

The present experiments were performed in anaesthetized, acutely prepared animals. Since, at least in theory, surgical stress may influence circulatory control differently in P and NP rabbits, the usefulness of observations made in such preparations may be questioned. However, in conscious, chronically-instrumented rabbits COI was around 200 ml min^{-1}. kg in both P and NP animals (Losonczy *et al.*, 1993; 1995) as

compared to the value of 150 ml min^{-1}. kg measured in both P and NP rabbits of the present study. Further, TPRI was proportionately higher by about 70%, in both P and NP rabbits, under anaesthesia than in conscious P and NP rabbits (Losonczy *et al.*, 1993; 1995). Thus, anaesthesia and surgery do not seem to influence differentially baseline haemodynamics in NP and P rabbits and the pregnancy-induced falls in MAP and TPR were well maintained.

In summary, we demonstrated that both the stimulation and the inhibition of NO synthesis have less and not more impact on vascular tone in P than in NP rabbits. Therefore, we speculate that although NO may contribute to the maintenance of reduced vessel tone in some vascular regions during pregnancy (e.g. uteroplacental (Conrad *et al.*, 1993a,b), renal (Danielson & Conrad, 1995)), the reduced total peripheral resistance, at least in rabbits, is mediated by an as yet unidentified mechanism.

This study was supported by OTKA (5187) and the Hungarian Kidney Foundation. The authors are grateful to Mrs Daisy Kertai for technical expertise.

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(Received December 19, 1995
Revised February 8, 1996
Accepted February 12, 1996)