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Lack of beneficial effects of the NO-donor, molsidomine, in the L-NAME-induced pre-eclamptic syndrome in pregnant rats

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1 In pregnant rats, chronic NO-synthase inhibition induces the development of a pre-eclamptic syndrome, characterized by an increase in maternal blood pressure, a loss of vascular refractoriness to pressor stimuli, a reduction in litter size and a decrease in pups (and maternal) weight. We investigated whether a NO-donor, molsidomine, administered during NO synthase inhibition, could restore a normal pregnancy.

2 Pregnant rats were given daily, starting from day 14 of gestation, saline (controls), or L-NAME (50 mg $kg^{-1} d^{-1}$), or molsidomine (15 or 30 mg $kg^{-1} d^{-1}$), or the L-NAME + molsidomine combinations. Maternal blood pressure and body weight, litter size, pups weight and vascular reactivity to pressor stimuli (angiotensin II, noradrenaline, electrical stimulation of the spinal cord) were investigated.

3 L-NAME alone, as compared to controls, increased maternal blood pressure, reduced litter size (−59%), increased foetal reabsorptions (+625%) and decreased foetal weight (−10%). Vascular reactivity to pressor stimuli was enhanced.

4 Molsidomine alone, as compared to controls, dose-dependently decreased maternal blood pressure but had no effect on vascular reactivity and, whatever the dose, on foetal outcome.

5 The L-NAME-molsidomine combinations dose (of molsidomine)-dependently limited the rise in maternal blood pressure induced by L-NAME alone but unexpectedly, dose-dependently and significantly worsened pregnancy evolution, e.g., at 30 mg $kg^{-1} d^{-1}$: litter size (−80%), foetal reabsorptions (+1025%), foetal weight (−24%). Vascular reactivity to pressor stimuli was paradoxically further enhanced.

6 Thus, in a chronic NO deprivation-induced model of pre-eclampsia in rats, molsidomine, possibly because of its hypotensive action, worsens the foetal outcome, which questions the usefulness of NO-donors in pre-eclamptic women.

Keywords: Pre-eclampsia; nitric oxide; L-NAME; molsidomine; vascular pressor reactivity; foetal outcome; pregnant rats

Introduction

Normal pregnancy is associated with a hyperdynamic state characterized by increased plasma volume and increased cardiac index and uterine artery blood flow, but, due to a decrease in systemic vascular resistance, arterial pressure is decreased. There is accumulating evidence that generation of nitric oxide (NO) by the vascular endothelium plays a crucial role in the control and modulation of vascular tone during normal pregnancy in human subjects (Myatt *et al.*, 1991; Weiner *et al.*, 1994) but also in rats (Conrad *et al.*, 1993; Goetz *et al.*, 1994). There is also evidence that endothelial cell dysfunction is present in women with a pre-eclamptic syndrome and that the resulting decrease in NO but also in prostacyclin synthesis is responsible for the clinical disorders characterizing pre-eclampsia, i.e., a reduced placental perfusion, an increased sensitivity to vasopressor agents, an activation of the coagulation cascade (Roberts *et al.*, 1991; Rutherford *et al.*, 1995; Baker *et al.*, 1996) and a progressive increase in peripheral resistance and in blood pressure. Thus, a reduced NO synthesis linked to a decreased NO synthase expression or activity has been reported in pre-eclamptic patients (Pinto *et al.*, 1991; Seligman *et al.*, 1994; Delacretaz *et al.*, 1995) although it is disputed by others (Baker *et al.*, 1995). Moreover, the plasma of pre-eclamptic patients has been shown (a) to contain a fraction that inhibits the ability of acetylcholine to relax pre-contracted rabbit aortic rings (Pinto *et al.*, 1992), but also (b) to reduce endothelial prostacyclin production (Ramsay *et al.*, 1994). Finally, raised plasma concentrations of an endogenous NO synthase inhibitor have also been described in pre-eclamptic women (Fickling *et al.*, 1993). In this context, supplementation with a NO donor might prove to alter favourably the evolution of the pre-eclamptic syndrome.

The present study was thus undertaken in order to test this hypothesis and investigate whether the administration of the endogenous NO synthase-independent NO donor, molsidomine, would be able, in a rat model of pre-eclampsia (Molnar & Hertelendy, 1992; Yallampalli & Garfield, 1993), (a) to oppose the increase in maternal blood pressure and to restore the reduced vascular responsiveness to vasopressor agents observed in normal pregnant rats, and (b) to normalize litter size and foetal weight.

Methods

Animals

Virgin Wistar rats (Iffa Credo, L'Arbresle, France) weighing 250-260 g were used and maintained at a constant temperature (24°C) in a 12-h light-dark cycle. All experiments were performed in accordance with the regulations of the French Ministry of Agriculture for animal health care.

After mating, the day of conception was determined by the presence of spermatozoa in the vaginal lavage. Two experimental protocols were conducted.

Protocol 1

On day 14 of gestation, the animals were randomized into four groups ($n = 4-5$ in each group). The first group received no treatment (controls), the second one was given N^G-nitro-L-arginine methylester (L-NAME, 50 mg $kg^{-1} d^{-1}$, by gavage), the third one received molsidomine (15 mg $kg^{-1} d^{-1}$, in the

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drinking fluid at a concentration based on liquid intake) and the fourth one was given the L-NAME (50 mg kg^{-1} d^{-1})-molsidomine (15 mg kg^{-1} d^{-1}) combination. Standard chow diet and water were available *ad libitum* for all animals and changed daily. Every day, all animals were weighed and systolic blood pressure (SBP) was measured in the conscious state using the tail cuff method and a photoelectric pulse detector (PC model 139 IITC, Woodland Hills, CA, U.S.A.) according to Bunag & Butterfield (1982). Gestation duration was measured and, after delivery, pups were removed, numbered and immediately weighed individually.

Protocol 2

Telemetric transmitters were implanted in female rats 7–10 days before mating to allow recovery of body weight. Mean (MBP), systolic and diastolic (DBP) blood pressures as well as heart rate (HR) were monitored during prepregnancy and pregnancy (Data Quest IV System, Data Sciences Inc., St-Paul, MI, U.S.A.). Gestation duration, maternal body weight, number of live pups born and pups body weight have previously been shown not to be affected by transmitter implantation (Azar & Sanchez-Pena, 1991). Data were collected every 20 min as a wave form curve for 10 s. Mean values were then calculated for 24-h intervals.

On day 13 of gestation, the pregnant animals were randomized into four groups ($n=4-5$ in each group). The first group received no treatment (controls), the second one was given L-NAME (50 mg kg^{-1} d^{-1}, by gavage), the third one received molsidomine (30 mg kg^{-1} d^{-1}, in the drinking fluid at a concentration based on liquid intake) and the fourth one was given the L-NAME (50 mg kg^{-1} d^{-1})-molsidomine (30 mg kg^{-1} d^{-1}) combination. Thirteen additional and age-matched non pregnant females received by gavage for 7 days either tap water (controls, $n=6$) or L-NAME (50 mg kg^{-1} d^{-1}, $n=7$).

On day 20 of gestation, the rats were weighed and anaesthetized with sodium pentobarbitone (30 mg kg^{-1}). They were pithed, bivagotomized and ventilated with room air (Harvard respirator, model 680, Southnatick, MA, U.S.A.). The carotid artery was cannulated (PE50) for blood pressure (Statham P10EZ, preamplifier model 134615–50, Gould Instruments, Ballainvilliers, France) and HR (Biotach amplifier, model 13–4615–66, Gould Instruments, Ballainvilliers, France) measurements. Blood pressure and HR were continuously recorded on a data management system (Graphtec Mark 12, Bioseb, Paris, France). The animals were then given atropine sulphate (1 mg kg^{-1}, i.v.) and gallamine (20 mg kg^{-1}, i.v.). Systemic pressor and cardiac responses to (a) increasing doses of noradrenaline (0.01–3 μg kg^{-1}, 1 ml kg^{-1}) and angiotensin II (10–300 ng kg^{-1}, 1 ml kg^{-1}), and (b) to electrical stimulation of the spinal cord (SSC) (0.25–2 Hz, 1 ms pulses, 60 V for 20 s, stimulator ST 198, Janssen Scientific Instruments, Paris, France) were recorded in the different experimental groups. The changes (from baseline) in MBP and HR were measured for each drug dose and for each stimulation frequency when the peak rise in pressure occurred. Parameters were allowed to return to baseline before each subsequent drug dose or stimulation frequency challenge.

At the end of the experiments, the uterus and their contents were removed, the number of foetuses, the number of foetal reabsorptions due to early foetal death, and finally the weights of individual foetuses were determined.

Statistical analysis

All data are expressed as mean±s.e.mean. Comparisons of mean values were carried out by analysis of variance followed by Student's *t* test. Mean absolute changes in MBP or HR induced by increasing frequencies of electrical stimulation of the spinal cord or increasing doses of noradrenaline or angiotensin II were analyzed by analysis of variance for repeated measurements using the Greenhouse-Geisser adjustment according to Ludbrook (1994). A *P* value less than 0.05 was considered to be statistically significant. Statistical analyses were performed using a BMDP Statistical Software (BMDP, Los Angeles, CA, U.S.A.).

Drugs

Drugs used were angiotensin II (Hypertensin, Ciba-Geigy, Basle, Switzerland), atropine sulphate (Sigma Chemical Co., Saint-Quentin-Fallavier, France), gallamine triiodide (Rhône-Poulenc Rorer, Vitry-sur-Seine, France), N^G-nitro-L-arginine methylester (L-NAME, Sigma Chemical Co., Saint-Quentin-Fallavier, France), molsidomine (Laboratoires Hoechst, Paris la Défense, France), pentobarbitone sodium (Abbott Laboratories, Saint-Rémy-sur-Avre, France) and (−)-noradrenaline bitartrate (Sigma Chemical Co., Saint-Quentin-Fallavier, France).

Doses are expressed in terms of the salt.

Results

Evolution of maternal parameters during pregnancy

Protocol 1: From day 14 of gestation until parturition, maternal body weight (BW) was less although not significantly so in L-NAME-treated rats than in controls (Figure 1). Although molsidomine had no effect *per se* on BW, the L-NAME-molsidomine combination further decreased BW, and the BW

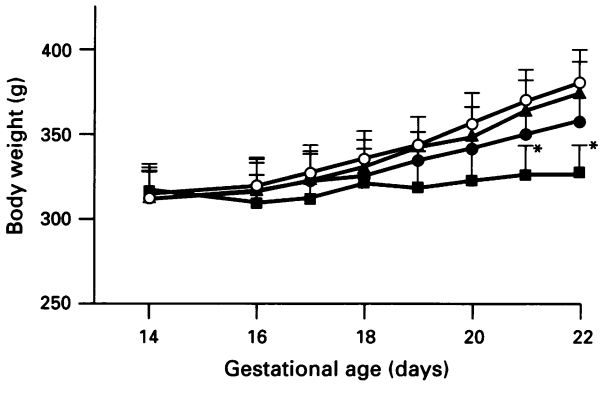

Figure 1 Evolution of maternal body weight in control pregnant rats (○) or in pregnant rats treated daily from day 14 of gestation with either L-NAME (50 mg kg^{-1} d^{-1}, ●), or molsidomine (15 mg kg^{-1} d^{-1}, ▲), or the L-NAME + molsidomine combination, ■) (Protocol 1). Values are means±s.e.mean. *$P<0.05$ vs controls.

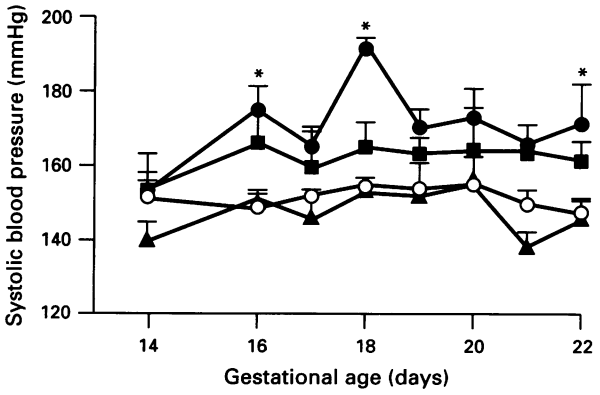

Figure 2 Evolution of maternal systolic blood pressure in control pregnant rats (○) or in pregnant rats treated daily from day 14 of gestation with L-NAME (50 mg kg^{-1} d^{-1}, ●), or molsidomine (15 mg kg^{-1} d^{-1}, ▲), or the L-NAME + molsidomine combination, ■) (Protocol 1). Values are means±s.e.mean. *$P<0.05$ vs controls.

values achieved at parturition were significantly lower than in controls (Figure 1).

SBP was significantly greater in L-NAME-treated rats than in controls. Molsidomine, 15 mg kg^{-1} d^{-1}, did not affect SBP. In rats treated with the L-NAME-molsidomine combination, SBP was intermediate between those of L-NAME-treated and of control rats, but not significantly different from either of them (Figure 2).

Protocol 2: Figure 3 illustrates the 24-h profiles of SBP and DBP values recorded by telemetry on day 19 of gestation in the four experimental groups. In control rats, the circadian SBP and DBP patterns showed a greater variability during dark-time. Blood pressure in L-NAME-treated rats was consistently greater than that of controls throughout the period of the experiment. Molsidomine reduced SBP and DBP during the dark period. Finally, and as compared to L-NAME-treated rats, blood pressure in animals treated with the L-NAME-molsidomine combination was almost similar during the light period but strongly reduced during the dark one (Figure 3). Table 1 summarizes the mean SBP and DBP values calculated on day 20 over the last 24-h recording period. As compared to controls, L-NAME significantly increased (+16/+17%) whereas molsidomine, 30 mg kg^{-1} d^{-1}, significantly decreased (−7/−9%) SBP and DBP. In the animals treated with the L-NAME-molsidomine combination, SBP and DBP were significantly greater (+6/+10%) than in controls but significantly smaller (−9/−6%) than in L-NAME-treated

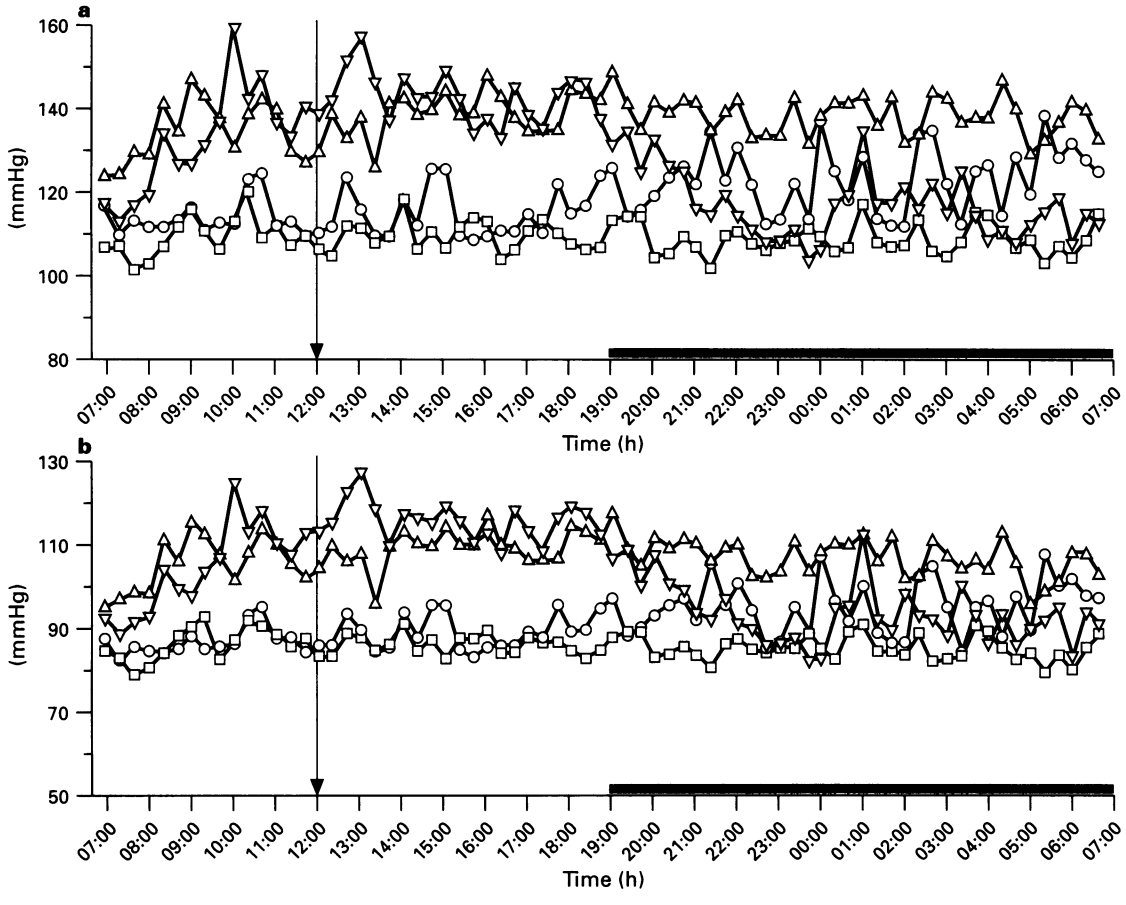

Figure 3 Evolution of maternal systolic (a) and diastolic (b) blood pressures as monitored by telemetry over a 24-h period on day 19 of gestation in control pregnant rats (○, $n=2$) and in pregnant rats treated daily from day 13 of gestation with L-NAME (50 mg kg^{-1} d^{-1}, △, $n=4$), or molsidomine (30 mg kg^{-1} d^{-1}, □, $n=3$), or the L-NAME + molsidomine combination, (▽, $n=3$) (Protocol 2). Arrow indicates the time of L-NAME or tap water oral administration. Solid line indicates the period of darkness (19h.00min to 07h 00min).

Table 1 Effects of a seven days-L-NAME (50 mg kg^{-1} d^{-1}), or molsidomine (30 mg kg^{-1} d^{-1}) or L-NAME (50 mg kg^{-1} d^{-1}) + molsidomine (30 mg kg^{-1} d^{-1}) administration to pregnant rats on day 20 of gestation (Protocol 2)

		Treatments		
Parameter	*0 (controls)*	*L-NAME*	*Molsidomine*	*L-NAME + Molsidomine*
Systolic blood pressure (mmHg)	119.3±1.0	138.8±0.8*	110.7±1.7*	126.7±1.5*,[a]
Diastolic blood pressure (mmHg)	91.3±0.8	107.0±0.6*	84.6±0.5*	100.6±1.3*,[a]
Litter size (*n*)	12.3±0.6	5.0±1.9*	11.5±1.3[a]	2.4±1.5*
Foetal reabsorptions (*n*)	0.8±0.5	5.8±2.8	1.3±0.6	9.0±2.2*
Foetal weight (g)	3.85±0.11	3.46±0.15	3.68±0.12	2.91±0.12*,[a]

Values are mean±s.e.mean. *Value significantly different from corresponding control group value: P at least <0.05. [a]Value significantly different from corresponding L-NAME group value: P at least <0.05.

animals. During the five preceding 24-h recording periods (from day 14 to day 19), similar findings were made regarding mean SBP and DBP values, e.g., as early as day 14, L-NAME increased (+16/+16%), molsidomine decreased (−6/−8%) and the L-NAME-molsidomine combination increased (+7/+9%) SBP and DBP as compared to control pregnant rats.

Interestingly, despite the higher SBP values provided by the tail-cuff method (Protocol 1) as compared to the telemetric method (Protocol 2), the blood pressure increasing effect of L-NAME estimated by the two methods was similar (approximately +16% after 6 days of treatment).

Foetal effects

Gestation duration, as compared to that measured in control animals, was not modified by any of the treatments (L-NAME, molsidomine or their combination) in Protocol 1 (data not shown).

In both protocols, and as compared to controls, litter size was reduced by L-NAME (Tables 1 and 2), the effect (−59%) being significant in Protocol 2 (Table 1). This reduction was even greater, dose (of molsidomine)-dependent and significant in both protocols (−65 and −80% in Protocols 1 and 2, respectively) in the L-NAME-molsidomine combination groups (Tables 1 and 2). Molsidomine, whichever the dose, had no effect on litter size.

Foetal reabsorption sites (Protocol 2) were strongly increased in the L-NAME (+625%) and in the L-NAME-molsidomine combination (+1025%) groups, the effect being significant in the latter (Table 1). Molsidomine *per se* had no effect.

Foetal weight measured at birth (Protocol 1, Table 2) was significantly reduced (as compared to controls) and to the same extent by both L-NAME (−16%) and the L-NAME-molsidomine combination (−17%), whereas molsidomine, 15 mg kg^{-1} d^{-1}, had no effect. When measured on day 20 of gestation (Protocol 2, Table 1), L-NAME (−10%, NS) and more markedly ($P<0.05$) the L-NAME-molsidomine combination (−24%, $P<0.05$) reduced foetal weight as compared to controls, whereas molsidomine, 30 mg kg^{-1} d^{-1}, had no effect.

Maternal vascular and cardiac reactivity

Table 3 summarizes the basal MBP and HR values measured in the anaesthetized pithed pregnant (on day 20 on gestation) and age-matched non pregnant rats from the six experimental groups. As can be seen, MBP values were almost identical in the four groups of pregnant rats and somewhat smaller, although not significantly so, than those measured in non pregnant rats. In addition, HR values measured in the six experimental groups were not significantly different from each other.

Tables 4 and 5 show the systemic pressor responses elicited by noradrenaline and angiotensin II and Figure 4 illustrates the systemic pressor and cardiac responses to SSC, in the six experimental groups.

It appears from these tables and figure that the systemic pressor (and tachycardia for SSC) responses to the three stimuli are consistently and significantly greater in the non pregnant control than in the pregnant control animals. L-NAME increased, although not significantly, the pressor responses to noradrenaline, angiotensin II and SSC in the pregnant rats, but these responses remained smaller, although not significantly so, than those elicited in control non pregnant animals. In non pregnant rats, L-NAME also slightly enhanced the pressor responses to noradrenaline and angiotensin II.

In pregnant animals, molsidomine had no effect on the noradrenaline and angiotensin II responses and slightly potentiated the SSC responses. However, the L-NAME-molsidomine combination strongly and significantly potentiated the pressor responses to noradrenaline, angiotensin II and SSC, the values achieved with noradrenaline and angiotensin being even greater, although not significantly so, than those obtained in non pregnant control animals (Tables 4 and 5, Figure 4).

Table 2 Mean (±s.e.mean) values of litter size and foetal weight in control pregnant rats and in pregnant rats treated daily from day 14 of gestation by L-NAME (50 mg kg^{-1} d^{-1}), or molsidomine (15 mg kg^{-1} d^{-1}) or L-NAME (50 mg kg^{-1} d^{-1}) + molsidomine (15 mg kg^{-1} d^{-1}) (Protocol 1)

	Treatments			
Parameter	*0 (controls)*	*L-NAME*	*Molsidomine*	*L-NAME+molsidomine*
Litter size (*n*)	11.4±1.5	9.3±2.5	11.8±1.0	4.0±2.4*
Foetal weight (*g*)	5.92±0.05	5.05±0.10*	5.91±0.04	4.97±0.07*

*Values significantly different from corresponding control group value: P at least <0.05.

Table 3 Basal mean (±s.e.mean) values of blood pressure and heart rate in the anaesthetized pithed animals of the six experimental groups (Protocol 2)

Groups	n	*Mean blood pressure* (mmHg)	*Heart rate* (beats min^{-1})
Pregnant rats			
Controls	4	58.3±4.5	356±16
L-NAME	3	58.7±2.3	360±6
Molsidomine	3	55.7±3.9	343±16
L-NAME+molsidomine	4	54.7±2.6	362±27
Non pregnant rats			
Controls	6	68.5±3.5	343±7
L-NAME	7	64.2±4.7	334±10

n = number of animals.

Table 4 Mean (±s.e.mean) variations of mean arterial pressure induced by noradrenaline in the control, L-NAME (50 mg kg^{-1} d^{-1})-, molsidomine (30 mg kg^{-1} d^{-1})- or the L-NAME (50 mg kg^{-1} d^{-1}) + molsidomine (30 mg kg^{-1} d^{-1}) combination-treated pregnant rats, and in the control and L-NAME-treated (50 mg kg^{-1} d^{-1}) non pregnant rats (Protocol 2)

	Noradrenaline (μg kg^{-1})					
Groups	*0.01*	*0.03*	*0.1*	*0.3*	*1*	*3*
Pregnant rats						
Controls	4.1±0.5	4.9±1.5	10.7±2.9	21.3±5.4	45.7±6.5	71.5±6.6
L-NAME	5.5±2.0	5.8±1.9	11.5±2.5	25.4±3.0	53.1±11.7	97.0±10.2
Molsidomine	5.0±0.6	4.1±1.1	10.5±2.8	16.1±2.0	48.7±5.3	82.9±6.9
L-NAME+molsidomine[a]	5.7±1.4	11.0±2.6	19.2±3.3	34.9±3.7	69.5±4.9	114.0±10.8
Non pregnant rats						
Controls[a]	4.6±1.5	3.6±1.0	16.0±2.1	34.3±3.3	66.6±4.5	104.8±5.9
L-NAME	7.0±1.5	8.1±1.8	19.0±2.5	38.4±3.8	75.5±4.8	119.0±4.2

[a]Dose-response curve significantly different from that obtained in control pregnant rats: $P<0.05$.

Table 5 Mean (±s.e.mean) variations of mean arterial pressure induced by angiotensin II in the control, L-NAME (50 mg kg^{-1} d^{-1})-, molsidomine (30 mg kg^{-1} d^{-1})- or the L-NAME (50 mg kg^{-1} d^{-1}) + molsidomine (30 mg kg^{-1} d^{-1}) combination-treated pregnant rats, and in the control and L-NAME-treated (50 mg kg^{-1} d^{-1}) non pregnant rats (Protocol 2)

Groups	*Angiotensin II (ng kg^{-1})* 10	30	100	300
Pregnant rats				
Controls	8.1±0.9	15.8±0.7	30.8±3.7	51.5±3.8
L-NAME	9.3±0.5	19.3±1.9	35.0±3.1	61.0±3.8
Molsidomine	8.4±1.5	14.0±2.8	27.9±3.9	56.6±4.8
L-NAME + molsidomine[a]	18.6±6.2	31.1±7.5	51.3±8.7	77.3±10.4
Non pregnant rats				
Controls[a]	17.7±1.7	27.8±1.9	47.0±3.0	66.8±5.1
L-NAME	22.8±5.2	34.6±5.7	54.8±6.1	79.3±5.0

[a]Dose-response curve significantly different from that obtained in control pregnant rats: $P<0.05$.

Regarding the SSC-induced tachycardia, the latter was affected neither by L-NAME (pregnant and non pregnant animals), nor by molsidomine nor the L-NAME-molsidomine combination (pregnant animals) (Figure 4).

Discussion

It appears from this study that, contrary to our hypothesis, co-administration to pregnant rats of a NO-donor, molsidomine, and of an inhibitor of NO-synthesis, L-NAME, does not suppress the pre-eclamptic syndrome induced by L-NAME alone, and even further worsens the foetal outcome.

The rat experimental pre-eclampsia model we chose was derived from that previously described (Molnar & Hertelendy, 1992; Yallampalli & Garfield, 1993) and consisted in the daily oral administration of L-NAME (50 mg kg^{-1} d^{-1}) to pregnant rats, starting on day 13–14 of gestation. This procedure mimics in pregnant rats the classical signs of the pre-eclamptic syndrome, i.e., a progressive increase in total peripheral resistance and in blood pressure (Molnar & Hertelendy, 1992; Yallampalli & Garfield, 1993; this study), a reduction in plasma volume expansion (Salas *et al.*, 1995) and, probably because of a vasoconstriction and a relative ischaemia in the foeto-placental unit, a reduction in placental blood flow (Ramsay *et al.*, 1994) with, as a result, intrauterine growth retardation, increase in foetal reabsorptions, and reductions in litter size and in foetal (and maternal) weight (Yallampalli & Garfield, 1993; Molnar *et al.*, 1994; Salas *et al.*, 1995; this study).

Regarding vascular reactivity, the vasopressor responses to angiotensin II and noradrenaline which are decreased during normal pregnancy (Gant *et al.*, 1987; Molnar & Hertelendy, 1992), have been shown to be restored to a large extent after NO synthesis blockade (Molnar & Hertelendy, 1992; Nathan *et al.*, 1995). Our study confirms this blunting of refractoriness to vasopressor agents after L-NAME and extends it to SSC, an endogenous vasoconstrictor stimulus. It should however be stressed that the vasopressor responses achieved in our L-NAME-treated pregnant rats always remained smaller than those obtained in untreated non pregnant rats and that, in another study, vascular refractoriness to phenylephrine was not suppressed at all after L-NAME (Nathan *et al.*, 1995). Thus, if NO appears to play an important role in the development of reduced vascular reactivity during pregnancy, other factors are probably also involved, e.g., vasodilator prostaglandins (Venuto *et al.*, 1984), oestrogens (Shan *et al.*, 1994), etc . . . Another important point in our study is the demonstration that not only the vasoconstrictor but also the tachycardic responses to SSC are attenuated in pregnant vs non pregnant rats (Protocol 2). But interestingly, this attenuated tachycardia is not affected at all by L-NAME, indicating that NO is not in-

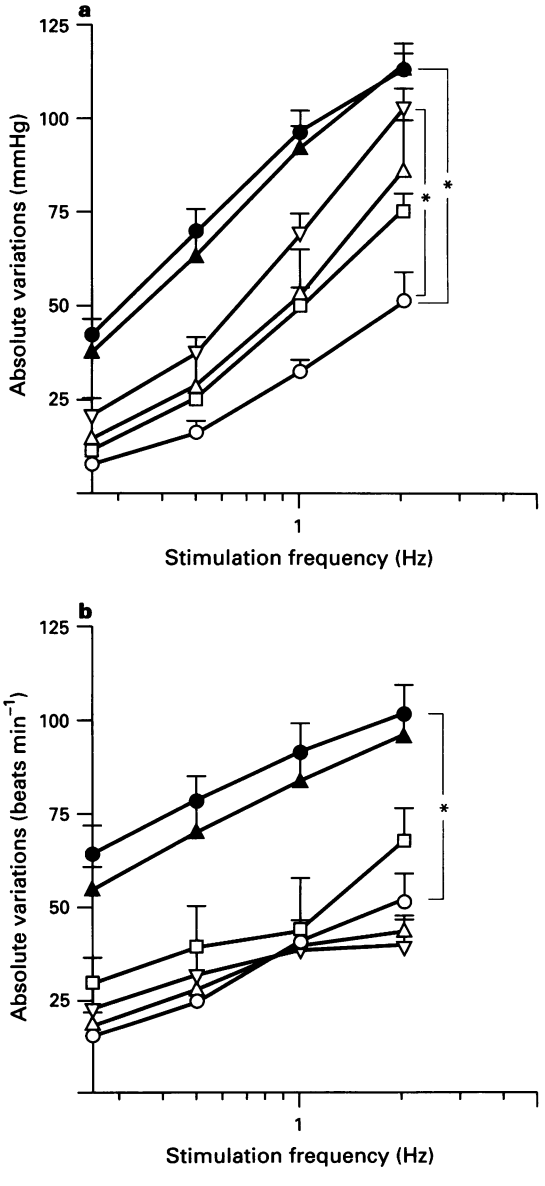

Figure 4 Mean±s.e.mean variations of mean arterial pressure (a) and heart rate (b) induced by electrical stimulation of the spinal cord in the control (○), L-NAME (50 mg kg^{-1} d^{-1}, △)-, molsidomine (30 mg kg^{-1} d^{-1}, □)-, or the L-NAME + molsidomine combination (▽)-treated pregnant rats, and in the control (●) and L-NAME-treated (50 mg kg^{-1} d^{-1}, ▲) non pregnant rats. *Stimulation frequency-response curve significantly different from that obtained in control pregnant rats, $P<0.05$.

volved in its mechanism and that other factors, e.g., a reduced cardiac sensitivity to catecholamines (Desimone *et al.*, 1988), possibly hormonally mediated, are responsible for its development during pregnancy.

In this experimental model of L-NAME-induced pre-eclampsia in rats, we investigated the effects of molsidomine. Our hypothesis was that adjunction of this drug might influence favourably, or even normalize, the course of pregnancy. This hypothesis was supported by the prior demonstration that, in pre-eclamptic women, infusion of glyceryl trinitrate improved uterine artery blood flow without simultaneously affecting SBP and HR (Ramsay *et al.*, 1994).

Molsidomine was chosen as it is a source of NO independent of the endogenous NO synthases and as it is not subject to the development of tolerance (Kuhn & Forstermann, 1989). Two doses were used which were clearly greater than that (3 mg kg^{-1}, orally) which induces in rats a significant increase in urinary nitrates and cyclic GMP excretion (Böger *et al.*, 1994). These doses were 15 mg kg^{-1} d^{-1}, which did not significantly reduce L-NAME-induced hypertension, and 30 mg kg^{-1} d^{-1}, which did significantly so, especially during the dark period when roaming behaviour is maximal. Furthermore, as shown by our data, these doses of molsidomine did not *per se* affect pregnancy duration, litter size and pup weight, a result in agreement with that previously reported with nitroglycerin (Yallampalli & Garfield, 1993).

Contrary to our hypothesis, molsidomine did not improve, and in fact worsened, the pre-eclamptic syndrome induced by L-NAME in pregnant rats. This worsening, which was already observed at the low dose of molsidomine (15 mg kg^{-1} d^{-1}), was clearly amplified at 30 mg kg^{-1} d^{-1}. It strikingly affected the litter size which was significantly reduced (vs L-NAME), the number of foetal reabsorptions which increased, and the foetal weight which was dose (of molsidomine)-dependently decreased, and significantly so at 30 mg kg^{-1} d^{-1} vs L-NAME alone. The mechanisms involved in this surprising finding are not clear. It may however be suggested that, because of the molsidomine-induced dose-dependent decreases in blood pressure (vs L-NAME), reductions in uteroplacental blood perfusion pressure occur, leading to relative placental ischaemia, foetal reabsorption and litter size reduction, intrauterine growth retardation and finally foetal weight decrease, all phenomenona that were observed in our study and which were all the more marked as the dose of molsidomine was greater. This hypothesis is in line with the clinical observation that in women with pre-eclampsia, it is important to maintain diastolic blood pressure above approximately 90 mmHg in order to preserve uteroplacental blood flow and foetal oxygenation and well being. Whether this explanation is correct needs further investigation, but if it were, it would support the view that, during pre-eclampsia, hypertension is at least partly useful in that it would ensure a sufficient uteroplacental perfusion pressure. Another explanation for the pregnancy worsening observed with the L-NAME-molsidomine combinations versus L-NAME alone might lie in the ability of molsidomine to generate not only nitric oxide but also superoxide anions (Feelisch *et al.*, 1989; Butler *et al.*, 1995), thereby increasing the likelihood of peroxynitrite generation. This could explain why molsidomine, in contrast to glycerol trinitrate, a NO donor that does not form peroxynitrite (Ramsay *et al.*, 1994), has no beneficial effects on uterine blood flow. A final explanation for the toxic interaction between L-NAME and molsidomine might be the following: in normal pregnant rats treated with molsidomine, the intact endothelium function helps to maintain uterine blood flow despite the drug-induced increase in venous capacitance, a compensatory mechanism that no longer operates in L-NAME-treated pregnant animals.

Whereas the L-NAME-molsidomine combinations were clearly devoid of any beneficial effect on pregnancy, they surprisingly suppressed vascular hyporeactivity to vasopressor stimuli in pregnant rats. If one admits that vascular refractoriness is linked to increased NO production during pregnancy and if partial recovery of reactivity thus appears logical during NO-synthase inhibition, one would have expected that molsidomine itself and in combination with L-NAME would also decrease vascular reactivity. In fact, molsidomine alone had no effect on reactivity. Furthermore, the molsidomine-L-NAME combination, as compared to L-NAME alone, increased the responses to vasopressor stimuli and even restored the responses usually observed in non pregnant rats. The differential reactivities to vasopressor stimuli exhibited in our study by the four groups of pregnant animals cannot be accounted for by differences in pre-existing vascular tone as in these pithed animals, basal MBP values were similar. Thus, the mechanism underlying the suppression of vascular refractoriness by the molsidomine-L-NAME combination in pregnant rats remains unknown at present and needs to be investigated further. Finally, it should be stressed that, in contrast to what is observed at the vascular level, the refractoriness to SSC-induced tachycardia observed in control pregnant rats versus control non pregnant rats, was not affected by L-NAME alone, by molsidomine alone, or by their combination, indicating that during pregnancy blunted vascular responses to pressor stimuli and reduced tachycardic responses to positive chronotropic stimuli do not develop through the same mechanism.

In conclusion, in a chronic NO deprivation-induced model of pre-eclampsia in rats, administration of the NO-donor, molsidomine, dramatically worsens the foetal outcome (litter size, pups weight, etc . . .) and opposes the loss of vascular but not of cardiac refractoriness. Whatever the mechanisms involved, the present data do not favour the administration of molsidomine to women with a pre-eclamptic syndrome.

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