ELSEVIER

European Journal of Pharmacology 371 (1999) 159–167

ejp

www.elsevier.nl/locate/ejphar

Adrenomedullin attenuates the hypertension in hypertensive pregnant rats induced by N^G-nitro-L-arginine methyl ester

Ikuko Makino [a,*], Kazuhiko Shibata [b], Yasuo Makino [a], Kenji Kangawa [c], Tatsuhiko Kawarabayashi [a]

[a] *Department of Obstetrics and Gynecology, School of Medicine, Fukuoka University, Fukuoka, 814-0150, Japan*
[b] *Research Laboratory of Biodynamics, School of Medicine, Fukuoka University, Fukuoka, 814-0150, Japan*
[c] *National Cardiovascular Center Research Institute, Fujishirodai, Suita, Osaka 565, Japan*

Received 3 December 1998; received in revised form 24 February 1999; accepted 2 March 1999

Abstract

We examined the effect of adrenomedullin on the cardiovascular system of an animal model for preeclampsia. An inhibitor of nitric oxide synthase, N^G-nitro-L-arginine methyl ester (L-NAME), was infused subcutaneously into rats at a constant rate from day 14 of pregnancy to make an animal model for preeclampsia. Adrenomedullin was continuously infused intravenously at a dose of 3 or 10 pmol/h from day 17 of pregnancy. The basal systolic blood pressure was significantly higher in the L-NAME treated rats than in the control rats. The adrenomedullin administration at day 19 of pregnancy showed a significant decrease in the blood pressure in the L-NAME treated rats than in vehicle rats during infusion. The blood pressure of normal pregnant rats did not significantly decrease by adrenomedullin infusion. The adrenomedullin decreased pup mortality of the L-NAME treated rats. Adrenomedullin attenuated the L-NAME induced hypertension and pup mortality. On the other hand, adrenomedullin administration in both pregnant rats in early gestation (5–11 days of pregnancy) and in non-pregnant rats did not show any significant effect on L-NAME-induced hypertension. The adrenomedullin mRNA level was predominantly expressed at high levels in the ovary, uterus and placenta, but at low levels in other tissues in pregnant rats in late gestation. The adrenomedullin mRNA level of the L-NAME treated rats in placenta decreased more than in the normal pregnant rats in late gestation ($P < 0.05$). These findings suggest that the adrenomedullin might play an important role in the regulation of the cardiovascular system of the mother and fetoplacental unit in rats.

Keywords: Adrenomedullin; Nitric oxide (NO); Preeclampsia; Pregnancy

1. Introduction

Preeclampsia is a common medical disorder of pregnancy. It is the leading cause of neonatal growth retardation, neonatal morbidity, mortality associated with premature delivery and maternal sickness. Pathophysiological changes, which include increases in the sensitivity to pressors and vascular permeability and activation of the coagulation cascade, suggest that vascular endothelial dysfunction might be an important component of this disorder.

Nitric oxide (NO), a potent vasodilator, is produced in the vascular endothelium and promotes vasodilation and also inhibits platelet aggregation (Moncada et al., 1991). A deficiency of NO could thus result in vasoconstriction, eventually leading to elevated blood pressure and local or disseminated intravascular coagulation, and thereby demonstrating the features of preeclampsia (Brown, 1991). Previous studies have demonstrated an important relationship between NO and blood pressure regulation in pregnancy. Several investigators reported the inhibition of NO synthesis with analogues of L-arginine such as N^G-nitro-L-arginine methyl ester (L-NAME) causes hypertension, proteinuria, fetal growth retardation, and increased fetal mortality without affecting gestational length (Yallampalli and Garfield, 1993; Molnar et al., 1994; Buhimschi et al., 1995). These phenomena are remarkably similar to

* Corresponding author. Tel.: +81-92-801-1011; Fax: +81-92-865-6032

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00151-X

preeclampsia. Therefore, L-NAME-treated rats has been recently used as an animal model for preeclampsia.

Adrenomedullin, a potent vasorelaxant/hypotensive peptide with 52 amino acid residues, was recently isolated from human pheochromocytoma by monitoring its activity to induce cAMP formation in platelets (Kitamura et al., 1993a). Adrenomedullin has a conserved structure among mammals such as rat and shows a partial homology with calcitonin gene-related peptide (CGRP). Human adrenomedullin has one intramolecular disulfide bond. The sequence homology of adrenomedullin with human CGRP and amylin is not so high. However, they share some structural features. A six-residue ring structure formed by an disulfide linkage and the carboxy-terminal amide structure were observed within the molecules (Kangawa et al., 1996). Thus, adrenomedullin is thought to belong to the CGRP superfamily. Subsequent studies have revealed that adrenomedullin immunoreactivity to be detected in the plasma (Nishikimi et al., 1994) while also being widely distributed in the lung, lung tumors, adrenal gland, heart atrium, kidney and pancreas (Martínez et al., 1995, 1996). The expression of mRNA for adrenomedullin has also been demonstrated in these tissues (Kitamura et al., 1993b). Endothelial cells are also known to produce adrenomedullin (Sugo et al., 1994). In addition, the plasma concentration of adrenomedullin has been demonstrated to increase in patients with essential hypertension compared with normotensive controls (Kitamura et al., 1994). Such evidence implies that adrenomedullin could participate in the physiological regulation of blood pressure and vascular homeostasis.

However, it has also recently been demonstrated that adrenomedullin suppresses the serum deprivation-induced apoptosis in rat endothelial cells (Kato et al., 1997). Isumi et al. (1998) have also demonstrated that adrenomedullin is synthesized and secreted from Swiss 3T3 cells while the adrenomedullin secreted from these cells stimulates the DNA synthesis of quiescent cells through the cAMP-mediated pathway. These findings suggest that adrenomedullin functions not only as a vascular tonus regulator, but also as a growth regulator.

Furthermore, recent preliminary report has demonstrated that the adrenomedullin exists in human amniotic fluid during the second trimester and its mRNA and protein were also found in the amniotic membranes (Macri et al., 1996). In addition, the plasma concentration of adrenomedullin has been shown to increase in human pregnancy (Di Iorio et al., 1997). These findings suggest that this peptide may play an important role in the reproductive physiology. However, no detailed tissue distribution of adrenomedullin mRNA in pregnancy has yet been reported. One purpose of this study is to clarify and examine the possible role of adrenomedullin in peeclampsia by using an animal model. Another purpose of this study is also to examine the precise distribution of adrenomedullin mRNA in pregnant rats.

2. Material and methods

2.1. Animals

Pregnant and non-pregnant rats (Wistar strain), 280–300 g, obtained from the Kyudo Animal Laboratory (Kumamoto, Japan) were maintained in an animal room with a 12 h light–dark cycle (0800–2000 h). The rats were divided into three groups including; pregnant rats in late gestation (13–20 days of pregnancy); pregnant rats in early-gestation (5–11 days of pregnancy), and non-pregnant rats. All animals were given free access to food and water. The guidelines approved by the animal research committee of Fukuoka University for the care and use of experimental animals were closely observed.

2.2. Induction of preeclampsia symptoms

Starting on day 5 (early gestation) or 14 (late gestation) of pregnancy, the rats received osmotic minipumps which delivered specific NO synthase inhibitor L-NAME (Nakarai Tesque, Kyoto, Japan), dissolved in a sterile saline solution, at a rate of 25 mg/day/rat, which was the same as that previously reported (Yallampalli and Garfield, 1993). In addition, non-pregnant rats were also administered L-NAME over an eight-day period. Osmotic minipumps (Model 2ML2, Alza, Palo Alto, CA, USA) were filled with saline with or without L-NAME and placed subcutaneously under light ether anaesthesia.

2.3. Administration of adrenomedullin

Osmotic minipumps (Model 2002, Alza) were filled with rat adrenomedullin (Peptide Institute, Osaka, Japan), and then were implanted subcutaneously from day 6 (early gestation) or 17 (late gestation) of pregnancy under light ether anaesthesia. In addition, non-pregnant rats were administered adrenomedullin over a six-day period. The pumps were connected to the left femoral vein by a polyethylene catheter (PE-10, Clay Adamus, USA) and were positioned in a pocket constructed with subcutaneous tissue just below the sub-scapular region, and then were continuously infused intravenously at a dose of 3 or 10 pmol/h. For the controls, sterile saline was infused in a similar manner.

2.4. Blood pressure measurement

The systolic blood pressure was measured with a pneumatic tail-cuff device (Riken Kaihatsu, Tokyo, Japan), after the animals were prewarmed on a heating blanket in a metal chamber maintained at 30°C. The rats were allowed to acclimate themselves to the restraining cage 2 times before the systolic blood pressure was measured. Each measurement was based on an average of five trials.

2.5. Northern blot analysis

The ovary, uterus, adrenal, placenta, liver, kidney, lung, heart, stomach, fetal brain, and mother brain were rapidly obtained on day 20 of pregnancy.

The total RNA was extracted using guanidine isothiocyanate according to the method used in a previous report (Makino et al., 1996). The RNA extract was quantified by measuring the absorbance at 260 nm.

The Northern blot analysis performed for adrenomedullin mRNA was essentially the same as that described in a previous report (Sakata et al., 1993).

Total RNA (10–30 μg) from various tissues were separated by formaldehyde/agarose gel electrophoresis and transferred to a nylon membrane. The 586 bp NaeI fragment derived from rat adrenomedullin cDNA (Sakata et al., 1993) was labeled by random primer labeling and used as a hybridization probe.

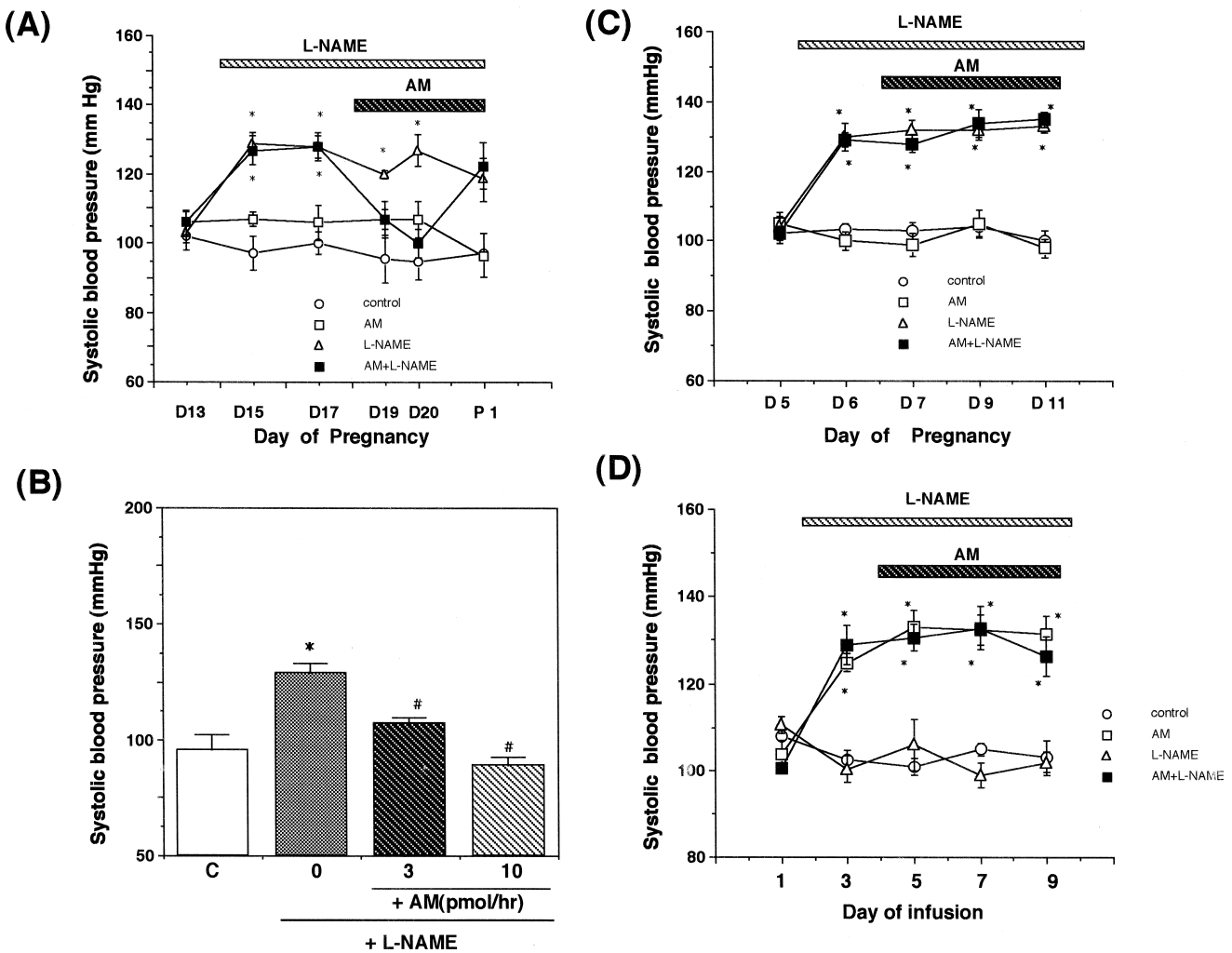

Fig. 1. (A) The effect of adrenomedullin, infused at a rate of 3 pmol/h either alone or in combination with 25 mg L-NAME per day, on the systolic blood pressure in pregnant rats in late gestation. L-NAME (25 mg/day) was administered subcutaneously with osmotic minipumps. Adrenomedullin was administered intravenously with osmotic minipumps. The control group was infused with saline alone. Blood pressure was measured on days 13, 15, 17, 19 and 20 of pregnancy. Each point represents the mean ± S.E.M. $^{*}P < 0.05$, compared with the control (Duncan multiple range test) ($n = 3$–8). AM: Adrenomedullin, L-NAME: N^{G}-nitro-L-arginine methyl ester, (○) control, (□) AM, (△) L- NAME, (■) AM + L-NAME. (B) Dose-dependent antagonistic effect of L-NAME induced hypertension on day 19 of pregnancy. $^{*}P < 0.05$, compared with control. $^{\#}P < 0.05$, compared with L-NAME treated group. (Duncan multiple range test) ($n = 3$–8). (C) The effect of AM, infused at a rate of 3 pmol/h either alone or in combination with 25 mg L-NAME per day, on systolic blood pressure in pregnant rats in early gestation. The blood pressure was measured on days 5, 6, 7, 9 and 11 of pregnancy. Each point represents the mean ± S.E.M. $^{*}P < 0.05$, compared with the control (Duncan multiple range test) ($n = 5$). AM: Adrenomedullin, L-NAME: N^{G}-nitro-L-arginine methyl ester, (○) control, (□) AM, (△) L-NAME, (■) AM + L-NAME. (D) The effect of AM, infused at a rate of 3 pmol/h either alone or in combination with 25 mg L-NAME per day, on systolic blood pressure in non-pregnant rats. The blood pressure was measured on days 1, 3, 5, 7 and 9 of infusion. Each point represents the mean ± S.E.M. $^{*}P < 0.05$, compared with the control (Duncan multiple range test) ($n = 4$–6). AM: Adrenomedullin, L-NAME: N^{G}-nitro-L-arginine methyl ester, (○) control, (□) AM, (△) L-NAME, (■) AM + L-NAME.

Table 1
Effects of adrenomedullin (AM) alone or in combination with N^G-nitro-L-arginine methyl ester (L-NAME) on the mortality rate and body weight of the pups

	Control	L-NAME (25 mg/rat)	L-NAME (25 mg/rat)		AM (3 pmol)
			AM (3 pmol)	AM (10 pmol)	
Body weight (g)	4.27 ± 0.40 ($n = 45$)	3.78 ± 0.20 ($n = 55$)	4.15 ± 0.13 ($n = 30$)	4.10 ± 0.10 ($n = 15$)	5.04 ± 0.60 ($n = 15$)
Pup mortality (%)	0.0 ± 0.0 ($n = 9$)	28.0 ± 6.4[a] ($n = 12$)	9.9 ± 4.6[b] ($n = 7$)	6.8 ± 2.1[b] ($n = 3$)	0.0 ± 0.0 ($n = 3$)

L-NAME dissolved in saline solution was administered s.c. from the osmotic minipumps, starting from day 14 of pregnancy. AM was administered i.v. by the osmotic minipumps. Pup mortality was expressed as the percentage of dead pups in each group.
[a] $P < 0.01$, compared with control.
[b] $P < 0.05$ compared with L-NAME treated group (Duncan multiple range test).

After hybridization, the blots were washed three times in 0.1 × SSC (15 mM NaCl, 1.5 mM sodium citrate)–0.1% SDS (sodium dodecyl sulfate) for 15 min at 65°C. The filters were then rehybridized with a radiolabelled G3PDH (glyceraldehyde 3-phosphate dehydrogenase) probe. The blots were exposed to X-ray film (Kodak, XAR-5) with an intensifying screen at −80°C for various lengths of time and then were scanned with a laser densitometer (Personal densitometer SI, Molecular Dynamics, USA) to determine the individual band density. The mean densitometric units derived from the controls were standardized to a value of 1.00.

2.6. Statistical analysis

All values were expressed as the mean ± S.E.M. The significance of the difference between the values from different groups was determined, using either Student's *t*-test or a one-way analysis of variance, followed by the Duncan multiple range test. A statistical analysis for repeated measurements was performed using the two-way analysis of variance.

3. Results

3.1. Effect of adrenomedullin on the systolic blood pressure measurements in L-NAME-induced hypertensive rats

The basal systolic blood pressure was 100.2 ± 8.2 mm Hg in animals in late gestation (day 14 of pregnancy), 102.5 ± 2.6 mm Hg in animals in early gestation (day 5 of pregnancy) and 103.9 ± 3.1 mm Hg in non-pregnant rats before treatment with saline or L-NAME, 25 mg/day. L-NAME produced a rise in the systolic blood pressure in all three groups. The blood pressure in the animals receiving dose of 25 mg/day was significantly higher than that of rats receiving saline solution only (130.0 ± 2.7 mm Hg in late gestation, 127.7 ± 3.1 mm Hg in early gestation, 133.0 ± 4.0 mm Hg in non-pregnant rats).

In animals in late gestation, as shown in Fig. 1(A), the administration of adrenomedullin (3 pmol/h) alone did not significantly affect the systolic blood pressure at any of the

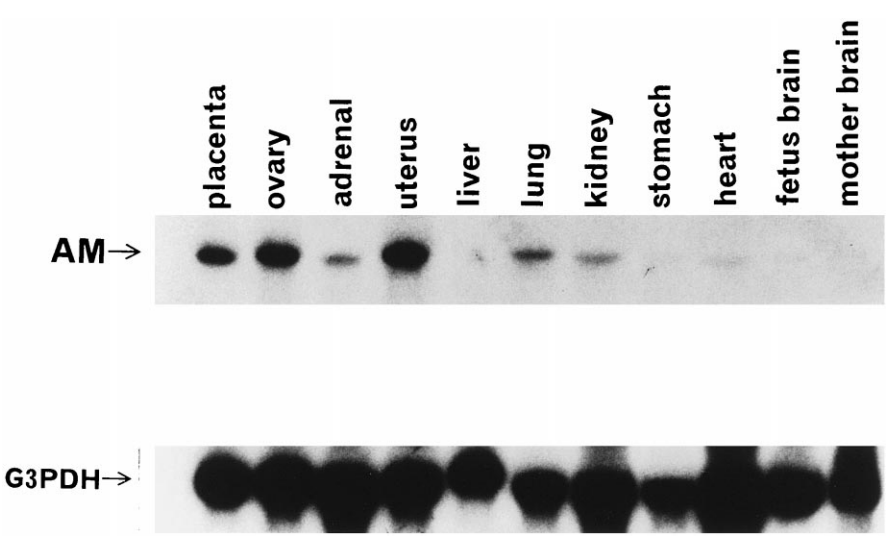

Fig. 2. A representative autoradiogram of a Northern blot analysis of adrenomedullin mRNA expression in various organs. A 30 μg of total RNA from each rat organs were loaded onto 1.5% agarose/formaldehyde gel. The gel was then blotted, hybridized with adrenomedullin cDNA and G3PDH cDNA probe. AM: adrenomedullin, G3PDH: glyceraldehyde 3-phosphate dehydrogenase.

Table 2
Relative amount of AM mRNA level in various tissues in pregnant rat (day 20 of pregnancy)

Areas	Relative amount of AM mRNA
Placenta	1.00
Ovary	1.49
Adrenal	0.18
Uterus	1.91
Liver	0.07
Lung	0.47
Kidney	0.19
Stomach	0.01
Heart	0.06
Fetus brain	0.03
Mother brain	0.11

AM mRNA level was determined by a Northern blot analysis.
The relative amounts of AM mRNA are expressed as the mean ratios in comparison with the corresponding mRNA levels in the placenta ($n = 3$).

five points when compared to the control group. The administration of 3 pmol/h adrenomedullin on days 18, 19 and 20 of pregnancy exerted a significant decrease in the systolic blood pressure in the L-NAME-treated rats. In addition, as shown in Fig. 1(B), adrenomedullin (3 or 10 pmol/h) produced a dose-related decrease in blood pressure in L-NAME-treated rats. However, the L-NAME-induced increase in blood pressure after delivery was not suppressed by adrenomedullin infusion. These findings indicated that adrenomedullin reversed the L-NAME-induced hypertension during pregnancy.

On the other hand, in either early gestation or non-pregnant rats, as shown in Fig. 1(C) and (D), the administration of adrenomedullin (3 pmol/h) did not significantly affect the L-NAME-induced hypertension.

3.2. Effect of adrenomedullin on the fetal mortality and body weight in animals in late gestation

The mortality of the fetuses was examined on day 20 of pregnancy. As shown in Table 1, the L-NAME-treated rats had a significantly higher fetal mortality than the controls. When adrenomedullin was administered to the L-NAME-treated rats, a substantial decrease in mortality was observed in a dose-dependent manner. No change in mortality was seen with adrenomedullin alone. However, the weight of the pups in L-NAME-treated rats was somewhat lower than in the control, but the difference was not significant.

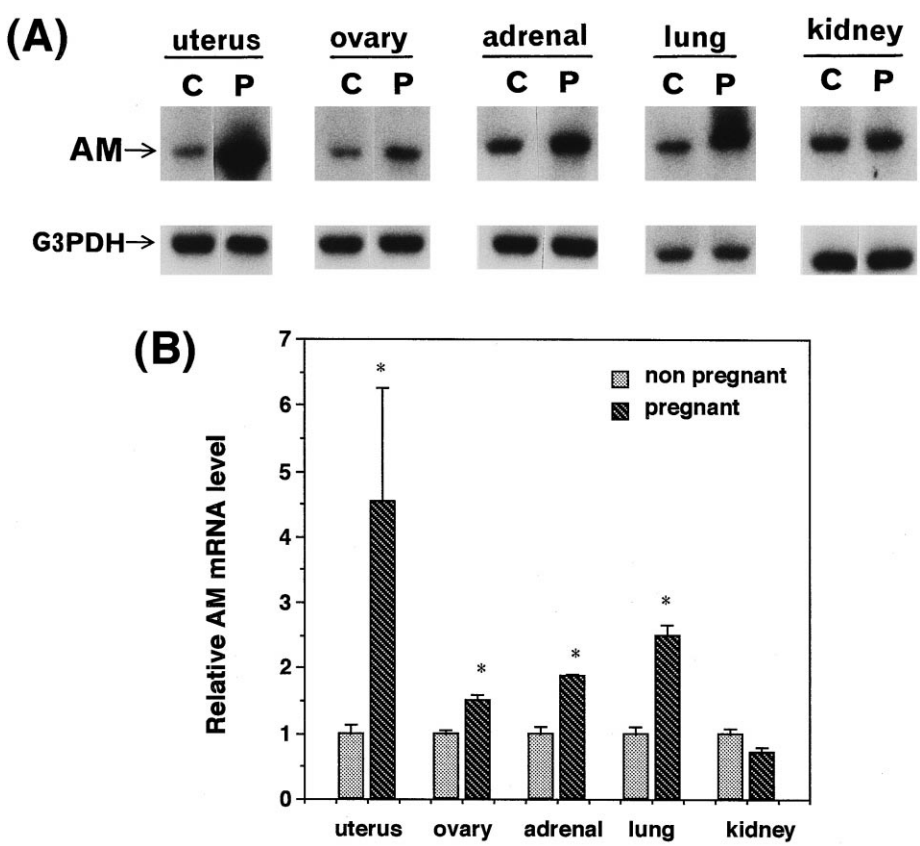

Fig. 3. The adrenomedullin mRNA expression in various organs in non- pregnant and pregnant rats. (A) A representative autoradiogram of adrenomedullin mRNA expression. C: Control (non-pregnant rat), P: Pregnant rat. (B) The relative amounts of adrenomedullin mRNA in various organs in non-pregnant and pregnant rats. The mRNA level was determined by a Northern blot analysis. The adrenomedullin mRNA level in non-pregnant rats in various organs were set at 1.00 and were compared with pregnant rats. Further details are shown in Fig. 1. $^{*}P < 0.05$ (Student's t-test) ($n = 6$).

3.3. Relative distribution of adrenomedullin mRNA in pregnant rats in late gestation

As shown in Fig. 2, adrenomedullin mRNA was shown as a clear single band and was the same size as that of all organs studied. These sizes, about 1.6 kb, were also consistent with those from a previous report (Sakata et al., 1993). However, the autoradiogram demonstrated adrenomedullin mRNA to be unevenly expressed at the respective organs studied. The placenta and uterus particularly contained a high amounts of adrenomedullin mRNA (Table 2). The adrenal also contained high amounts of adrenomedullin mRNA as reported previously (Sakata et al., 1993). However, other organs contained comparatively lower amounts.

We also compared the adrenomedullin mRNA expression of the uterus, ovary, kidney, adrenal and lung between pregnant (day 20 of pregnancy) and non-pregnant rats. As shown in Fig. 3(A) and (B), the adrenomedullin mRNA level of the uterus was extremely higher in pregnant rats than that in non-pregnant rats. In addition, the mRNA levels of the ovary, adrenal and lung were significantly higher in pregnant rats than that in non-pregnant rats. However, the levels in the kidney did not show any differences between these groups.

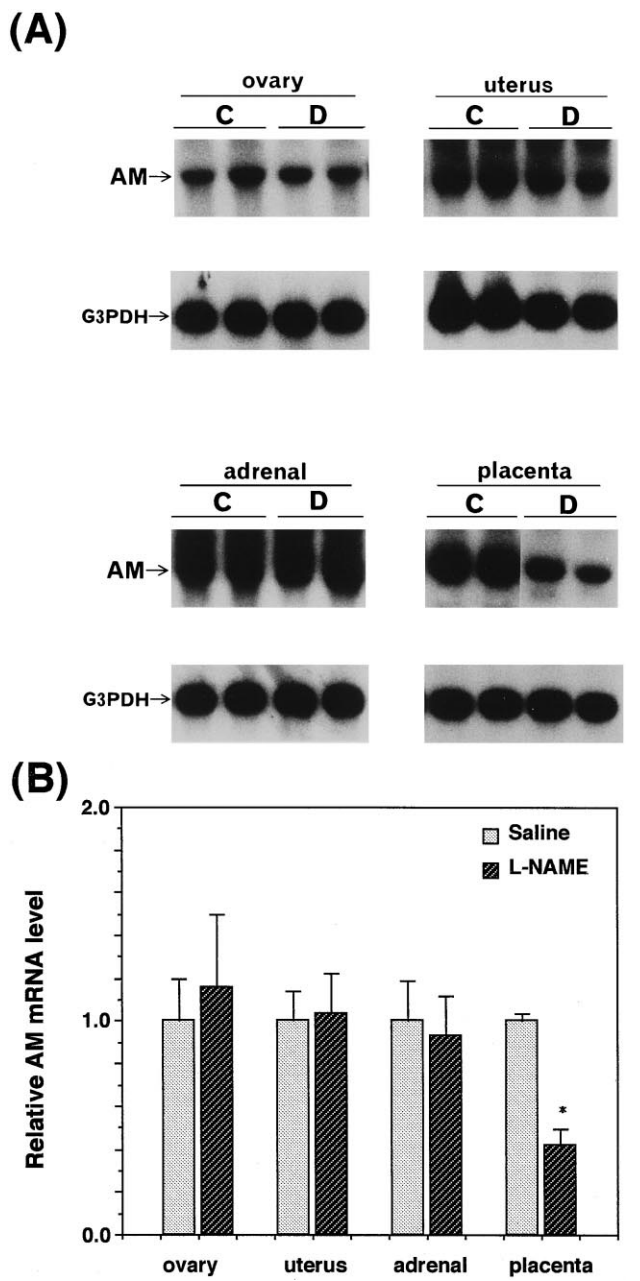

Fig. 4. Effect of L-NAME infusion on the adrenomedullin mRNA level in pregnant rats. L-NAME (25 mg/day) was administered subcutaneously with the osmotic minipumps. (A) A representative autoradiogram of adrenomedullin mRNA expression. C: Control (Saline treated rat), D: Drug (L-NAME treated rat). (B) The relative amounts of the adrenomedullin mRNA level in various organs. The mRNA level was determined by a Northern blot analysis. The mRNA levels of the control at each organ were set at 1.00 and then were compared with those of the L-NAME treated group. Further details are shown in Fig. 1. $^{*}P < 0.05$ (Student's *t*-test) ($n = 5$).

3.4. Relative distributions of adrenomedullin mRNA in normal and L-NAME-treated pregnant rats (day 20 of pregnancy)

As shown in Fig. 4(A) and (B), the adrenomedullin mRNA levels of the L-NAME-treated rats in the placenta decreased more than that in the normal pregnant rats ($P < 0.05$), however, the levels did not show any changes in the other organs studied.

4. Discussion

In this study, we used the animal model of preeclampsia. Several investigators found the L-NAME treated pregnant rats to show preeclampsia-like symptoms consisting of hypertension, intrauterine growth restriction, proteinuria and renal glomerulus injury (Yallampalli and Garfield, 1993; Molnar et al., 1994). In this animal model, the infusion of L-arginine can prevent the onset of this condition (Buhimschi et al., 1995). These findings are remarkably similar to those seen in human preeclampsia and support the hypothesis that a decrease in the bioavailability of NO might thus play a role in the development of this disease. In our study, the blood pressure in animals receiving L-NAME at 25 mg/day were significantly higher than that in rats receiving saline solution only. In addition, the L-NAME treated rats showed a higher fetal mortality than did intact rats. These findings were consistent with previous reports (Buhimschi et al., 1995). As a result, these animals seem to be appropriate model for preeclampsia.

On the other hand, the plasma concentration of adrenomedullin has been recently reported to increase in human pregnancy (Di Iorio et al., 1997) and high levels of

adrenomedullin are found in amniotic fluid and fetal membranes (Macri et al., 1996), thus suggesting that this peptide plays a role both in the adaptation of the vascular system during pregnancy and in the regulation of the placental vascular tone.

However, the biological activity and the sources of adrenomedullin synthesis in pregnancy have not yet been defined. In the present study, we showed the adrenomedullin mRNA levels to predominantly be at high level in the placenta, uterus and ovary in comparison to other tissues. These results demonstrated for the first time that the reproductive organs in pregnant rats exhibit an intensive expression of adrenomedullin, which surpassed the levels expressed in the adrenal. In addition, the adrenomedullin mRNA level in uterus was higher in pregnant rat than that in non-pregnant rats as previously reported (Upton et al., 1997). Such evidence suggests that adrenomedullin may thus possibly play an important role in pregnancy.

We also demonstrated for the first time that the infusion of adrenomedullin reversed hypertension and decreased the pup mortality induced by L-NAME in animals in late gestation, but not in animals in early gestation and in non-pregnant rats. We used a lower dose of adrenomedullin (3–10 pmol/h) than that in our previous infusion study (about 50 pmol/h) in SHR and WKY (Khan et al., 1997). In previous report, we measured the plasma concentration of adrenomedullin after infusion. The increase in the plasma concentration of adrenomedullin was found to be 0.6–0.9 fmol/ml. As the plasma adrenomedullin level in rats has been reported to be 3.6 fmol/ml (Sakata et al., 1994). The results concluded that chronically infused adrenomedullin has a hypotensive effect in hypertensive model rat, at a plasma adrenomedullin concentration within the physiological limits. Therefore, in the present study, we consider the plasma concentration of adrenomedullin to also be within the physiological limits. The present data thus suggest the significance of such a small increase of plasma adrenomedullin within the physiological limit for the regulation of blood pressure in pregnant rats.

In addition, adrenomedullin did not affect the basal blood pressure or pup mortality in normal pregnant rat. However, the L-NAME-induced hypertension after delivery was not reversed by adrenomedullin infusion. One possible explanation for this is that the rise in blood pressure after delivery may have been the direct effect of the termination of pregnancy. However, the control rats and L-NAME-treated rats did not increase the blood pressure after delivery. Thus, it is not conceivable that the increased blood pressure after delivery could have been the direct effect of the termination of pregnancy. Another possibility is that the hypotensive effect of adrenomedullin apparently depends on the presence of the fetoplacental complex. This may imply that the hypotensive effect of adrenomedullin was not direct, but indirect action by the induction of/interaction with some other vasorelaxant substances by the placenta. However, in animals in early gestation, the administration of adrenomedullin did not affect the L-NAME-induced hypertension. Therefore, it is not conceivable that the increased blood pressure after delivery is caused by the induction of some other mechanism by the placenta. In our preliminary observation, we observed that the adrenomedullin receptor mRNA level in the descending thoracic aorta increased in animals in late gestation in comparison to the non-pregnant rats, but no such increase was seen in either early gestation or after delivery (unpublished observation). It is therefore possible that the increase in the adrenomedullin receptor mRNA level in late gestation contributes to the decrease in blood pressure in L-NAME-treated pregnant rats.

Such evidence suggests that adrenomedullin may play an important role in the regulation of blood pressure and placental perfusion in animals in late gestation. In addition, it has been recently demonstrated that CGRP reverses the hypertension and decreases the fetal mortality in pregnant rats induced by L-NAME same as adrenomedullin (Yallampalli et al., 1996). Adrenomedullin is thought to belong to the CGRP superfamily. Therefore, the present results suggest that these peptide family may have similar biological activity.

We also observed that the adrenomedullin mRNA level in the placenta in L-NAME treated rats was decreased in comparison to the intact rats, but not in any other organs. This finding suggests that the decrease in adrenomedullin in the placenta may be related to the generation of such preeclampsia symptoms as hypertension and fetal mortality. However, although the adrenomedullin mRNA level are still very high in uterus, ovaries and placenta even after L-NAME treatment, in situ produced adrenomedullin does not seem to be able to affect blood pressure directly. In addition, we do not have radioimmunoassay data on the adrenomedullin levels in the tissues. Therefore, further experiments are needed to clarify at this point.

At present, the precise mechanism of the antagonistic effect of adrenomedullin is unclear. cAMP has been suggested to be a second messenger for adrenomedullin, because this peptide was isolated based on its increase in platelet cAMP and it also increases in cAMP in vascular smooth muscle cells, endothelial cells, and mesangial cells (Kitamura et al., 1993a). Adrenomedullin is thought to dilate blood vessels by increasing the cAMP level in smooth muscle cells of the vascular wall (Kitamura et al., 1993a). However, Itahara et al. (1994) found the hypotensive activity of adrenomedullin to be diminished in anaesthetized rats by pretreatment with L-NAME, a NO synthase inhibitor, a finding that suggests endothelium-dependent vasodilation by adrenomedullin. In addition, adrenomedullin has been recently reported to increase NO release from the kidney and bovine aortic endothelial cells (Shimekake et al., 1995). Furthermore, adrenomedullin has been demonstrated to increase the intracellular content of cGMP, a second messenger of NO, in cardiac ventricular

myocytes (Ikenouchi et al., 1997). Previous reports also demonstrated that adrenomedullin augments the cytokine-induced NO synthase expression in cardiac myocytes and vascular smooth muscle cells (Ikeda et al., 1996a,b). Furthermore, previous report suggested that a increased NO synthesis occurs during late gestation, but not in mid gestation or non-pregnant rats (Nathan et al., 1995). Therefore, the mechanism of hypotensive effect of adrenomedullin in late gestation may be related in part to the NO system. However, further studies are required to clarify the precise mechanisms involved in the correction of hypertension and the improvement in fetal mortality.

In conclusion, the present study suggests that L-NAME-induced elevated blood pressure and increased fetal mortality can be reversed by the low dose administration of adrenomedullin in late gestation. Adrenomedullin may thus play an important role in the regulation of blood pressure, the blood supply to the utero-placental unit and fetal development in late gestation.

Acknowledgements

We thank Mr. Brian Quinn for reading the manuscript. This work was supported in part by a Grant-in-Aid for general Scientific Research (No. 09671725) from the Ministry of Education, Science, Sports and Culture of Japan.

References

Brown, M.A., 1991. Pregnancy-induced hypertension: pathogenesis and management. Aust. N.Z. J. Med. 21, 257–273.

Buhimschi, I., Yallampalli, C., Chwalisz, K., Garfield, R.E., 1995. Preeclampsia-like conditions produced by nitric oxide inhibition: effect of L-arginine and D-arginine and steroid hormones. Hum. Reprod. 10, 2723–2730.

Di Iorio, R., Merinoni, E., Scab, D., Letizia, C., Cosmi, E.V., 1997. Adrenomedullin in pregnancy. Lancet 349, 328.

Ikeda, U., Kanbe, T., Kawahara, Y., Yokoyama, M., Shimada, K., 1996a. Adrenomedullin augments inducible nitric oxide synthase expression in cytokine-stimulated cardiac myocytes. Circulation 94, 2560–2565.

Ikeda, U., Kanbe, T., Shimada, K., 1996b. Adrenomedullin increase inducible nitric oxide synthase in rat vascular smooth muscle cells stimulates with interleukin-1. Hypertension 27, 1240–1244.

Ikenouchi, H., Kangawa, K., Matsuo, H., Hirata, Y., 1997. Negative inotropic effect of adrenomedullin in isolated adult rabbit cardiac ventricular myocytes. Circulation 95, 2319–2324.

Isumi, Y., Minamino, N., Katafuchi, T., Yoshioka, M., Tsuji, T., Kangawa, K., Matsuo, H., 1998. Adrenomedullin production in fibroblasts: its possible function as a growth regulator of Swiss 3T3 cells. Endocrinology 139, 2552–2563.

Itahara, Y., Watanabe, T.X., Inui, T., Chino, N., Kimura, T., Sakakibara, S., 1994. Analysis of hypotensive mechanisms of human and rat adrenomedullin in anesthetized rat. Jpn. J. Pharmacol. 64, 145, Suppl. 1.

Kangawa, K., Kitamura, K., Minamino, N., Eto, T., Matsuo, H., 1996. Adrenomedullin: a new hypotensive peptide. J. Hypertens. 14, S105–S110, Suppl. 5.

Kato, K., Shichiri, M., Marumo, F., Hirata, Y., 1997. Adrenomedullin as an autocrine/paracrine apoptosis survival factor for rat endothelial cell. Endocrinology 138, 2615–2620.

Khan, A.I., Kato, J., Kitamura, K., Kangawa, K., Eto, T., 1997. Hypotensive effect of chronically infused adrenomedullin in conscious Wistar–Kyoto and spontaneously hypertensive rats. Clin. Exp. Pharmacol. Physiol. 24, 139–142.

Kitamura, K., Kangawa, K., Kawamoto, M., Ichiki, Y., Nakamura, S., Matsuo, H., Eto, H., 1993a. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem. Biophys. Res. Commun. 192, 553–560.

Kitamura, K., Sakata, J., Kangawa, K., Kojima, M., Matsuo, H., Eto, T., 1993b. Cloning and characterization of cDNA encoding a precursor for human adrenomedullin. Biochem. Biophys. Res. Commun. 194, 720–725.

Kitamura, K., Ichiki, Y., Tanaka, M., Kawamota, M., Emura, J., Sakakibara, S., Kangawa, K., Matsuo, H., Eto, T., 1994. Immunoreactive adrenomedullin in human plasma. FEBS Lett. 342, 288–290.

Macri, C.J., Martínez, A., Moody, T.W., Gray, B.K.D., Miller, M.-J., Gallagher, M., Cuttitta, F., 1996. Detection of adrenomedullin, a hypotensive peptide, in amniotic fluid and fetal membranes. Am. J. Obstet. Gynecol. 175, 906–911.

Makino, I., Shibata, K., Ohgami, Y., Fujiwara, M., Furukawa, T., 1996. Transient up-regulation of the AT2 receptor mRNA level after global ischemia in rat brain. Neuropeptides 30, 596–601.

Martínez, A., MIller, M.-J., Unsworth, E.J., Siegfried, J.M., Cuttitta, F., 1995. Expression of adrenomedullin in normal human lung and in pulmonary tumors. Endocrinology 136, 4099–4105.

Martínez, A., Weaver, C., López, J., Bhathena, S.J., Elsasser, T.H., MIller, M.-J., Moody, T.W., Unsworth, E.J., Cuttitta, F., 1996. Regulation of insulin secretion and blood glucose metabolism by adrenomedullin. Endocrinology 137, 2626–2632.

Molnar, M., Suto, T., Toth, T., Hertelendy, F., 1994. Prolonged blockade of nitric oxide synthesis in gravid rats produced sustained hypertension, proteinuria, thrombocytopenia and intrauterin growth retardation. Am. J. Obstet. Gynecol. 170, 1459–1466.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev. 4343, 109–142.

Nathan, L., Cuevas, J., Chaudhuri, G., 1995. The role of nitric oxide in the altered vascular reactivity of pregnancy in the rat. Br. J. Pharmacol. 114, 955–960.

Nishikimi, T., Kitamura, K., Saito, Y., Shimada, K., Ishimitsu, T., Takamiya, M., Kangawa, K., Matsuo, H., Eto, T., Omae, T., Matsuoka, H., 1994. Clinical studies on the sites of production and clearance of circulating adrenomedullin in human subjects. Hypertension 24, 600–604.

Sakata, J., Shimokubo, T., Kitamura, K., Nakamura, S., Kangawa, K., Matsuo, H., 1993. Molecular cloning and biological activities of rat adrenomedullin, a hypotensive peptide. Biochem. Biophys. Res. Commun. 195, 921–927.

Sakata, J., Shimokubo, T., Kitamura, K., Nishizono, M., Iehiki, Y., Kangawa, K., Matsuo, H., Eto, T., 1994. Distribution and characterization of immunoreactive rat adrenomedullin in tissue and plasma. FEBS Lett. 352, 105–108.

Shimekake, Y., Nagata, K., Ohta, S., Kambayashi, Y., Teraoka, H., Kitamura, K., Eto, T., Kangawa, K., Matsuo, H., 1995. Adrenomedullin stimulates two signal transduction pathways, cAMP accumulation and Ca^{2+} mobilization, in bovine aortic endothelial cells. J. Biol. Chem. 270, 4412–4417.

Sugo, S., Minamino, N., Kangawa, K., Miyamoto, K., Kitamura, K., Sakata, J., Eto, T., Matsuo, H., 1994. Endothelial cells activity synthesize and secrete adrenomedullin. Biochem. Biophys. Res. Commun. 201, 1160–1166.

Upton, P.D., Austin, C., Taylor, G.M., Nanddha, K.A., Clark, A.J.,

Ghatei, M.A., 1997. Expression of Adrenomedullin (ADM) and its binding site and ADM messenger ribonucleic acid in 20-day pregnant rats compared with nonpregnant rats. Endocrinology 139, 2508–2514.

Yallampalli, C., Garfield, R.E., 1993. Inhibition of nitric oxide synthesis in rats during pregnancy produces symptom identical to preeclampsia. Am. J. Obstet. Gynecol. 169, 1316–1320.

Yallampalli, C., Dong, Y.L., Wimalawansa, S.J., 1996. Calcitonin gene-related peptide reverses the hypertension and significantly decreases the fetal mortality in pre-eclampsia rats induced by N^{G}-nitro-L-arginine methyl ester. Human Reprod. 11, 895–899.