Physiol. Res. 49: 175-182, 2000

Changes in Plasma Angiotensin-Converting Enzyme Activity and Noradrenaline Responses to Long-Term Nitric Oxide Inhibition Vary Depending on Their Basal Values in Chickens

H. E. AKSULU[1], I. BINGÖL[1], F. KARATAS[2], H. SAGMANLIGIL[1], B. ÜSTÜNDAG[3]

Fırat University, Faculty of Medicine, Department of Pharmacology[1] and Biochemistry[3] and Faculty of Science Department of Chemistry[2], Elazıg, Turkey

Received August 15, 1999
Accepted September 21, 1999

Summary

In this study, we investigated the effects of N^{ω}-nitro-L-arginine (L-NNA) on arterial blood pressure (BP), plasma noradrenaline (NA) and adrenaline (A) levels and angiotensin-converting enzyme (ACE) activity. L-NNA was applied with tap water (1 mg/ml) from the 3rd to the 8th week of age (group L-NNA_1). In Experiment 1, long-term L-NNA application increased BP compared to the control group (group C_1) (L-NNA_1 = 131.4 ± 6.3, n=6; C_1= 82.7 ± 4.7 mm Hg, n=7) but decreased plasma noradrenaline and adrenaline levels and ACE activity (NA levels: C_1 = 15.5 ± 0.8, n=7; L-NNA_1= 8,6 ± 0.5 ng/ml, n=7; A levels: C_1 = 15.5 ± 0.8, n=7; L-NNA_1 = 6.0 ± 0.5 ng/ml, n=7; ACE activities: C_1= 87.3 ± 3.1, n=6; L-NNA_1 = 46.2 ± 1.9 U/l, n=5). On the other hand, in Experiment 2 (carried out under the same conditions and in age-matched chickens), blood pressure, plasma noradrenaline levels and ACE activity were found to differ in the control group (C_2) (BP=141.4 ± 15.5 mm Hg, n=7; NA =1.1 ± 0.4 ng/ml, n=7; ACE = 57.2 ± 5.3 U/l, n=7) as compared to C_1, while plasma adrenaline levels were similar. In this series, long-term L-NNA application (group L-NNA_2) did not change the BP, but surprisingly increased noradrenaline and ACE values (values of L-NNA_2: BP = 165.7 ± 15.6 mm Hg, n=7; NA = 9.3 ± 1.3 ng/ml, n=8; ACE = 149.4 ± 16 U/l, n=8) while decreasing plasma adrenaline levels. L-arginine addition to L-NNA treatment completely reversed plasma noradrenaline and ACE activity values. These results indicate the modulatory activity of an L-arginine-NO pathway on adrenaline release as well as on the renin-angiotensin system in chickens.

Key words

Blood pressure • N^{ω}-nitro-L-arginine • adrenaline • noradrenaline • angiotensin-converting enzyme •chicken

Introduction

Endothelium-dependent relaxation and its attenuation by hypercholesterolemia have been observed in chickens (Aksulu *et al.* 1986). It was also reported that the chickens' endothelium releases an endothelium-derived relaxing factor (EDRF) that pharmacologically partially resembles EDRF of mammalian blood vessels (Hasegawa *et al.* 1993). The mammalian EDRF is nitric oxide (NO) which is enzymatically formed from

Fax +420 2 293 894
E-mail physres@biomed.cas.cz http://www.lf2.cuni.cz/physiolres/physiolres.htm

L-arginine (L-ARG). Its formation is inhibited by certain analogues such as L-N^G-monomethyl-arginine (L-NMMA) and N^{ω}-nitro-L-arginine (L-NNA) (Palmer *et al.* 1988, Rees *et al.* 1990). Inhibition of NO synthesis causes vasoconstriction and systemic hypertension, indicating an involvement of the L-arginine-NO pathway in the regulation of blood flow and pressure in mammals (Gardiner *et al.* 1990). Halbrügge *et al.* (1991) reported that acute inhibition of NO synthesis by L-NMMA inhibits noradrenaline (NA) and adrenaline (A) release in rabbits. They suggested that NA inhibition by L-NMMA is mediated by the baroreflex mechanism, but A inhibition is related to the abolition of NO synthesis in the adrenal medulla. The physiological importance of NO and its contribution to the pathophysiological processes have become at the center of interest in mammals. But there is scantly knowledge about the L-arginine-NO system in chickens. The sympathoadrenergic activity is high in chickens and it has propensity to naturally occurring early atherosclerosis and high blood pressure (BP) (Clarkson *et al.* 1965). Therefore, the chicken seems to be a useful model for investigating the relationship between the sympathoadrenergic system and blood pressure, including the regulatory influence of the systems such as renin-angiotensin and L-arginine-NO. In the present study, we aimed at investigating the effect of long-term application of L-NNA from an early age on plasma NA and A levels and their relationship with blood pressure in chickens.

Methods

Animals

Male white leghorn chickens, *Gallus gallus*, aged one day were purchased from Çetinkaya Farm, Çumra-Konya, Turkey. They were housed freely in a wide and day-lighted room (340x360x350 cm) divided into 4 compartments (110x180x187 cm) with a wire fence and were fed with commercial chicken chow (Köy-Tür Ltd, Elazig, Turkey).

Experimental Protocols

The experiments were performed according to two protocols at different seasons of the year. In Experiment 1, 3-week-old chickens (in early spring) were divided into 2 groups. L-NNA was applied to group 1 (group L-NNA_1, n=15) with tap water in 0.1 % concentration from the third week of age till the eight week, i.e. for 5 weeks. The other group (group C_1, n=15) received only normal tap water *ad libitum.* At the end of week 8, some of the chickens (n=7) in each group were used for blood pressure measurements and the rest for collecting blood samples. In Experiment 2 (carried out in late autumn), the animals were bred under the same conditions as in Experiment 1. The age-matched chickens were divided into four groups receiving either L-NNA, L-arginine (L-ARG) in 0.3 % concentration or L-NNA plus L-ARG, while the fourth group received tap water only. The groups in Experiment 2 comprised the control group (group C_2, n=15), the L-NNA treated group (group L-NNA_2, n=15), L-NNA + L-ARG treated group (group L-NNA + L-ARG, n=15) and L-ARG treated group (n=15).

Measurement of blood pressure

The chickens were anesthetized with ketamine (Ketalar, Parke-Davis, Turkey; 75 mg/kg, i.m.) on the day of the experiment. The surgical procedure and the cannulation were described elsewhere (Nishimura *et al.* 1982). Briefly, the chickens were laid down in the supine position, the feathers inside the right leg were plucked, and an incision was made on the skin. A polyethylene catheter (4FG, Fortex, 4 cm in length) was inserted via the femoral artery into the aorta. The arterial BP was measured directly by means of the manometric system that was filled with saline (0.9 % NaCl) containing heparin (100 IU/ml); after allowing 3-4 min for stabilization, BP was recorded. All BP measurements were performed under normal light.

Collection of blood samples

Some of the chickens in each group were used for the collection of blood samples. Conscious chickens were taken to a room with normal light, gently handled not to alarm the animal unduly and quickly decapitated. Arterial and venous blood was collected together from the decapitated site into ice-cold tubes containing Na-EDTA (0.2 ml for 5 ml blood). In this manner, 8 to 10 ml of blood could be collected within 10 s. The collected blood samples were centrifuged immediately (2500 *g*, 10 min) and the obtained plasma was stored at –70 °C until assayed. The heart was removed for determining the heart weight/body weight ratio in all the groups.

Analytical methods

Plasma renin activity (PRA) was measured by radioimmunoassay using renin MAIA kit (Biodata Diag., Polymedco Inc., USA). Briefly, in an ice-cooled bath, 1 ml of plasma was pipetted into a plastic tube, then 10 µl

phenylmethylsulfonyl fluoride and 100 µl maleate generation buffer were added and mixed. After a 90 min incubation period, PRA was determined according to the radioimmunassay method modified by Haber *et al.* (1969). PRA was expressed in nanograms of angiotensin per ml and hour of incubation.

Plasma angiotensin-converting enzyme activity (ACE) measurements were performed using ACE kits (Sigma Diagnostic, USA) according to the method of Holmquist *et al.* (1979). Lyophilized reagents containing normal, control and calibrator 2-furylacryloyl-L-phenylalanilyl-glycylglycine (FAPGG) substrate were dissolved (pH 8.2 and 0.5 mmol/l FAPGG concentration). Reagents were put into blank, standard or sample tubes and were incubated for 5 min at 37 °C in a water bath, later the absorbancy values was measured and calculated. A 340 nm filter was used in the spectrophotometer (Shimadzu UV-1201, Japan). Plasma ACE activity was expressed in U/l.

Plasma noradrenaline and adrenaline levels were determined by HPLC (Cecil 1100, UK) according to the method described by Hallbrügge *et al.* (1991) but using UV detection (data module, HP, USA). Samples and standards were separated by supercosil LC-ABZ (5 cm x 4.6 mm ID; 5 µm particles, Supelco). The mobile phase was acetonitrile + 25 mM $K_2H_2PO_4$ (5/95, v/v). On the day of analysis, samples were transferred into filtration units and thoroughly mixed for 10 min with 0.2 ml of 2 M Tris HCl (pH 8.7) and 20 mg Al_2O_3. A filter (Whatman GF/C) replaced the bottom stopper of the filtration unit and the filtration unit was centrifuged (1000 *g*, 1 min, 4 °C) to remove the supernatant. The Al_2O_3 within the filtration unit was washed three times with 1 ml of distilled water and the catechols were finally desorbed from Al_2O_3 with 2 x 75 µl of 0.1 M $HClO_4$, each washing and desorption step being followed by centrifugation (1000 x *g*, 1 min, 4 °C). Finally, samples and the mobile phase of 150 µl of 0.1 M $HClO_4$ eluted from Al_2O_3 and degassed in ultrasonic water bath were then injected (20 µl) for analysis in the HPLC system. The standards and samples were applied at a flow rate of 1 ml/min and read at 270 nm wavelength with UV detection. The concentrations of catecholamines were determined from their respective peak areas by comparison to the standard curve. The values were expressed in ng/ml. The recovery was 68-81 %.

Drugs and reagents

ACE kit was obtained from Sigma Diagnostic, renin MAIA kit from Biodata Diag., Polymedco Inc. (USA), and Al_2O_3 from Merck (Darmstadt, Germany). N^{ω}-nitro-L-arginine and all other reagents and solvents, which were obtained from Sigma Chem. Co (Steinheim, Germany), were of analytical grade.

Statistics

Results are given as means ± S.E.M. Differences between the means of groups were evaluated by Student's t -test.

Table 1. The body weight (BW) and heart weight/body weight (HW/BW) ratio of the groups in Experiments 1 and 2, and effects of L-NNA, L-ARG and L-NNA + L-ARG applications on arterial blood pressure

	Experiment 1		**Experiment 2**			
	Control	**L-NNA**	**Control**	**L-NNA**	**L-ARG**	**L-NNA + L-ARG**
	(n=15)	(n=15)	(n=15)	(n=15)	(n=15)	(n=15)
BW at 4 weeks of age (g)	260.1±18	280.2±32	342.6±26	342±27	345±17	345±12
BW at 8 weeks of age (g)	726.4±72	820±76	998±61	1002±74	1011±46	1014±45
HW/BW (g/kg)	0.52±0.04	0.55±0.08	0.51±0.03	0.49±0.03	0.50±0.04	0.46±0.04
	(n=6)	(n=7)	(n=7)	(n=7)	(n=7)	(n=7)
Arterial BP (mm Hg)	82.0±4.7	131.4±6.3*	141.4±15.5	165.7±15.6	147.9±17.5	161.4±15.5

** $p<0.01$, when a comparison was made between control and L-NNA treated groups in Experiment 1.*

Results

Effects of L-NNA, L-ARG and L-NNA + L-ARG applications on body weight and the heart weight/body weight ratio

The body weight of chickens in Experiments 1 and 2 were different at the beginning (4th week) and at the end (8th week) of the experiments; but no treatment changed the growth rate or development of the heart weight/body weight ratio in the chickens of all groups in both experiments (Table 1).

Effects of L-NNA, L-ARG and L-NNA + L-ARG applications on arterial blood pressure

The basal arterial BP values of the control groups of each experiment were different (C_1: 82.0 ± 4.7, n=6 ; C_2: 141.4 ± 15.5 mm Hg, n=7). In the L-NNA treated group of Experiment 1, arterial BP was significantly increased compared to the corresponding control group (L-NNA_1: 131.4 ± 6.3, n=7; C_1: 82.0 ± 4.7 mm Hg, n=6, $p<0.01$), whereas no significant changes were found in the L-NNA treated group of Experiment 2 compared to their controls (L-NNA_2: 165.7 ± 15.6, n=7; C_2: 141.43 ± 15.47, mm Hg, n=7). The animals treated according to Experiment 2, neither L-ARG nor L-NNA + L-ARG changed the arterial BP significantly compared to C_2 and L-NNA_2 groups (Table 1, Fig. 1).

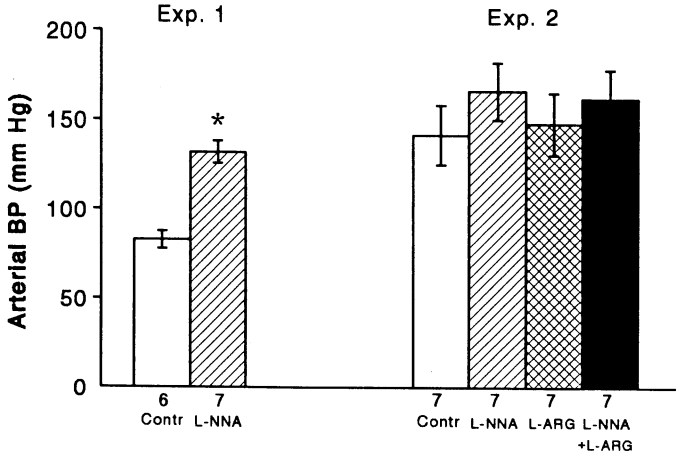


Fig 1. *Effect of L-NNA, L-ARG and L-NNA + L-ARG treatments on arterial blood pressure (BP) in chickens. There are differences in basal BP between the two experiments. L-NNA treatment increased the BP only in Experiment 1 (* p<0.01).*

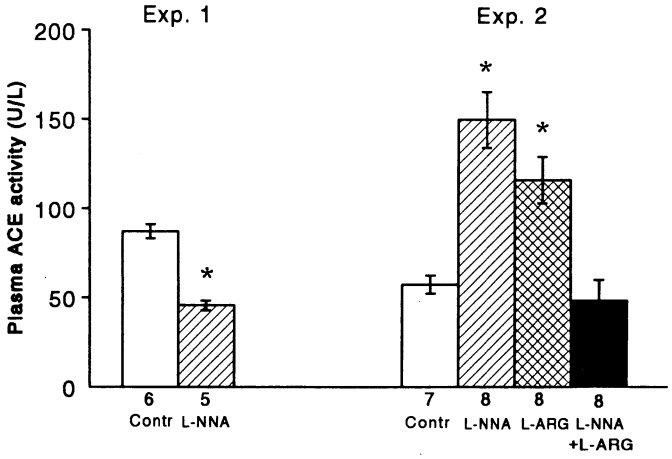


Fig 2. *Effects of L-NNA, L-ARG and L-NNA + L-ARG treatments on plasma angiotensin-converting enzyme (ACE) activity in chickens. There are differences between the basal values of the two experiments. In Experiment 1, L-NNA treatment decreased plasma ACE activity. In Experiment 2, L-NNA treatment increased plasma ACE activity significantly, While L-ARG addition completely abolished the effects of L-NNA treatment (* p<0.001).*

Effects of L-NNA and L-NNA + L-ARG applications on PRA.

PRA measurements were performed only in animals of Experiment 1. The values of PRA were relatively low due to the human AI antibody used for the measurements and they did not differ significantly in the L-NNA treated and control groups (0.17 ± 0.02 and 0.20 ± 0.01 ng ANG I ml^{-1} h^{-1}, respectively).

Effects of L-NNA, L-ARG and L-NNA + L-ARG applications on ACE activity

The basal values of ACE activity in the control groups of each experiment were also different (C_1: 87.27 ± 3.12, n = 6; C_2: 57.23 ± 5.30 U/l, n=7). Interestingly, L-NNA treatment in these two experiments changed ACE activity values inversely. In Experiment 1, ACE activity was significantly decreased in the L-NNA treated group as compared to the controls (46.16 ± 1.90 and 87.27 ± 3.12 U/l, respectively, $p<0.001$) (Fig. 2). On the other hand, the ACE activity in Experiment 2 was significantly increased after L-NNA treatment in comparison to their controls. This increase in ACE activity was completely reversed after adding L-ARG to the L-NNA treatment (C_2: 57.23 ± 5.30, n=7; L-NNA$_2$: 149.36 ± 15.97, n=8; L-NNA + L-ARG: 48.25 ± 11.52 U/l, n=8, $p<0.001$) (Fig. 2). L-ARG treatment alone also enhanced plasma ACE activity significantly (Fig. 2).

Effects of L-NNA, L-ARG and L-NNA + L-ARG applications on plasma NA and A levels

Basal plasma NA levels were also different in the control groups of each experiment (C_1: 15.56 ± 1.24, n=7; C_2: 1.09 ± 0.37 ng/ml, n=7), whereas plasma A levels were not different (C_1: 15.51 ± 0.79, n=7; C_2: 13.16 ± 1.41 ng/ml, n=7) (Figs 3 and 4). Interestingly, L-NNA treatments also changed plasma NA levels inversely in each experiment (C_1: 15.56 ± 1.24, n=7; L-NNA$_1$: 8.59 ± 0.72, n=7, $p<0.01$; C_2: 1.09 ± 0.37, n=7 ; L-NNA$_2$: 9.33 ± 1.29 ng/ml, n=8, $p<0.001$), but plasma A levels were decreased in both groups. The addition of L-ARG to L-NNA treatment completely reversed plasma NA and A responses in Experiment (1.56 ± 0.89, n=8 and 10.30 ± 2.07 ng/ml, n=8, respectively) (Fig. 4). L-ARG treatment alone increased plasma NA levels, but decreased plasma A levels (Fig. 4).

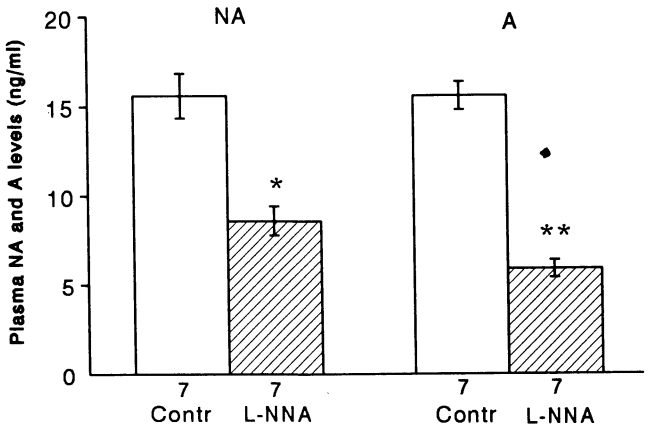
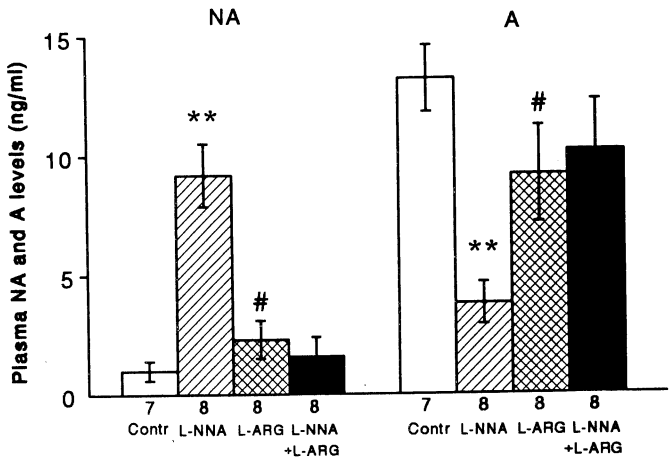


Fig 3. *Effects of L-NNA treatment on plasma noradrenaline (NA) and adrenaline (A) levels in Experiment 1. L-NNA treatment significantly decreased both noradrenaline and adrenaline levels (* $p<0.01$, ** $p<0.001$).*

Fig 4. *Effects of L-NNA, L-ARG and L-NNA + L-ARG treatments on plasma noradrenaline (NA) and adrenaline(A) levels in Experiment 2. L-NNA treatment increased plasma noradrenaline level but decreased plasma adrenaline level significantly (** $p<0.001$). L-ARG addition to the L-NNA treatment completely abolished the effects of L-NNA treatment both on plasma noradrenaline and adrenaline levels (# $p<0.01$).*



Discussion

The present study was aimed at investigating the long-term effects of L-NNA, NO synthase inhibitor, on plasma NA and A levels and plasma ACE activity and their relationship to blood pressure in chickens to which L-NNA was applied from an early age. Endothelium-dependent relaxation has been observed in isolated blood vessels of chicken (Aksulu *et al.* 1986). It was also reported that chicken endothelium releases EDRF that pharmacologically partially resembles EDRF of mammalian blood vessels (Hasegawa *et al.* 1993). In mammals, EDRF was identified as NO and its physiological importance in the regulation of blood pressure and regional blood flow is well known (Moncada *et al.* 1991). But there is no report on this subject in chickens.

In the preliminary study (Experiment 1), we have found that long-term L-NNA application increased arterial BP and decreased both plasma NA and A levels and ACE activity but did not change the heart weight/body weight ratio. We furthermore assessed that these effects of long-term L-NNA application are related to a possible L-arginine-NO pathway in chickens. Therefore, in an another series (Experiment 2) we repeated our studies with additional L-arginine application. Surprisingly, in spite of special attention to maintain the same experimental conditions, we found that in this series of experiments the chickens had higher arterial BP than in the preceding series. The only difference between the chickens of the two experiments concerned the season of the year. High sympatho-adrenergic activity, a tendency to high BP and to early atherosclerosis in several avian species and the influence of age, sex, environment and diet on BP in some species were reported (Sturkie 1986, Kamimura *et al.* 1995). In the present study, the chickens of Experiment 2 appeared to be influenced probably by the seasonal difference and/or diet with regard to their body weight and elevated BP.

The results of Experiment 1 have shown that long-term L-NNA application decreases plasma NA and A levels and ACE activity while increasing arterial BP in chickens. The decrease of plasma A levels was greater than that of NA. We could not decide whether the decrease of NA and A plasma levels and ACE activity is due to a direct effect of L-NNA or is secondary to the developed hypertension. However, it seems that the decrease in plasma NA levels is due to a baroreflex mechanism, as was demonstrated in the study of Halbrügge *et al.* (1991). These authors reported that acutely applied NO synthase inhibitor, L-NMMA, inhibited NA and A release in anesthetized rabbits. They suggested that inhibition of NA release by L-NMMA is due to a baroreflex mechanism but its inhibitory effect on A release is due to a direct action on the adrenal medulla. The reduced synthesis and release of NA in spontaneously hypertensive rats and in essential hypertension in man have been reported (Louis *et al.* 1969, 1975). However, an increased release of NA as the cause of hypertension in rats or an increased release of NA in hypertensive turkeys have also been reported (De Champlain *et al.* 1967, El-Halawani *et al.* 1972).

Interestingly, L-NNA treatment in Experiment 2 significantly increased plasma NA levels and ACE activity while decreasing plasma A levels and without changing BP. Several reports, however, have indicated that EDRF-like substances inhibited NA release in mammalian arteries (Cohen and Weisbrod 1988, Greenberg *et al.* 1990). Nevertheless, it has also been shown that inhibition of NO synthesis did not affect NA release (Toda *et al.* 1991, Thorin and Atkinsson 1994). We cannot speculate on these results, but it should also be taken into consideration that the elevated BP may influence this process. It seems certain that these effects of L-NNA treatment are closely related to an L-arginine-NO pathway, while the addition of L-arginine to L-NNA treatment completely reversed these changes (Moncada *et al.* 1991). However, it can be clearly seen that the increased plasma NA levels and ACE activity could not influence the BP. This may serve as evidence supporting the view that sympathoadrenergic activity does not contribute directly to sustained BP elevation.

It should be stressed that only plasma A levels did not undergo changes at different BP and it was significantly decreased by L-NNA treatment in both experiments. Especially, the decrease with L-NNA treatment in Experiment 2 in the absence of BP changes and its complete reversal with the addition of L-arginine to the L-NNA treatment are consistent with the study of Halbrügge *et al.* (1991) and indicate a modulatory role of the L-arginine-NO pathway on the release of adrenaline from the adrenal medulla in chickens.

In conclusion, we have shown in these two series of experiments that 1) long-term inhibition of NOS also

elevates blood pressure in normotensive chickens, 2) plasma noradrenaline levels and ACE activity values show a difference depending on the blood pressure (since long-term NOS inhibition could change these parameters inversely in the chicken, the basal BP values must be taken into account when evaluating these parameters), 3) plasma adrenaline levels are very stable in the chicken and there is evidence suggesting that the L-arginine-NO pathway modulates adrenaline release from the adrenal medulla in chickens.

Acknowledgement

The authors are grateful to Dr. Ahmet Ayar for reviewing the manuscript. This study was supported by grants from Fırat University Research Foundation (FÜNAF-253) and Eczacıbaşı Scientific Research and Award Fund, Istanbul, Turkey.

This work was presented at the International Symposium "*Nitric Oxide: From Molecular Level to Clinical Application*" held in Bratislava, June 27-29, 1999 and published as abstracts in *Physiol Res* **48:** 27P, 1999.

References

AKSULU HE, CELLEK S, TÜRKER RK: Cholesterol feeding attenuates endothelium-dependent relaxation response to acethylcholine in the main pulmonary artery of chickens. *Eur J Pharmacol* **129**: 397-400, 1986.

AKSULU HE, BINGÖL I, KARATAŞ F, SAĞMANLIGIL H, ÜSTÜNDAĞ B: Changes in plasma angiotensin-converting enzyme activity (ACE) and noradrenaline responses to long-term nitric oxide inhibition vary depending on their basal values in chickens. *Physiol Res* **48:** 27P, 1999.

AKSULU HE, BINGÖL I, SAĞMANLIGIL H, ÜSTÜNDAĞ B, KARATAŞ F, KÖKSEL O: N^{ω}-nitro-L-arginine decreases plasma renin (PRA) and angiotensin-converting enzyme (ACE) activities and plasma noradrenaline level without changing blood pressure in chickens. *Physiol Res* **48:** 27P, 1999.

CLARKSON TB, MIDDLETON CC, PRICHARD RW, LOFLAND HB: Naturally-occurring atherosclerosis in birds. *Ann NY Acad Sci* **127**: 685-705, 1965.

COHEN RA, WEISBROD RM: Endothelium inhibits norepinephrine release from adrenergic nerves of rabbit carotid artery. *Am J Physiol* **254**: H871-H878, 1988.

DE CHAMPLAIN J, KRAKOFF LR, AXELROD J: Catecholamine metabolism in experimental hypertension in the rat. *Circ Res* **20**: 136-145, 1967.

EL-HALAWANI ME, WAIBEL PE, APPEL JR, GOOD AL, DUKE GE: Catecholamines in turkeys with high or low blood pressure: effects of tyrosine hydroxylase and ganglionic blocker. *Can J Physiol Pharmacol* **50**: 697-702, 1972.

GARDINER SM, COMPTON AM, BENNETT T, PALMER RMJ, MONCADA S: Control of regional blood flow by endothelium-derived nitric oxide. *Hypertension* **15**: 486-492, 1990.

GREENBERG SS, DIECKE FP, REEVY K, TANAKA TP: Release of norephinephrine from adrenergic nerve endings of blood vessels mediated by endothelium-derived relaxing factor. *Am J Hypertens* **3**: 211-218, 1990.

HABER E, KOERNER L, PAGE LB, KRIMAN B, PURNODE A: Application of radioimmunoassay for angiotensin I to the physiologic measurement of plasma renin activity in normal subjects. *J Clin Endocrinol Metab* **29**: 1349-1355, 1969.

HALBRÜGGE T, LÜTSCH K, THYEN A, GRAEFE K-H: Role of nitric oxide formation in the regulation of haemodynamics and the release of noradrenaline and adrenaline. *Naunyn-Schmiedeberg's Arch Pharmacol* **344**: 720-727, 1991.

HASEGAWA K, NISHIMURA H, KHOSLA MC: Angiotensin II-induced endothelium-dependent relaxation of fowl aorta. *Am J Physiol* **264**: R903-R911, 1993.

HOLMQUIST B, BÜNNING P, RIORDAN JF: A continuous spectrophotometric assay for angiotensin converting enzyme. *Anal Biochem* **95**: 540-554, 1979.

KAMIMURA K, NISHIMURA H, BAILEY JR: Blockade of β-adrenoceptor in control of blood pressure in fowl. *Am J Physiol* **269**: R914-R922, 1995.

LOUIS WJ, SPECTOR S, TABEI R, SJOERDSMA A : Synthesis and turnover of norepinephrinein the heart of the spontaneously hypertensive rat. *Circ Res* **24**: 85-91, 1969.

LOUIS WJ, DOYLE AE, ANAVEKAR SN: Plasma noradrenaline concentration and blood pressure in essential hypertension, phaeochromocytoma and depression. *Clin Sci Mol Med* **48** (suppl 2): 229s-242s, 1975.

MONCADA S, PALMER RMJ, HIGGS EA: Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* **43**: 109-142, 1991.

NISHIMURA H, NAKAMURA Y, SUMNER RP, KHOSLA MC: Vasopressor and depressor actions of angiotensin in the anesthetized fowl. *Am J Physiol* **242**: H314-H324, 1982.

PALMER RMJ, ASHTON DS, MONCADA S: Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333**: 664-666, 1988.

REES DD, PALMER RMJ, SCHULZ R, HODSON HF, MONCADA S: Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br J Pharmacol* **101**: 746-752, 1990.

STURKIE PD: Heart and circulation: anatomy, hemodynamics, blood pressure, blood flow. In: *Avian Physiology*. PD STURKIE (ed), Springler, New York, 1986, pp 130-166.

THORIN E, ATKINSSON J: Modulation by endothelium of sympathetic vasoconstriction in an in vitro preparation of the rat tail artery. *Br J Pharmacol* **111**: 351-357, 1994.

TODA N, YOSHIDA K, OKAMURA T: Analysis of potentiating action of N^G-nitro-L-arginine on the contraction of the dog temporal artery elicited by transmural stimulation of noradrenergic nerves. *Naunyn-Schiedeberg's Arch Pharmacol* **343**: 221-224, 1991.

Reprint requests

Hakki Engin Aksulu, Firat University, Faculty of Medicine, Department of Pharmacology, TR-23200, Elaziğ, Turkey.