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Long-Term Lisinopril Dihydrate Application Decreases Plasma Noradrenaline But Not Adrenaline Levels in Chickens

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Summary

Little is known about the effect of chronic angiotensin-converting enzyme inhibition on the catecholamine levels in fowls. In this study, we investigated the effects of chronic lisinopril dihydrate (Ld) application on the plasma levels of adrenaline and noradrenaline and on the blood pressure. Lisinopril was given in different concentrations (25, 75 and 250 mg/l drinking water) to the white Leghorn chickens for 9 weeks, while the control group drank tap water only. Twenty-eight hours after the last lisinopril application, arterial blood pressure (BP), plasma adrenaline and noradrenaline levels, plasma renin (PRA) and plasma angiotensin-converting enzyme (ACE) activities were determined. In all concentrations, lisinopril significantly increased PRA and decreased ACE activities. Arterial BP was decreased only in the group receiving high lisinopril concentration (Controls 119±10.27, Ld3 98±5.4 mm Hg). However, the lower lisinopril concentrations did not alter arterial BP compared to the control group. Plasma noradrenaline levels were decreased in a concentration-dependent manner (47-58 %), but plasma adrenaline levels remained unchanged. The heart weight/body weight ratio was not changed in any of the lisinopril-treated groups. The persistent decrease in the blood pressure after lisinopril treatment was not directly related to a decrease of plasma ACE activity or plasma noradrenaline levels. Its mechanism still remains to be elucidated.

Key words

Chicken • Blood pressure • Adrenaline • Noradrenaline • Lisinopril dihydrate

Introduction

With its high sympathoadrenergic activity and propensity to the occurrence of naturally occurring early atherosclerosis and high blood pressure (BP), the chicken seems to be a useful model for investigating the relation between the sympathoadrenergic and renin-angiotensin system and their influence on the development of early hypertension and atherosclerosis (Clarkson *et al.* 1965). Angiotensin II (AII) has a biphasic effect on BP in the chicken: the depressor response is mediated by an endothelium-derived relaxing factor (EDRF) that pharmacologically partially resembles the mammalian EDRF, whereas the pressor effect is due to the release of catecholamines from the adrenal medulla and/or adrenergic nerve terminals (Nishimura *et al.* 1982, Wilson and Butler 1983, Hasegawa *et al.* 1993). The presence of specific AII binding sites in vascular smooth muscle and in the endothelium of the chicken has also been reported (Takei *et al.* 1988, Stallone *et al.* 1990). Interestingly, AII does not contract isolated aortic preparations in the chicken (Moore *et al.* 1981), but it

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relaxes them in an endothelium-dependent manner (Yamaguchi and Nishimura 1986, 1988). The physiological importance of vascular angiotensin receptors and the contribution of the renin-angiotensin system to BP maintenance in chickens are not yet sufficiently understood. Kamimura *et al.* (1995) showed that β-adrenoceptor blockers decreased BP without any effect on plasma renin activity (PRA) while angiotensin antagonists failed to decrease the BP. They suggested that, in chickens, the elevation of the activity of the sympatho-adrenomedullary system may be the primary mechanism responsible for elevated BP rather than the renin-angiotensin system. In an earlier study, Nishimura *et al.* (1981) also reported that the acute AII infusion increases plasma norepinephrine levels, but the angiotensin-converting enzyme (ACE) inhibitor SQ 14,225 produces no consistent change in plasma catecholamine levels during chronic treatment in mature chickens. All these studies with the ACE inhibitor were performed on mature chickens in which high BP and arteriosclerosis had already been established. Therefore, our aim was to investigate the effects of an ACE inhibitor, lisinopril dihydrate, which was applied from an early age to maturity, on blood pressure and plasma catecholamine levels in chickens.

Methods

Animals

Male white leghorn chickens, *Gallus gallus,* aged 1 day, were purchased in May from Cetinkaya Farm, Cumra-Konya, Turkey. They were housed freely in a wide and day-lighted room (340x360 x 350 cm) divided into four compartments (110x180x187cm) with a wire fence and were fed with commercial chicken chow (Köy-Tür Ltd, Elazig) containing 0.35%.NaCl

Experimental Protocols

Dose regimes of long-term lisinopril application. At the age of 3 weeks, the chickens were divided into 4 groups. Lisinopril dihydrate (Ld) was applied to three groups in following concentrations: 25 mg/l tap water (group Ld1, n = 15), 75 mg/l (group Ld2, n = 15) and 250 mg/l (group Ld3, n = 15). In all groups these lisinopril concentrations were given once a day at 08:00 h in the amount of one liter for weeks 4-5 of age, 1.5 l for weeks 6-7 and 2 l for weeks 8-12 of age. The lisinopril solutions were consumed approximately within one hour by the chickens of all groups, after which the normal tap water was allowed *ad libitum* until 20:00 h every day. The fourth group (group C, n = 14) received normal tap water *ad libitum* from 08:00 h until 20:00 h. At the end of week 12, some of the chickens (n = 7) in each group were used for the measurement of blood pressure and the rest for collecting blood samples.

Surgical Procedures

Measurement of blood pressure. Twenty-eight hours after the last lisinopril application the chickens were anesthetized with ketamine (Ketalar, Parke-Davis, Turkey; 75 mg/kg, i.m.). The surgical procedure and cannulation were described in detail elsewhere. Briefly, the chickens were laid down in the supine position, the feathers inside the right leg were plucked and a skin incision was performed (Nishimura *et al.* 1982). A polyethylene catheter (4FG, Fortex) was inserted into the femoral artery. The arterial BP was measured directly *via* a manometric system that was filled with saline (0.9 % NaCl containing heparin 100 IU/ml). BP was recorded after it had become stabilized.

Blood sample collection

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

Analytical Methods

Plasma renin activity was measured as the rate of angiotensin I generation. Blood (5 ml) was collected into tubes containing 0.2 ml Na-EDTA and centrifuged (10 min, 2500-3000 *g*, 4 °C). Plasma was removed and stored at -20° C until assayed for PRA. Renin MAIA kit (based on the radioimmunoassay method) was used for PRA measurement. In an ice bath, 1 ml plasma was pipetted into uncoated glass or plastic tubes and 10 µl of phenylmethylsulfonyl fluoride (PMSF) and 100 µl of a maleate generation buffer (pH 6, containing Na-EDTA,

neomycin sulphate and inert blue dye with 0.1 % sodium azide as preservative) were added, mixed and placed into an ice bath. Plasma renin activity was determined by radioimmunossay according to the modified method of Haber *et al.* (1969).

Total counts were assessed using paired tubes. Angiotensin I, blank and standard (100 µl) were put into tubes followed by 100 µl of plasma given into renin control and sample tubes (37 °C and 4 °C series). One ml tracer-buffer was added to all tubes and 0.1 ml renin MAIA antiserum was added to all tubes except total counts tubes and incubated for 18-20 h in an ice bath. After incubation, 1 ml renin separation reagent was added into all tubes except the total count tubes. Tubes were mixed for 10 min. The content of the tubes was dried on filter paper before the values were determined. PRA levels (ng/ml/h) were calculated with graphic representation prepared on semilogaritmic paper.

Plasma angiotensin-converting enzyme activity was determined using ACE kits according to the method of Holmquist *et al.* (1979). Lyophilized reagents containing normal, control and calibrator N-3-[2-furyl]acryloyl)-phe-gly-gly (FAPGG) substrate were dissolved (pH 8.2 and FAPGG concentration 0.5 mmol/l). 340 nm filter was used in spectrophotometer Shimadzu UV-1201. Reagents were put into blank, standard and sample tubes which were incubated for 5 min at 37 °C in a water bath, later the absorbance value was read. Plasma ACE activity was expressed in U/l.

Plasma noradrenaline and adrenaline levels were determined by HPLC (Cecil 1100, UK) with UV detection (data module, HP, USA). Samples and standards were separated by supelcosil LC-ABZ (5 cm x 4.6 mm ID; 5 µm particles, Supelco). The mobile phase was acetonitrile plus 25 mM $K_2H_2PO_4$ (Merck) (pH 2 with phosphoric acid and 5.4 with heptansulfonic acid) (5/95, v/v). Venous and arterial blood were collected in ice-cold plastic tubes containing EDTA and centrifuged (15 min, 2600 *g*, 4 °C). Plasma samples (1.5 ml) were mixed and stored at –70 °C until assayed. 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 by means of a rotatory mixer. Thereafter, the bottom stopper of the filtration unit was replaced by a filter (Whatman GF/C) and the filtration unit was centrifuged (1000 x *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 water, and the catechols were finally desorbed from Al_2O_3 with 2 x 75 µl 0.1 M $HClO_4$, each washing and desorption step being followed by centrifugation (1000 *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 an ultrasonic water bath were then injected (20 µl) into the HPLC system (Halbrügge *et al.* 1988, 1991). 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 comparing with the standard curve. Values were expressed as ng/ml. Recovery was found to be 68-81 %.

Drugs and Reagents

Lisinopril dihydrate was obtained from Eczacibaşi, (Istanbul, Turkey), adrenaline hydrochloride, noradrenaline bitartrate and all other reagents and solvents from Sigma (Steinheim, Germany). Al_2O_3 was obtained from Merck (Darmstadt, Germany), renin MAIA kit from Biodata Diag., Polymedco Inc. (USA).

Statistics

Results are expressed as means ± SEM. Differences between group means were determined by Students t-test (paired and unpaired where appropriate).

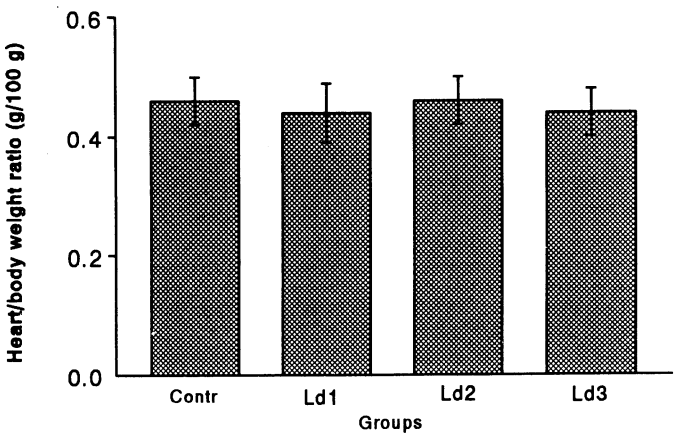

Fig. 1. *The distribution of the heart weight/body weight ratio according to groups. The heart weight/body weight ratio are not significantly different in any group treated with lisinopril compared to the control group (Mean ± SD).*

Results

Effect of long-term lisinopril application on body weight, heart weight/body weight ratio, plasma renin activity and angiotensin-converting enzyme activity

In chickens, lisinopril application (from the 3rd to the 12th week of age) had no effect on the growth rate. Body weight increased by 330 % over the 9 weeks. At the beginning of lisinopril application and at the end of the experiment, body weights were 276±3 and 1183±16 g in the group exposed to the highest dose of lisinopril (250 mg/l drinking water), whereas they were 257±17 and 1130±25 g in controls. The heart weight/body weight ratio also did not differ significantly in all the groups treated with lisinopril compared to the control group, despite the tendency to a dose-dependent decrease (Fig. 1).

The measurements of PRA and ACE activities were performed in the blood samples collected 28 h after the last lisinopril application. PRA values were relatively low because human AI antibody was used for the measurement in this study. However, PRA values in lisinopril-treated groups were found to be increased in a dose-dependent manner. ACE activities remained inhibited in all lisinopril-treated groups 28 h after the last lisinopril application, but this inhibition was not dose-dependent.

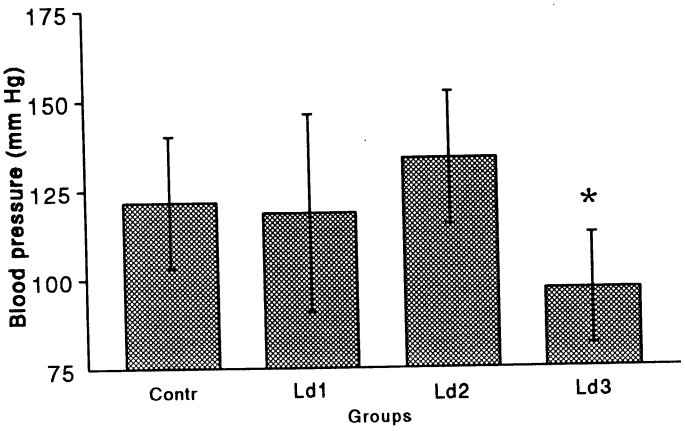

Fig. 2. *Arterial blood pressure (mm Hg, mean ± SD) after long-term lisinopril dihydrate (Ld) application in different concentrations [Ld1 (n = 7), Ld2 (n = 7), Ld3 (n = 7)] and control (C, n = 5) groups. BP was decreased only in the group receiving the highest dose of lisinopril. * P<0.05.*

Effect of long-term lisinopril application on arterial blood pressure

The arterial BP measured 28 h after the last lisinopril application was found to be decreased only in the group receiving the highest dose of lisinopril. The arterial BP values (means ± SEM) in control and Ld1, Ld2 and Ld3 groups were 122±7.82, 119± 10.27, 135± 6.54 and 98±5.43 mm Hg, respectively (Fig. 2).

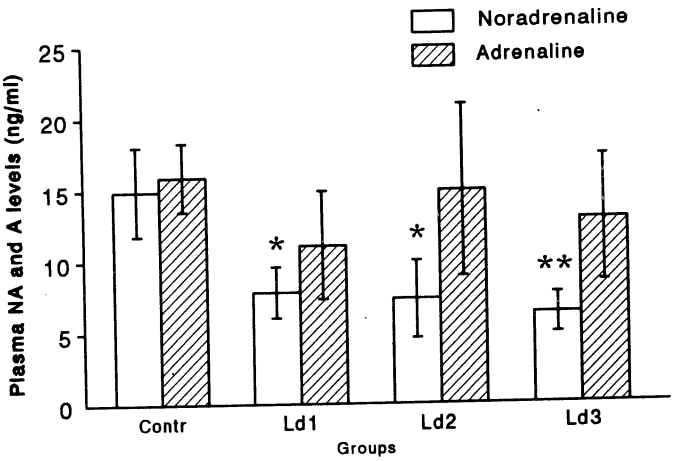

Fig. 3. *Plasma noradrenaline (NA) and adrenaline (A) levels (Mean ± SD) in control (C, n=7) and lisinopril dihydrate (Ld) treated groups. [Ld1 (n = 8), Ld2 (n = 8), Ld3 (n = 8)]. The lisinopril application significantly decreased plasma noradrenaline levels compared to control group, but plasma adrenaline levels remained unchanged. * P<0.01,** P<0.001.*

Effect of long-term lisinopril application on plasma noradrenaline and adrenaline levels

Plasma norepinephrine levels determined in blood samples collected 28 h after the last lisinopril application were found to be decreased significantly in a dose-dependent manner as compared to the control group, but plasma epinephrine levels remained unchanged (Fig. 3). The decrease of plasma norepinephrine levels in Ldl, Ld2 and Ld3 groups were 47, 51 and 58 %, respectively.

Discussion

The effects of AII on BP and its probable mechanisms is well studied in chickens (Nishimura *et al.* 1982, Moore *et al.* 1981, Yamaguchi and Nishimura 1986, Kamimura *et al.* 1995, Nishimura *et al.* 1981). Acutely infused AII increases both plasma NA and A levels but acute and chronic angiotensin antagonism has no effect on the levels of these amines in mature chickens (Kamimura *et al.* 1995, Nishimura *et al.* 1981, Moore 1980).

In the present study, chronic angiotensin antagonism from an early to mature age (from the 3rd to the 12th week of age) was induced by long-term lisinopril application. With the dose regimes used in this study, it is not possible to define the exact daily lisinopril amounts per kg to which the chickens were exposed. Nevertheless, it is evident that there is a dose-dependent difference between groups in the effects of lisinopril on PRA and ACE activity. The values of PRA obtained in this study were low due to the heterologous antibody used for the measurement, but there was a dose-dependent increase of PRA in the groups treated with lisinopril, indicating the validity of dose regimes used (Nishimura *et al.* 1981).

However, our findings on the inhibition of plasma ACE activities did not disclose a parallelism with the applied doses in spite of the ACE inhibition induced in all groups. This may be related to a possible rebound activation of ACE because blood samples were collected 28 h after the last dose of lisinopril. However, it is difficult to explain why PRA remains dose-dependently inhibited, while ACE activity was reactivated.

In our long-term experiments with different lisinopril dosage blood pressure was significantly decreased only in the highest lisinopril group. Intermediate and low lisinopril doses were insufficient to decrease blood pressure. The most important results obtained in this study concern the effects of long-term lisinopril application on NA and A levels. Although acute lisinopril applications produced contradictory changes in NA and A levels (data not shown), chronic lisinopril administration did not affect plasma adrenaline levels in either group, but decreased noradrenaline levels significantly, persistently and dose-dependently in all the groups.

In the present study, values of plasma NA and A levels in chickens were higher compared with the other reports (Nishimura *et al.* 1981, Vanhoutte 1989, 1993). These differences may depend on the type of experiment, blood sampling and measurement conditions. Since plasma NA levels were decreased in all lisinopril-treated groups, but BP was only reduced in the high lisinopril-treated group, we suggest that long-term lisinopril application can decrease plasma NA levels without affecting the BP. Therefore, the decrease of BP in the group with the highest lisinopril dose could not be related to a decrease of plasma NA levels. Furthermore, we could not find any changes in heart weight/body weight ratio and in the histopathological examination of the arterial tissue.

Makki *et al.* (1994) reported that plasma ACE inhibition is not the primary factor in arterial and cardiac structural change which were caused by long-term lisinopril application in rats. While long-term lisinopril treatments could not affect plasma adrenaline levels, but did decrease plasma noradrenaline levels, it can be proposed that the modulatory role of AII is exerted on the adrenergic nerve terminals rather than on the adrenal medulla.

It seems that the effect of high concentration of lisinopril on blood pressure, is related to its effect on renin-angiotensin system. This indicates the important role of RAS in the maintenance of BP in chickens, which is contrary to the reports of Nishimura *et al.* (1981). These authors reported the failure of AII antagonist to cause a sustained BP lowering or a decrease in plasma catecholamines, suggesting that the renin-angiotensin system does not play a primary role in maintaining the BP in chickens (Nishimura *et al.* 1981).

In conclusion, we have shown for the first time that the long-term ACE inhibition decreases plasma noradrenaline levels without changing the blood pressure and adrenaline levels. This suggests that in chickens the modulatory role of angiotensin II involves adrenergic nerve terminals rather than the adrenal medulla.

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