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Cardiovascular System in Offsprings of Hypertensive Rats with Defective Nitric Oxide Production

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Summary

The question was addressed of how nitric oxide synthase (NO synthase) inhibition-induced hypertension in rat parents would affect the cardiovascular system in their offsprings. Two experimental groups were set up: Group I – offsprings of parents who had both been administered NO synthase inhibitor L-nitro-arginine methyl ester (L-NAME 40 mg/kg/day) for 5 weeks, the treatment of dams continued till week 12. Group II – offsprings fed by dams administered L-NAME after delivery only for a period of 4 weeks. Control age-matched offsprings formed the third group. Blood pressure and heart rate in parents and in 3-week-old offsprings were determined noninvasively. In the offsprings, body and heart weight were measured and the heart/body weight ratio (HW/BW) was calculated. The NO synthase activity, and also ornithine decarboxylase activity as a marker of polyamine production, were determined in the heart. The acetylcholine-induced relaxation of aortic rings was also followed. A marked blood pressure increase with a tendency to a decreased heart rate was found in the offsprings of Group I. A significant decrease in heart weight and body weight with a decreased HW/BW ratio indicated cardiac hypotrophy that contrasted with the decrease in NO synthase activity and increase in ornithine decarboxylase activity in the heart. Noteworthy was also the finding of completely preserved relaxation of the aorta to acetylcholine. Offsprings of Group II were similarly characterized by significantly higher blood pressure, a tendency to decreased heart rate, a decrease in heart weight, but not of the HW/BW ratio. The contrasting findings of heart weight decrease on the one hand and NO synthase activity decrease and ornithine decarboxylase increase on the other, were also found in this group. Full relaxation of the aorta to acetylcholine was preserved. It can be concluded that remarkable alterations in the cardiovascular system were found in offsprings of hypertensive NO compromised parents.

Key words
Offsprings • Hypertension • Cardiac development • Nitric oxide • Ornithine decarboxylase

Introduction

Soon after the endothelium-derived relaxing factor (EDRF) had been discovered, Radomski (1991) described the metabolic pathway arginine → citrulline + NO in *Limulus polyphemus*, suggesting this pathway as a phylogenetic archetype. In parallel, attention was paid to the ontogenetic dynamics of arginine metabolism and NO production in pulmonary vessels (Abman *et al.* 1991, Shaul *et al.* 1993). Endothelial NO synthase was detected in the microcirculatory area of respiratory muscles in fetuses during late pregnancy (El Dwairi *et al.* 1998).

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Conduit arteries of canine fetuses and newborns were proven to respond remarkably to acetylcholine (Török and Gerová 1996, 1997). The compromised function of the endothelium in offsprings of parents with essential hypertension was repeatedly addressed, the results are, however, controversial (Taddei *et al.* 1996, Miyamoto *et al.* 1998, Kato *et al.* 1999). Thus the aim of the study was to focus on the cardiovascular system of offsprings of hypertensive parents due to definitely compromised arginine metabolism and NO production. Two experimental models were set up. In one model, before fertilization was allowed to occur, a sustained NO compromised hypertension was induced by inhibition of NO synthase lasting 4 weeks both in females and males. The inhibition of NO synthase was then continued in females throughout pregnancy and breast feeding. In the second model, NO synthase was inhibited in dams only, immediately after delivery, and the treatment was continued during breast feeding up to the end of the experiment.

Basal cardiovascular parameters (blood pressure and heart rate) were measured noninvasively in the offsprings. At the end of the experiment, heart and body weight were determined and the HW/BW ratio was calculated. Data concerning the cardiac response to compromised NO production followed by hypertension were controversial in adult animals (Arnal *et al.* 1993, Bernátová *et al.* 1996, Sládek *et al.* 1996, Banting *et al.* 1997). Contrary to other models of experimental hypertension, the results of experiments from various laboratories cast doubt on the cardiac hypertrophy in the experimental model of NO-deficient hypertension (Arnal *et al.* 1993, Bartunek *et al.* 2000, De Oliveira *et al.* 2000). To acquire more information on the mechanisms involved in the growth of the heart in our experimental model, the primary parameter – activity of NO synthase in the heart – was followed. Furthermore, the involvement of one of the growth supporting pathways was studied, namely the conversion of ornithine to polyamines, known to contribute to cardiac growth (Bartolomé *et al.* 1980, Zimmer and Peffer 1986, Gerová *et al.* 1995). Ornithine decarboxylase, as the rate limiting enzyme of the above conversion, was determined. This pathway is rather interesting since ornithine is one of the direct products of arginine metabolism, governed by the enzyme arginase.

Finally, to acquire further information on the dilation ability of systemic vessels induced by activation of NO synthase, the vasomotor activity of aortic rings from offsprings of hypertensive NO compromised parents was assessed *in vitro*.

Methods

Wistar-Kyoto rats were used for the experiments. Procedures and the experimental protocol used in this study was approved by the Animal Care Committee of the Slovak Academy of Sciences.

Parents

Ten-week-old Wistar-Kyoto rats of both sexes were taken for the experiments. They were housed under a 12 hour dark-light cycle at constant temperature (22-24 ºC) and free access to pellet food and water. For the first four weeks, they were housed in individual cages.

Experimental group I consisted of 8 females and 8 males. The animals received the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) in a dose 40 mg/kg/day in drinking water. The eight pairs in this group were used because preliminary experiments revealed a rather high mortality in offsprings of parents administered L-NAME (Gerová *et al.* 2000).

Experimental group II consisted of three females fertilized by untreated males. L-NAME 40 mg/kg/day was administered to dams after delivery only, over a period of 4 weeks.

Control group consisted of three females and three males, age-matched to the above experimental groups.

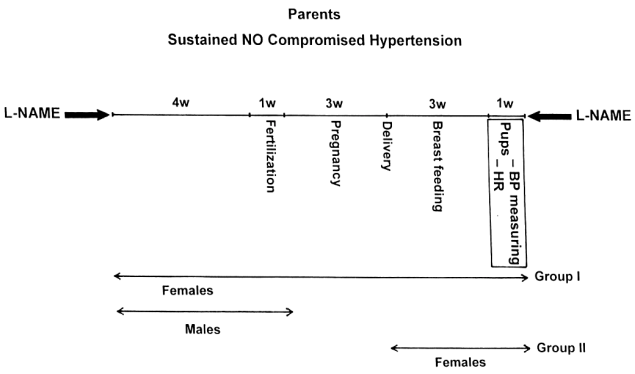

Fig. 1. *Scheme and time course of the experiment.*

Experimental protocol

The course of experiment is shown in Figure 1. The animals of *experimental group I* were administered L-NAME at the age of 10 weeks for a period of 5 weeks when NO compromised hypertension developed and became stabilized. Fertilization occurred in the 5th week. In females, the administration of L-NAME was continued

in the course of pregnancy, delivery, breast-feeding and even in the period of blood pressure measurements in the offsprings till the end of the experiment.

In the experimental group II, the females, fertilized by untreated males, were administered L-NAME immediately after delivery and the treatment continued throughout breast feeding and blood pressure measurements in the offsprings (Fig. 1).

The age-matched control group of animals was fertilized at the same time and under the same conditions as the above experimental groups, yet without inhibition of NO synthase (Fig. 1).

Blood pressure and heart rate in all adult animals were measured weekly by the tail-cuff plethysmographic method. A break in the measurements was made during pregnancy and early breast-feeding. Blood pressure and heart rate were measured again in the second week after delivery and at the end of the experiment. Each value of blood pressure and heart rate represented a mean of five consecutive measurements during one session. Body weight was assessed each week.

Offsprings

Control group: Thirty offsprings were delivered by three control females. Twenty-two randomly selected newborns were taken for the experiments.

Experimental group I consisted of offsprings born from both parents with NO compromised hypertension. Out of 8 females, two died in the course of 4 weeks of L-NAME treatment. Thus, six females delivered a total of 49 offsprings. Only 37 offsprings survived till the third week. Finally, 30 offsprings, randomly selected, were taken for the measurements.

Experimental group II consisted of 27 offsprings born from untreated three females and three males. The females were administered L-NAME only after delivery. All offsprings survived and were used for the measurements.

Experimental protocol

Blood pressure and heart rate were measured in all three groups after the animals had attained three weeks, twice at three-day intervals. The above parameters were measured on the tail artery using the plethysmographic method, with a miniaturized size-adjusted fine rubber cuff and plexiglass ring. Similarly as in adults, each value of blood pressure and heart rate represented the mean of five measurements during one session.

The body weight was determined at the end of the experiment. The offsprings were sacrificed by decapitation. The heart was weighed and the HW/BW ratio was calculated and the heart was divided into two halves for estimation of NO synthase activity and ornithine decarboxylase activity. Each half contained both left and right ventricle.

The thoracic aorta was excised and the upper part was used for ODC activity determination. The middle part of the thoracic aorta was used for studying the vasomotor activity in organ bath experiments.

NO synthase activity

Total NO synthase activity was determined in crude homogenates of tissues by measuring the formation of [^{3}H]-L-citrulline from [^{3}H]-L-arginine (Amersham, UK), as previously described by Bredt and Snyder (1990) with some modifications (Pecháňová *et al.* 1997). A segment of the tissue (75 mg) was homogenized (2x30 sec, Ultra-Turrax homogenizer) in 425 µl of ice-cold 50 mM Tris-HCl buffer, pH 7.4. Then 50 µl of crude homogenate of the sample was incubated in the presence of 50 mM Tris-HCl, pH 7.4, containing 1 µM L-[^{3}H]arginine (specific activity 5 GBq/mM, about 100 000 DPM), 30 nM calmodulin, 1 mM β-NADPH, 3 µM tetrahydrobiopterin, and 2 mM Ca^{2+}, in a total volume of 100 µl. After 10-min incubation at 37 °C, the reaction was stopped by addition of 1 ml of 20 mM HEPES buffer, pH 5.5, containing 2 mM EDTA, 2 mM EGTA and 1 mM L-citrulline. The samples were centrifuged at 10 000 x *g* for 1 min at 4 °C and the supernatant was applied to 1 ml Dowex 50WX-8 columns (Na^+ form). [^{3}H]-L-citrulline was eluted with 2 ml of water and measured by liquid scintillation counting. NO synthase activity was expressed as pkat.g^{-1} of proteins. Protein concentration was determined according to Lowry *et al.* (1951).

Ornithine decarboxylase activity

The ODC activity was assayed as $^{14}CO_2$ released from [^{14}C]-ornithine using the method of Slotkin and Bartolomé (1983). The total incubation volume was 250 µl. DL-[1-^{14}C]-ornithine hydrochloride (specific activity 520 MBq/mmol, Hungarian Academy of Sciences, Hungary) was purified prior to use by thin-layer chromatography on silica gel plates. After developing with chloroform : methanol : 17 % ammonium hydroxide (4:4:2) a spot corresponding to [^{14}C]-ornithine was eluted with aqueous solution of 2 % ethanol.

Organ bath studies

The middle part of the thoracic aorta was excised, cleaned of excess of adhering tissue and cut into 3-4 mm long rings. Each ring was vertically mounted on stainless steel hooks and transferred to a jacket organ chamber filled with Krebs solution of the following composition (mM): NaCl 118, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, CaNa.EDTA 0.3, ascorbic acid 0.55. The solution was maintained at 37 ºC and continuously gassed with 95 % O_2 + 5 % CO_2, pH 7.2-7.4. The tension of the ring was recorded isometrically with an electromechanical transducer (Sanborn FT10) on a potentionic recorder (Labora TZ 4200). The resting tension of the aortic ring was 10 mN. The tissue was allowed to equilibrate for a period of 60-90 min before being contracted submaximally by adding phenylephrine (10^{-6} M). After the contractile response was stabilized, relaxation was induced using cumulative addition of acetylcholine (10^{-9}-10^{-5} M). The stabilized value of contraction with phenylephrine was considered to be 100 % and the relaxation responses were calculated as a percentage of this contraction.

Statistical evaluation

Data were expressed as the mean + S.E.M. Significant differences among groups were evaluated by one-way analysis of variance (ANOVA).

Results

Parents

Control group

The time course of blood pressure in the control group is given in Figure 2. The litter size was 7-12 offsprings and all survived till the end of the experiment.

Experimental group I: The blood pressure values are given in Figure 2. Blood pressure after the first week of L-NAME administration increased in females from 102.8±1.1 mm Hg to 129.9±4.3 mm Hg (P<0.001) and fluctuated around the value of 146.8±7.9 mm Hg (P<0.001) established at the end of the experiment. In males, the blood pressure increased from 107.4±2.8 mm Hg to 137.0±3.2 mm Hg (P<0.001) in the first week and to 154.8±5.2 mm Hg (P<0.001) in the fifth week of the experiment.

In females administered L-NAME the heart rate was 245.5±6.3 beats/min at the start of the experiment and 234.6±6.8 beats/min in the second week after delivery, i.e. it did not change significantly, although there was a trend to lower values. It increased to 330 beats/min (P<0.01) at the end of the experiment. In males, the heart rate decreased significantly from 252.4±8.8 beats/min to 229.3±3.6 beats/min (P<0.001) in the fifth week of treatment only.

The litter size was 4-11 offsprings. Only 75.5 % survived by the third week.

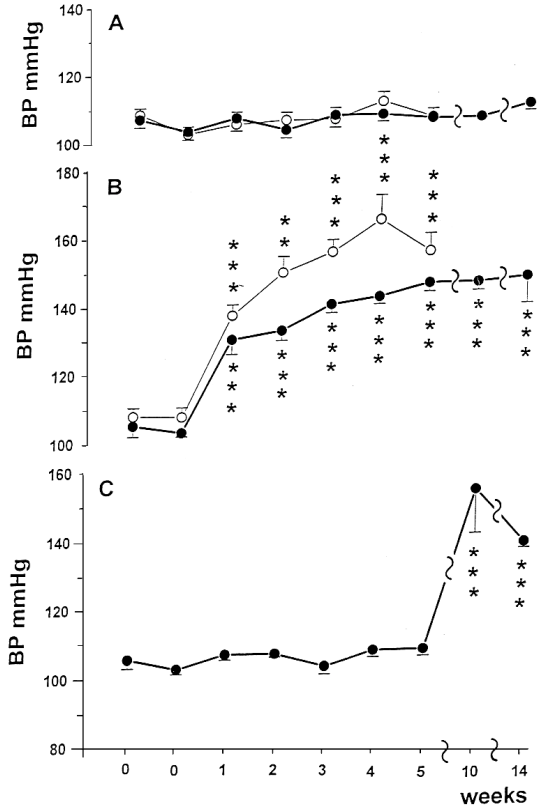

Fig. 2. (*A) Blood pressure of control rat parents, (B) blood pressure of parents with NO synthase inhibition by administration of L-NAME (40 mg/kg/day), (C) blood pressure of dams with L-NAME administration after delivery only. Mean values ± .S.E.M of females ●-●, mean values ± S.E.M. of males ○-○, ** P<0.001 vs control values.*

Experimental group II

In females administered L-NAME after delivery only, the blood pressure increased from 106.8±2 mm Hg measured before delivery to 153.0±12.7 mm Hg (P<0.001) and 137.5±1.8 mm Hg (P<0.001) after the second week of L-NAME administration and at the end of the experiment, respectively.

Before delivery the heart rate was 268.6±5.0 beats/min, in the second week after delivery it was 259.0±16.7, and at the end of experiment it increased to 377.8±20.3 beats/min (P<0.01).

The litters contained 7-12 offsprings, all surviving till the end of the experiment.

Offsprings

Control group

Blood pressure in the controls in two subsequent measurements within the interval of 3 days was 94.6±4.5 mm Hg and 104.6±2.1 mm Hg, respectively. The heart rate was 409.0±17.7 beats/min and 388.3±20.2 beats/min, respectively (Fig. 3).

Heart weight measured at the end of the experiment was 367.7±11.4 mg, body weight was 83.6±2.7 g and calculated HW/BW ratio was 4.4±0.2 (Fig. 4).

The activity of NO synthase in the heart of 7 offsprings represented 0.523±0.05 pkat/g (Fig. 5).

Ornithine decarboxylase activity (n=11) was 412±33 pmol CO_2/mg protein/h in the heart and 281±24 pmol CO_2/mg protein/h in the aorta of control offsprings (Fig. 6).

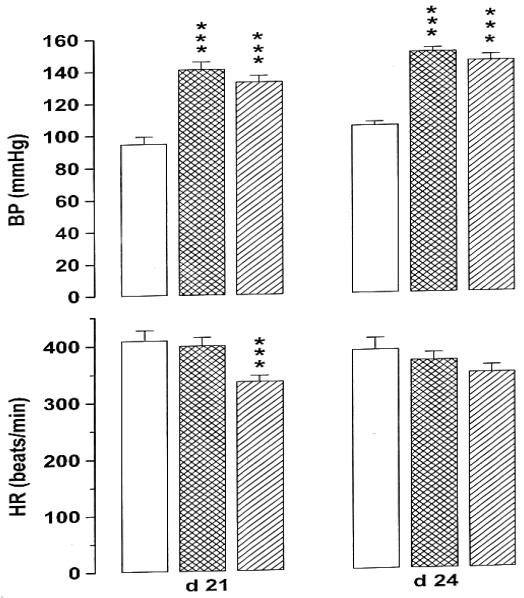

Fig. 3. *Blood pressure and heart rate in offsprings aged 21 and 24 days. Mean values ± S.E.M. of control group of offsprings (white columns), of experimental group I - offsprings of both hypertensive parents with NO synthase inhibited by L-NAME 40 mg/kg/day (cross-hatched columns), and of experimental group II - offsprings of untreated parents and dams administered L-NAME 40 mg/kg/day after delivery only (hatched columns). *** P<0.001 vs respective value in offsprings of control group.*

Experimental group I

The blood pressure in 3-week-old offsprings was 140.9±4.6 mm Hg in the first measurement and 150.0±2.3 mm Hg three days later. In both measurements, the values were significantly higher (P<0.001) than in control offsprings, the heart rate being 399.4±14.3 beats/min and 368.7 beats/min, respectively (Fig. 3).

Figure 4 presents the results on heart and body weight. The heart weight of offsprings in experimental group I was only 276.8±15.4 mg, which was significantly lower than in control animals (367.7±11.4 mg, P<0.05). The body weight was 68.7±3.2 g, which is a value significantly lower compared with controls (83.6±2.7 g, P<0.01). The HW/BW ratio in these offsprings was 3.9±0.1, which was significantly lower than the corresponding value in controls (4.4±0.2, P<0.05).

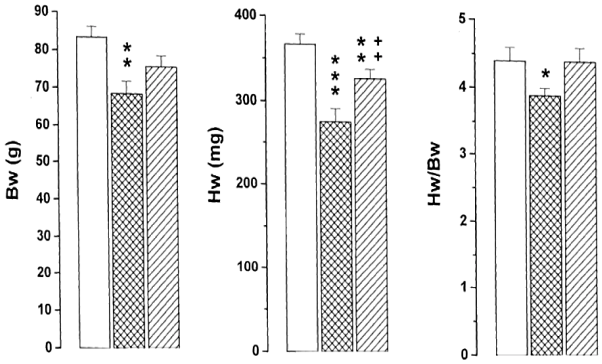

Fig. 4. *Body weight, heart weight and HW/BW ratio in offsprings of control group (white columns), experimental group I (cross-hatched columns), and experimental group II (hatched columns). Mean values ± .S.E.M., ** P<0.01, *** P<0.001, vs control group, ++ P<0.01 vs experimental group I.*

Activity of NO synthase in the heart of these offsprings (n=7) was markedly decreased to 0.26±0.02 pkat/g (P<0.05) (Fig. 5).

The activity of ornithine decarboxylase in the heart and aorta of offsprings in experimental group I (n=11) was 542±42 pmol CO_2/mg protein/h (P<0.05) and 392±32 pmol CO_2/mg protein/h (P<0.05), respectively. The values were significantly higher than the corresponding values found in the controls (Fig. 6).

Experimental group II

Blood pressure in offsprings of this group was 132.6±4.1 (P<0.001) in the first measurement, which was significantly higher in comparison to the control group. Significantly higher was also the value of the second measurement three days later, i.e. 144.2±3.6 mm Hg (P<0.001). No significant differences were found in the

blood pressure measurements between experimental group I and experimental group II (Fig. 3).

Heart rate in offsprings of experimental group II was 335.5±10.6 beats/min (P<0.001) and 345.9±12.9 beats/min. The value of the first measurement was significantly lower in comparison to the controls (Fig. 3).

The heart weight of the offsprings fed by mothers with inhibited NO synthase after delivery was 327.9±10.7 mg (P<0.01), i.e. significantly lower than in the controls. Body weight of 75.9±2.9 g indicated a decline, however, this was not significant in comparison with the controls (Fig. 4). The HW/BW ratio 4.3±0.17 did not differ significantly from the respective value of control animals (Fig. 4).

NO synthase activity in the heart of offsprings in the experimental group II (n=6) was 0.21±0.019 pkat/g (P<0.05), which was significantly lower than in the controls. The value was close to the value found in the heart of offsprings of the experimental group I (Fig. 5).

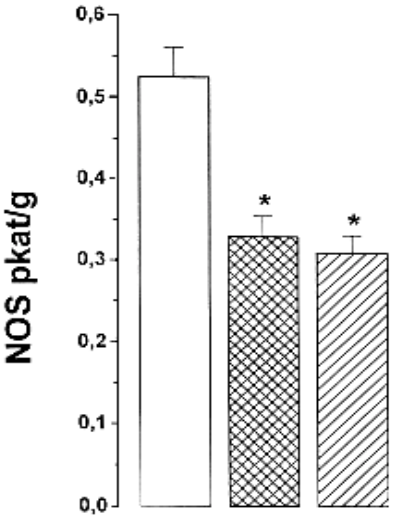

Fig. 5. *NO synthase activity in the heart of offsprings at the age of 4 weeks. Mean values ±. S.E.M. of control group (white column), of experimental group I (cross-hatched column), and of experimental group II (hatched column). * P<0.05 vs control group.*

Ornithine decarboxylase activity in the heart of these offsprings (n=10) was 432±24 pmol CO_2/mg protein/h and in the aorta 325±18 pmol CO_2/mg protein/h. In both tissues these values were not significantly higher than in the tissues of control offsprings (Fig. 6).

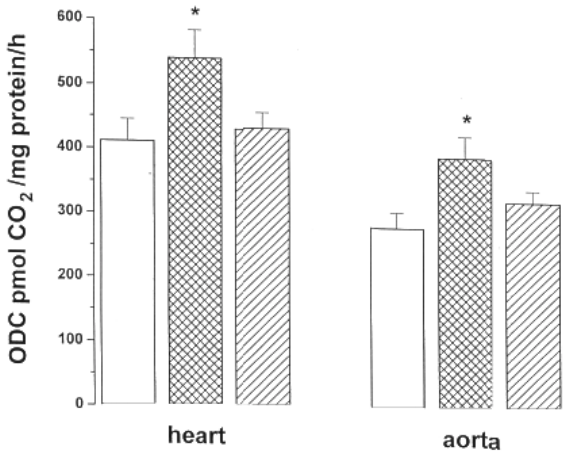

Fig. 6. *ODC activity in the heart and aorta of offsprings at the age of 4 weeks. Mean values ± S.E.M. of control group (white columns), of experimental group I (cross-hatched columns), and of experimental group II (hatched columns) * P<0.05 vs control group.*

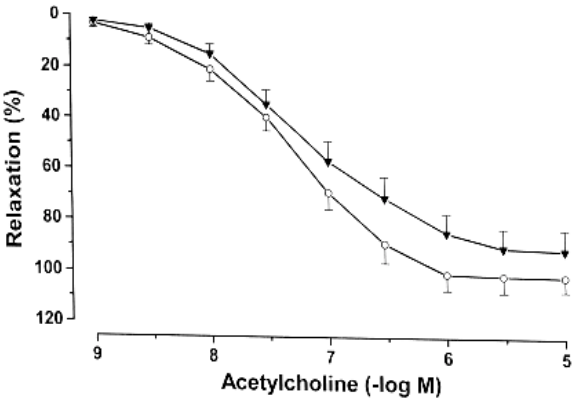

Fig. 7. *Concentration-response curves to acetylcholine in phenylephrine-precontracted (10^{-6} M) aortic rings from offsprings of control group (○) and from offsprings of experimental group I (▲). Values are means ± S.E.M.*

Acetylcholine-induced relaxation of offsprings aortic rings

Acetylcholine (10^{-9}-10^{-5} M) induced concentration-dependent relaxations in aortic rings from control rats, precontracted with 10^{-6} M phenylephrine. Complete relaxation (102.2±7.4 %, n=12) was reached at 10^{-6} M acetylcholine.

Acetylcholine also induced relaxations in aortic rings from hypertensive offsprings of the experimental group I (n=14). The dose-response curve was similar to that of control offsprings, with the maximal value of 91.2±8.1 % achieved at 10^{-6} M acetylcholine (Fig. 7).

Acetylcholine-induced relaxations of aortic rings from offsprings of the experimental group II were similar to those found in hypertensive offsprings of the experimental group I. The course the dose-response curve, as well as the maximal value of relaxation (93.9±7.9 %, n=11), did not differ significantly when compared to either the corresponding values of the experimental group I or to control offsprings (Fig. 8).

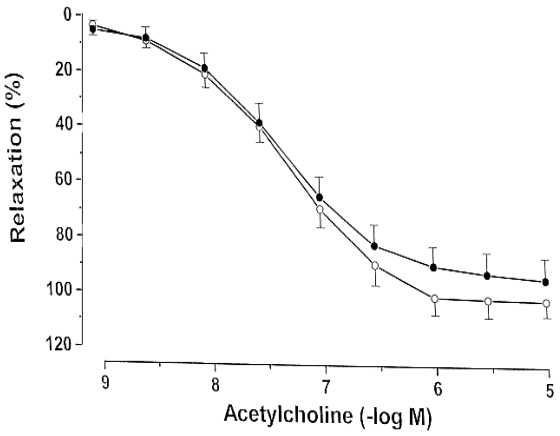

Fig. 8. *Concentration-response curves to acetylcholine in phenylephrine-precontracted (10^{-6} M) aortic rings from offsprings of control group (○) and from offsprings of experimental group II (●). Values are means ± S.E.M.*

Discussion

After the inhibition of NO synthase by L-NAME administration (experimental group I) the blood pressure of the parents increased in the first week and fluctuated around the enhanced value during the whole experimental period, in agreement with other authors (Ribeiro *et al.* 1992, Arnal *et al.* 1993, Gerová and Kristek 2001). The heart rate tended to decline. The litter size, when both parents had inhibited NO synthase (experimental group I), was lower (4-11 pups) than that of control parents (7-12 pups). Moreover, only 75.5 % of offsprings survived till the third week.

Blood pressure of dams administered L-NAME after delivery only (experimental group II) increased similarly as in animals of experimental group I. The litter size equal to the control group did not change in the course of breast feeding. All offsprings survived the third week. In our previous experiments (Török and Gerová 1996) canine pups administered L-NAME for 6 weeks survived till the end of experiment. Contrary to our findings in both rats and dogs, Prickaerts *et al.* (1998) found a relatively high mortality in rat offsprings administered L-NAME postnatally. These authors, however, administered L-NAME intraperitoneally to newborn rats daily.

The offsprings born to parents, which both had sustained NO compromised hypertension, yielded a pattern of a distinctly compromised cardiovascular system. They had a remarkably higher blood pressure and a tendency to a decreased heart rate. The heart weight was lower in comparison with control offsprings. Even when taking into account the retardation of general body growth, the slower rate of heart growth was still remarkable. Indeed, the HW/BW ratio was significantly lower in these offsprings than in the controls, indicating obvious hypotrophy of the heart. This finding is the more surprising if one further considers two other experimental results. The first concerns the remarkable decline of NO synthase activity in the heart, which dropped down to about 60 % of the value found in the hearts of control offsprings. These data testify about the severe interference into the arginine metabolism, implying that growth acceleration could be expected at a low level of nitric oxide (Garg and Hassid 1989). In contrast, a distinct attenuation of growth of the heart was found. The second result, concerning ornithine decarboxylase activity, is in concert with our hypothetical consideration, namely: arginine, besides its conversion to citrulline + nitric oxide, governed by NO synthase, is also metabolized by the Krebs pathway to ornithine + urea controlled by arginase. A negative feedback was revealed between the two pathways (Cynober *et al.* 1995, Boucher *et al.* 1999). As far as our experiments are concerned, a low level of NO synthase activity in the myocardium, implying a low level of intermediates and end-products, should affect positively the activity of arginase and thus increase ornithine production, which is the substrate for polyamines – supporters of growth. The above consideration justified to expect cardiac hypertrophy. However, completely contrary findings were seen in offsprings of parents with NO compromised hypertension: not only a decreased heart weight but also a low HW/BW ratio. Both indicators of growth support, i.e. (i) the decrease of NO synthase activity and (ii) the increase of ornithine decarboxylase activity, are completely controversial to the hypotrophy of the heart. Does this mean that other principles of growth support of the heart are operative in NO deficiency? Recently, de Oliveira *et al.* (2000) offered convincing evidence on cardiac hypotrophy by measuring the size of myocytes in adult rats with inhibition of NO synthase induced even by low doses of L-NAME administered for several months.

The data of Pignatti *et al.* (1999), obtained in a completely different experimental model – cultured chick embryo cardiomyocytes – yielded the same message.

Although our findings do not allow us to define the underlying mechanisms, they allow to state unequivocally that heart hypothrophy is present in the offsprings of hypertensive NO compromised parents.

Given the cardiac hypotrophy with implied low cardiac efficiency, the high blood pressure at least suggests a very marked ”narrowing” of the resistance vessels. There are no sufficient data to decide whether the ”narrowing” is in line with the results of Folkow *et al.* (1958) or with the opinion of Heagerty *et al.* (1993).

Concerning the offsprings born from mothers with NO synthase inhibited by L-NAME after delivery only, a similar increase of blood pressure was found in both measurements at the beginning of the fourth week. The findings are in concert with our previous experiments with canine pups (Török and Gerová 1996) and those of Voelker *et al.* (1995) who inhibited NO synthase by L-NAME administration postnatally. There was no quantitative difference in the blood pressure increase of offsprings born either from NO-deficient hypertensive parents or from normal parents but fed by NO-deficient hypertensive mothers.

The tendency of heart rate to decline was evident in both measurements, the decrease being even more marked in the first measurement. The lower heart weight was not yet accompanied by a change in the HW/BW ratio. Similarly to the experimental group I, a low heart weight was found despite remarkably decreased NO synthase activity. The increased ornithine decarboxylase activity was not yet significant in both the heart and the aorta. Thus no substantial differences between the two experimental groups were found concerning the alterations of cardiovascular system.

The results of our *in vitro* studies with thoracic aorta preparation of offsprings from parents with NO compromised hypertension were suprising. The experiments demonstrated that endothelium-dependent relaxation to acetylcholine was present in aortic rings and the relaxation was quantitatively of the same range as in aortic rings from control offsprings. This was true for both the S-shaped dose-response curve and the value of maximum relaxation.

The relaxation ability of aortic rings to acetylcholine is surprising as the sustained high blood pressure accompanied with hypothrophy of the heart found in offsprings can only be explained by a severe constriction of resistant vessels. Does this mean that different even divergent mechanisms govern the tonus of smooth muscle cells in resistant and conduit arteries of offsprings with NO compromised hypertension?

Comparison of data from both experimental groups showed that there was no significant difference between the responses of aortic rings to acetylcholine in hypertensive offsprings originating from parents, who were both L-NAME treated, and hypertensive offsprings fed by mothers with NO synthase inhibition after delivery. The results are in agreement with those obtained in our previous studies with fetal and newborn dogs (Török and Gerová 1996). However, they are in a strong contrast with the remarkably attenuated acetylcholine relaxation of aortic preparations from adult rats with NO compromised hypertension, as well as in other models of experimental hypertension (Dominiczak and Bohr 1995, Török and Kristek 2000).

The extensive relaxation of the aorta of offsprings from hypertensive NO compromised parents could be explained: (i) by a high level of NO synthase and/or its activity in the wall of major arteries even after L-NAME treatment. This is supported by the findings of high endothelial NO production in the pulmonary artery during the fetal and early postnatal period (Abman *et al.* 1991, Shaul *et al.* 1993); or (ii) by a high level of endothelium-derived hyperpolarizing factor (Feletou and Vanhoutte 1998), or (iii) by a low level of superoxide production and/or by a high level of superoxide dismutase. Further analysis would be necessary to decide which of the three above issues underlies the extensive acetylcholine relaxation of the aorta of offsprings from parents with NO compromised hypertension.

Finally, it is necessary to keep in mind the lower litter size and early mortality of offsprings from parents with NO compromised hypertension.

It may thus be concluded that hypertensive rat parents with NO compromised production have smaller litter sizes and higher newborn mortality. The cardiovascular system of offspring was characterized by high blood pressure and hypotrophy of the heart. Cardiac hypotrophy is hard to understand in the light of the underlying decrease of NO synthase activity and increase in ornithine decarboxylase activity in the myocardium. The relaxation ability of the thoracic aorta was preserved even quantitatively, and contrasted with the maintained high blood pressure. The offsprings born of normal parents and fed by hypertensive NO deficient mothers yielded a similarly compromised cardiovascular system, with lower heart weight but unaltered HW/BW ratio.

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