INTRODUCTION

Long-term decreases in nitric oxide (NO) production result in sustained increases in blood pressure, accompanied by hypertrophy of the heart and the arterial walls of both resistance and conduit arteries (1-4). In most experiments, NO deficiency has been achieved by NG-nitro-L-arginine metylester (L-NAME) administration. Although L-NAME is not a specific NO synthase inhibitor, it is proposed to preferentially inhibit endothelial NOS (eNOS). This proposed selectivity is supported by the observation that the endothelium-dependent relaxation in response to Ach is significantly decreased following both acute and chronic L-NAME administration (5, 6). The hypothesis that a decrease in endogenous NO production is a major contributor to the cardiovascular alterations that lead to hypertension (or vice versa) strongly supports the administration of exogenous NO donors to prevent blood pressure increases and hypertrophy of the heart and arterial walls of conduit arteries (7, 8). The effect of neuronal NO synthase (nNOS) inhibition on the cardiovascular system is unclear. However, there is relative agreement in the literature that the inhibition of nNOS with the specific inhibitor 7NI evokes BP-independent hypertrophy of the heart, kidneys, and conduit arterial walls in normotensive Wistar rats.

The aim of this study was to investigate the long-term effects of 7-nitroindazole on the heart, kidneys, thoracic aorta, and carotid arteries from the progeny of mothers that had been treated with 7-nitroindazole (7NI) (10 mg/kg/day in drinking water) during gestation and nursing. The offspring were also treated with 7NI (10 mg/kg/day in drinking water) until 10 weeks of age. Mean arterial pressure (BP) was measured by tail-cuff plethysmography starting at 4 weeks of age. After perfusion fixation with glutaraldehyde at 120 mmHg, the heart and kidneys were weighed and the thoracic aorta and carotid arteries were processed for morphological investigation. The BP and body weight of treated rats did not differ from age-matched control rats during the course of the experiment. In the experimental group, at the end of the experiment, the heart weight/body weight and kidney weight/body weight ratios were decreased. In addition, the wall thickness (intima + media), cross sectional area (intima + media), and wall thickness/inner diameter ratio were significantly decreased in both the thoracic aorta and carotid arteries without a change in the inner vessel diameter. Circumferential wall tension was increased in both arteries. The data clearly indicate that long-term inhibition of neuronal nitric oxide (NO) synthase with the specific inhibitor 7NI evokes BP-independent hypertrophy of the heart, kidneys, and conduit arterial walls in normotensive Wistar rats.

Key words: nitric oxide, 7-nitroindazole, ontogeny, neuronal nitric oxide synthase, heart, kidney, thoracic aorta, carotid arteries
rats after 7NI administration. Study of the regulatory systems that influence cardiovascular function, particularly early in ontogeny, could provide an opportunity to better understand and/or develop treatments to prevent the development of pathological conditions in the cardiovascular system.

MATERIALS AND METHODS

Animals

During the experiments, the animals were housed at room temperature (22–24°C) with a 12 h light/dark cycle and fed with a regular pellet diet. The procedures were performed in accordance with institutional guidelines and were approved by the State Veterinary and Food Administration of the Slovak Republic and by an Ethics Committee according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Directive 2010/63/EU of the European Parliament).

The parents consisted of 3 females Wistar rats, aged ten weeks, that received the NO synthase inhibitor 7NI at a dose of 10–12 mg/kg/day in drinking water. Fertilization occurred in the second week of 7NI administration. 7NI administration continued during pregnancy and nursing up to 4 weeks postpartum. The control group consisted of 3 age-matched females housed under the same conditions.

The experimental group of offspring consisted of 10 newborns from 3 parents fed by dams receiving 7NI continuously. The control group of offspring consisted of 9 newborns from 3 control parents fed by control mothers. The offspring in the experimental group were treated with 7NI (10 mg/kg/day in drinking water) until 10 weeks of age.

Experimental procedures

The mean arterial pressure of the offspring was measured noninvasively in the tail artery weekly (starting at 28 days postpartum using a miniaturized cuff). At 10 weeks of age, the animals were sacrificed by administering an overdose of anesthetic (zoletil and ketamin). The chest was opened, and the cardiovascular system was perfused via the left ventricle with 300 mM glutaraldehyde in 100 mM sodium phosphate buffer (pH 7.2–7.4) at a perfusion pressure of 120 mmHg for 10 min. After perfusion, the hearts and both kidneys were removed and weighed. The middle parts of the carotid arteries and thoracic aorta were excised, cleaned, divided into segments of approximately 1 mm in length, and processed for electron microscopy (for details, see 7).

Two randomly selected segments of each artery from each animal were cut perpendicular to their longitudinal axis. The inner circumference and arterial wall thickness (tunica intima + tunica media) of individual vessels were measured in semithin sections under a light microscope. Arterial wall thickness (WT) was measured at approximately 45° intervals around the vessel circumference. The inner radius (r) and the wall thickness/inner diameter ratio of the vessels were then calculated from these data. The cross sectional area (tunica intima + tunica media) of the arterial wall was calculated - \( \pi \left[ (r + WT)^2 - r^2 \right] \).

Statistical analysis

The data obtained are expressed as the mean ±S.E.M. The statistical significance was assessed using ANOVA and Bonferroni’s post hoc test for unpaired variables. The results were considered to be statistically significant when P<0.05.

RESULTS

All rats in both the control and experimental groups survived until the end of the experiment. The weekly mean arterial pressure and body weight measurements of the animals did not differ significantly between control and experimental groups throughout the course of the experiment (Fig. 1).

At the end of the experiment (when the animals reached 10 weeks of age), the body weight (254±11.79 g) and the heart weight (738±36.16 mg) of the experimental rats did not differ significantly from the body weight (231±9.06 g) and heart weight (768±27.46 mg) of the age-matched control rats. The heart weight/body weight ratio (mg/g) in the experimental group (2.90±0.047) was significantly lower (P<0.01) than that in the control group (3.33±0.130) (Fig. 2).

The kidney weight/body weight ratio (mg/g) in the rats treated with 7NI (7.72±0.18) was significantly lower (P<0.01) than that in the control group (9.55±0.44).

Fig. 1. Mean arterial pressure measurements throughout the course of the experiment in conscious control Wistar rats (full circles) and in Wistar rats chronically treated with 7-nitroindazole (open circles).
The general characteristics of the thoracic aorta and carotid arteries are given in Table 1. The wall thicknesses (tunica intima + tunica media) of the thoracic aorta and carotid artery were decreased in 7NI-treated rats to 91% (P<0.05) and 88% (P<0.01), respectively, of the control values. A similar decrease in the cross-sectional area of the arterial wall (tunica intima + tunica media) was observed in both the thoracic aorta and carotid artery of 7NI-treated rats, to 88% (P<0.05) and 91% (P<0.05), respectively, of the control values. The inner diameter of the thoracic aorta and carotid artery was not affected by long-term 7NI administration.

Table 1. Wall thickness (WT), cross sectional area (CSA), inner diameter (ID), and wall thickness/inner diameter ratio (WT/ID) of thoracic aorta and carotid artery of control Wistar rats (Wistar) and Wistar rats treated with 7-nitroindazole (Wistar + 7NI). *p<0.05 vs. control, **p<0.01 vs. control.

<table>
<thead>
<tr>
<th>Artery</th>
<th>WT (µm)</th>
<th>CSA (µm²)</th>
<th>ID (µm)</th>
<th>WT/ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>60.77±1.60</td>
<td>281890±8180</td>
<td>1394±38</td>
<td>4.563±0.207</td>
</tr>
<tr>
<td>Wistar+7NI</td>
<td>55.58±1.13*</td>
<td>250130±8407*</td>
<td>1382±29</td>
<td>3.917±0.118**</td>
</tr>
<tr>
<td>Carotid artery</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wistar</td>
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<td>5525±1141</td>
<td>695±25</td>
<td>3.62±0.271</td>
</tr>
<tr>
<td>Wistar+7NI</td>
<td>21.69±0.54**</td>
<td>50494±1665*</td>
<td>721±15</td>
<td>3.025±0.094**</td>
</tr>
</tbody>
</table>

Fig. 2. Body weight (BW), heart weight (HW), and heart weight/body weight ratio (HW/BW) of control Wistar rats (Wistar) and Wistar rats treated with 7-nitroindazole (Wistar + 7NI). **p<0.01 vs. control.

Fig. 3. Circumferential wall tension (Tension) in carotid artery (CA W) and thoracic aorta (TA W) of control Wistar rats and in carotid artery (CA W + 7NI) and thoracic aorta (TA W + 7NI) of Wistar rats treated with 7 nitroindazole. *p<0.05 vs. control.
The wall thickness/inner diameter ratio was significantly (P<0.01) decreased in both the thoracic aorta and carotid artery, to 86% and 84%, respectively, of the control values.

The circumferential wall tension in the thoracic aorta (1241±49 dyn/cm²) and carotid artery (163±109 dyn/cm²) was significantly increased after 7NI administration to 1398±41 dyn/cm² (P<0.05) and 1872±60 dyn/cm² (P<0.05), respectively (Fig. 3).

DISCUSSION

The main finding of this study is that the administration of 7NI to Wistar rats from the prenatal period to adulthood resulted in blood pressure-independent hypertrophy of the heart, kidneys, and conduit arterial walls.

From the 4th week of age (the age at which blood pressure measurements were initiated) to the end of the experiments, the mean arterial pressure of the progeny of mothers that had been treated with 7NI did not differ from the mean arterial pressure of untreated age-matched Wistar rats. There are no data in the literature to which our results can be compared. Nevertheless, our data are in good agreement with the results of experiments in which nNOS inhibition with 7NI administered for several days or weeks had no significant effect on the BP of adult normotensive rats (9–13). In contrast to the effect of 7NI on blood pressure, the long-term administration of L-NAME evoked a BP increase in the 4-week-old offspring of mothers that had been treated with L-NAME during pregnancy and nursing (15) and in adult normotensive Wistar rats (5, 6).

During the whole course of the experiment 7NI treatment did not evoke changes in body weight. After 7NI treatment, no significant changes in heart weight was observed. Because the numerical value of body weight was increased and heart weight was decreased (albeit not significantly), the heart weight/body weight ratio was significantly decreased, indicating hypotrophy of the heart. We propose that disruption of the equilibrium between BP (vascular resistance) and myocardial mass could lead to a decline in cardiac function over time.

The hypotrophic effect of 7NI treatment on wall thickness and cross sectional area in the absence of alterations in the inner diameter was observed in the thoracic aorta and carotid artery. These findings are similar to those observed after 7NI administration to adult Wistar rats for 6 weeks (12, 13). In contrast, L-NAME administration to adult Wistar rats has been associated with hypertrophy of the heart and conduit arterial walls (16). Surprisingly, in the 4-week-old offspring of Wistar mothers treated with L-NAME, the increase in BP was accompanied by hypotrophy of the heart and conduit arterial walls (15). We propose that the hypotrophy of the heart and arterial walls in the aforementioned study was likely an indirect result of NO deficiency. The primary cause of the heart and arterial wall hypotrophy could be fetal malnutrition during the prenatal period resulting from the treatment of the mothers with L-NAME; the significantly lower body weight of the newborns in this study supports this hypothesis. It was shown by Barker (17) that fetal malnutrition can affect the function and structure of the cardiovascular system.

Long-term 7NI administration decreases the wall thickness/inner diameter ratio of the conduit arteries. The equilibrium between blood pressure, wall thickness, and the inner diameter of arteries ensures the adequate supply of blood to surrounding tissues to meet their metabolic needs. The alteration of any of these parameters in the absence of compensatory changes in the other parameters results in a change in circumferential wall tension (BP × radius/WT). Therefore, the ratio between blood pressure, wall thickness and the inner diameter of arteries can strongly influence cardiovascular function and structure. The blood pressure-independent decrease in arterial wall thickness without a change in the inner arterial diameter observed after 7NI administration resulted in an increase in circumferential wall tension. If the amount of circumferential wall tension observed in control arteries represents an optimal value (evolutionarily optimized) in that it provides tissues with sufficient nutrition, an increase in circumferential wall tension could evoke harmful effects on the cardiovascular system. It has been suggested that increased circumferential wall tension is a primary factor contributing to the hypertrophic and atrophic responses of the arterial wall (18, 19).

It is still unclear which stimulus is responsible for hypotrophy of the heart and arterial walls after 7NI administration. It is seems to be clear that BP is not affected by 7NI administration. In the current study and in previous studies (12, 13), we did not observe changes in the inner diameter of conduit arteries. Therefore, 7NI administration primarily affects the trophicity of the heart and arterial walls. Moreover, nNOS inhibition and eNOS inhibition exert opposing effects. Because NO cannot be stored in reserves (it is synthesized "on-demand") and cannot be transported to distant sites of action, it is likely that the concentration of NO at its site of synthesis and its effect on local regulatory systems can evoke different responses of cardiovascular system. The tissue distribution of eNOS and nNOS is different. Both enzymes are present in various tissues at different concentrations (20). While eNOS is primarily localized to endothelial cells in the cardiovascular system, nNOS is found predominantly in the nervous system (21). A high concentration of nNOS was also observed in the kidneys, particularly in the macula densa (22, 23). It has been documented that nNOS participates in the stimulation of renin synthesis and that nNOS is the major NOS isoform regulating renal hemodynamics (9). We speculate that nNOS inhibition could be at least partially responsible for the 7NI-induced hypotrophy of the heart and arterial wall via a decrease in angiotensin II production resulting from a decrease in renin synthesis after 7NI administration. Angiotensin II is a multifunctional peptide that can increase BP, stimulate growth factors, and evoke a strong proliferative effect on cells; decreased ang II production would have the opposite effect. We propose that if NO deficiency due to nNOS inhibition results in increased blood pressure, this effect could be masked by the consequences of decreased angiotensin II production. The decreased kidney weight/body weight ratio observed in our study supports this hypothesis. This hypothesis, however, requires further study.

The data from this study clearly suggest that the responses of the cardiovascular system are markedly different after inhibition of NO synthases with 7NI and L-NAME. The mechanisms of action of these compounds are not clear. Further experiments are needed to elucidate the various roles of NO in the cardiovascular system and to determine its exact role in different physiological processes. A better understanding of the relationship between the factors that control cardiovascular function may help to more accurately explain the functional differences observed in blood vessels in various types of hypertension.

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