SHORT COMMUNICATION

Nitric Oxide and Salt Resistance in Dahl Rats: No Role of Inducible NO Synthase

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Summary

Inducible NO synthase (NOS II) was proposed to play an important role in salt resistance of Dahl salt-resistant (SR/Jr) rats. Its chronic inhibition by specific inhibitors was accompanied by blood pressure (BP) elevation in animals subjected to high salt intake. The aim of our study was to evaluate 1) whether such inhibitors affect BP and/or its particular components (sympathetic tone and NO-dependent vasodilation) only under the conditions of high salt intake, and 2) whether similar BP effects are elicited after systemic or intracerebroventricular (icv) application of these inhibitors. Wistar rats fed Altromin diet (0.45 % NaCl) and SR/Jr rats fed either a low-salt (LS, 0.3 % NaCl) or a high-salt (HS, 4 % NaCl) diet were studied. Aminoguanidine (AMG) and 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT) were used as NOS II inhibitors. BP and its responses to acute blockade of renin-angiotensin system (captopril), sympathetic nervous system (pentolinium) and NO synthase (L-NAME) were measured in conscious cannulated rats. There were no significant changes of BP or its components in either Wistar rats or SR/Jr rats subjected to chronic inhibition of NOS II by peroral aminoguanidine administration (50 mg/kg/day for 4 weeks). This was true for SR/Jr rats fed either LS or HS diets. Furthermore, we have studied BP effects of chronic icv administration of both NOS II inhibitors in SR/Jr rats fed HS diet, but we failed to find any BP changes elicited by such treatment. In conclusion, inducible NO synthase does not participate in the resistance of SR/Jr rats to hypertensive effects of excess salt intake.

Key words

High salt intake • Dahl salt-resistant rats • Blood pressure • NO synthase II • Aminoguanidine • AMT • Renin-angiotensin system • Sympathetic nervous system • Nitric oxide

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The absolute or relative reduction of nitric oxide (NO) production by endothelial NO synthase (NOS III) causes the elevation of blood pressure (BP) because NO-dependent vasodilation is counterbalancing the sympathetic vasoconstriction. Thus, endothelial dysfunction is considered an important factor in the pathogenesis of various forms of hypertension.

The role of NO in the pathogenesis of salt hypertension has been repeatedly studied. There are reports on both absolute [1] and relative NO deficiency [2] in this form of experimental hypertension. A limited attention has been paid to the involvement of particular NOS isoforms in this hypertensive model. Deng and Rapp [3] proposed the importance of inducible NOS (NOS II) because a quantitative trait locus on chromosome 10 seemed to include *Nos2* gene. However, their later study [4], which used a linkage map for rat chromosome 10, indicated that the respective QTL does not include *Nos2* gene. Moreover, we did not find the polymorphism of *Nos2* gene in our colony of inbred Dahl/Rapp rats [5], although this colony was established from the breeding pairs provided by Prof. John P. Rapp.

Nevertheless, Rudd *et al.* [6] and Tan *et al.* [7] reported that chronic administration of selective NOS II inhibitors (aminoguanidine, AMT or 1400W) enabled a salt-induced BP elevation in salt-resistant rats. In the

present study we tried to confirm that chronic NOS II inhibition induced by either peroral or intracerebroventricular administration of the above NOS II inhibitors can modify BP of normotensive Wistar rats and to test whether chronic NOS inhibition can permit the development of salt hypertension in salt-resistant Dahl rats.

Male 8-week-old outbred Wistar rats fed an Altromin diet (0.45 % NaCl) and inbred salt-resistant (SR/Jr) Dahl rats fed a low-salt diet (0.3 % NaCl) were obtained from the breeding facility of the Institute of Physiology CAS in Prague. The animals were housed at 23 °C under a 12 h light/dark cycle, given tap water *ad libitum* and fed a low-salt diet (0.3 % NaCl) prior to the experiment. During the experiment some SR/Jr rats were switched to high-salt diet (4 % NaCl) for four weeks. All procedures and experimental protocols were approved by the *Ethical Committee of the Institute of Physiology, Czech Academy of Sciences* (Protocol Nr. 90/2019) and conform to the *European Convention on Animal Protection and Guidelines on Research Animal Use* (Directive 2010/63/EU).

In chronic experiments, the animals were subjected to either systemic (peroral, po) or central (intracerebroventricular, icv) administration of NOS II inhibitors for 4 weeks. At the end of the experiment we determined the participation of major vasoactive systems (RAS, SNS and NO) in BP maintenance of conscious cannulated animals. The effects of chronic systemic administration of aminoguanidine (AMG, 50 mg/kg/day in the drinking fluid) were compared with those of chronic icv application of AMG (2 mg/kg/day) or 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT, 0.5 mg/kg/day). The doses of these inhibitors were selected on the basis of our preliminary experiments which indicated that higher icv doses were not tolerated by chronically infused animals.

For chronic icv AMG or AMT application the osmotic minipumps (Alzet, type 2004) were placed on the back of the animals and connected by polyethylene catheter with the fixed application icv cannula which was implanted into the lateral cerebral ventricle 24 h before the experiments [8]. Briefly, animals were anesthetized with 2.5 % isoflurane. Permanent cannula was placed in a position for later injections into the right lateral ventricle using stereotactic apparatus (AP=-1.0, L=1.8, V=4.2; according to Paxinos and Watson [9]) and fixed to the skull with synthetic resin (Duracrol, Pentron, Czech Republic). The icv infusions into lateral cerebral ventricle lasted 4 weeks.

The changes of mean arterial pressure (MAP) elicited by the sequential blockade of major vasoconstrictor and vasodilator systems (RAS, SNS and NO) were determined in conscious rats cannulated one day prior to the experiment. Two polyethylene cannulas which were implanted under 2.5 % isoflurane anesthesia (PE 50 for BP measurement in the left carotid artery, PE 10 for the infusion of drugs to the jugular vein), were exteriorized in the interscapular region. Blood pressure and heart rate were recorded using a pressure transducer and a multichannel recorder (ADInstruments, Bella Vista, Australia) in conscious animals placed in small plastic cages. All measurements were carried out between 8:00-12:30 to reduce the circadian variations in BP levels.

We used a modified protocol of Minami *et al.* [10] which was adapted in our laboratory [11]. Briefly, baseline MAP levels were recorded in conscious rats after a 30-min adaptation period in transparent measuring plastic cages. Then, we started with RAS blockade (captopril 10 mg/kg iv) which was followed 15 min later by ganglionic blockade of SNS (pentolinium 5 mg/kg iv). Five min later, NO synthase inhibitor (30 mg/kg L-NAME iv) was injected and BP was monitored for further 20 min. BP levels before and after particular blockades were determined and the respective BP changes were calculated.

All drugs were obtained from Sigma (St. Louis, Missouri, USA). Captopril, pentolinium and L-NAME were dissolved in saline and given as intravenous bolus injections (1 ml/kg). Aminoguanidine was administered either in the drinking water (50 mg/kg/day) or intracerebroventricularly in saline (2 mg/kg/day). AMT (2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine) was also dissolved in saline.

The data are presented as the means ± SEM. One-way ANOVA was used to analyze BP and HR as well as their response to the sequential blockade of the above mentioned vasoactive systems. The differences were considered to be significant at $p<0.05$ level.

Chronic treatment with NOS II inhibitor aminoguanidine did not affect BP or its particular components (angiotensin II-dependent, sympathetic and NO-dependent) in Wistar rats fed 0.45 % NaCl diet (Fig. 1). This was true for the animals subjected to the peroral administration of high AMG doses (50 mg/kg/day) as well as for the rats receiving AMG through icv infusion (2 mg/kg/day). It should be noted that chronic AMG treatment of Wistar rats did not induce

sympathetic hyperexcitation. Moreover, this treatment did not affect NO-dependent vasodilation, indicating that inducible NO synthase does not participate in BP regulation under normal conditions.

In a further experiment, we compared the effects of chronic peroral administration of high AMG doses (50 mg/kg/day) on BP or its component in Dahl SR/Jr rats fed either a low-salt or high-salt diet (Fig. 2) There were no significant BP effects in either group of SR/Jr rats indicating that this NOS II inhibitor did not cause salt-induced BP elevation in this rat strain.

Finally, we tried to elicit BP elevation and/or sympathoexcitation in salt-loaded SR/Jr rats by 4-week-lasting icv administration of AMG (2 mg/kg/day) or AMT (0.5 mg/kg/day) which are the highest tolerable doses of these drugs for icv infusion in this rat strain. Figure 3 shows that none of the drugs used elicited any significant changes in BP or its particular components in salt-resistant Dahl rats.

Our study did not confirm previous reports [6,7] that chronic inhibition of NOS II restored the ability of salt-resistant Dahl/Rapp rats to respond by BP elevation to excess salt intake. Indeed, we did not observe any significant change in blood pressure or its sympathetic or NO-dependent component in salt-loaded SR/Jr subjected to chronic treatment with NOS II inhibitors, although we gave these inhibitors through either peroral or intracerebroventricular administration. The absence of changes in sympathetic BP component in our SR/Jr rats treated with aminoguanidine or AMT is a rather important finding because sympathetic hyperexcitation is a typical hallmark of both salt hypertension in Dahl salt-sensitive rats [2,12] and NO-deficient hypertension in Sprague-Dawley or Wistar rats [13,14].

It is rather difficult to explain the discordance of our study with previous reports [6,7] which differed in the origin of salt-resistant Dahl rats, their age, the composition of the diet, the duration of NOS II inhibition, the mode of inhibitor administration, etc. Future experiments should examine the role of NOS II in salt-sensitive Dahl rats in which NO production by the inducible NOS isoform seems to attenuate the development of salt hypertension [1,6,7].

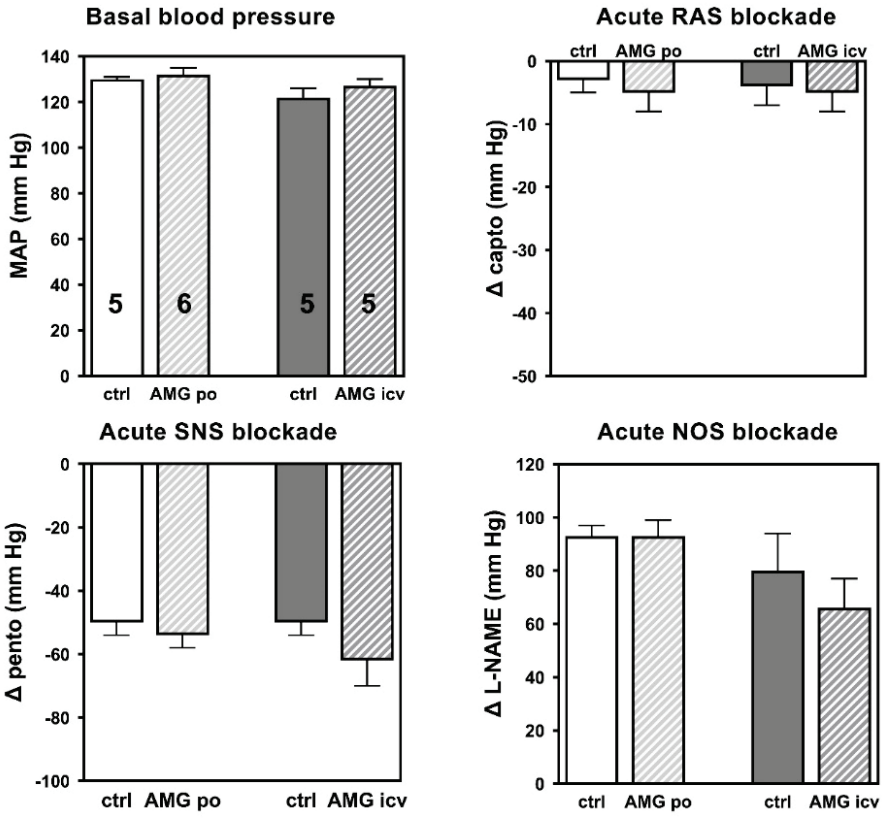

Fig. 1. Blood pressure effects of chronic peroral (po, 50 mg/kg/day) or intracerebroventricular (icv, 2 mg/kg/day) administration of aminoguanidine to male Wistar rats fed an Altromin diet (0.45 % NaCl). MAP – basal mean arterial pressure, RAS blockade – MAP change after acute captopril injection (10 mg/kg iv), SNS blockade – MAP change after acute pentolinium injection (5 mg/kg iv), NOS blockade – MAP change after acute L-NAME injection (30 mg/kg iv). Data are means ± SEM, number of animals are given at the bottom of the columns.

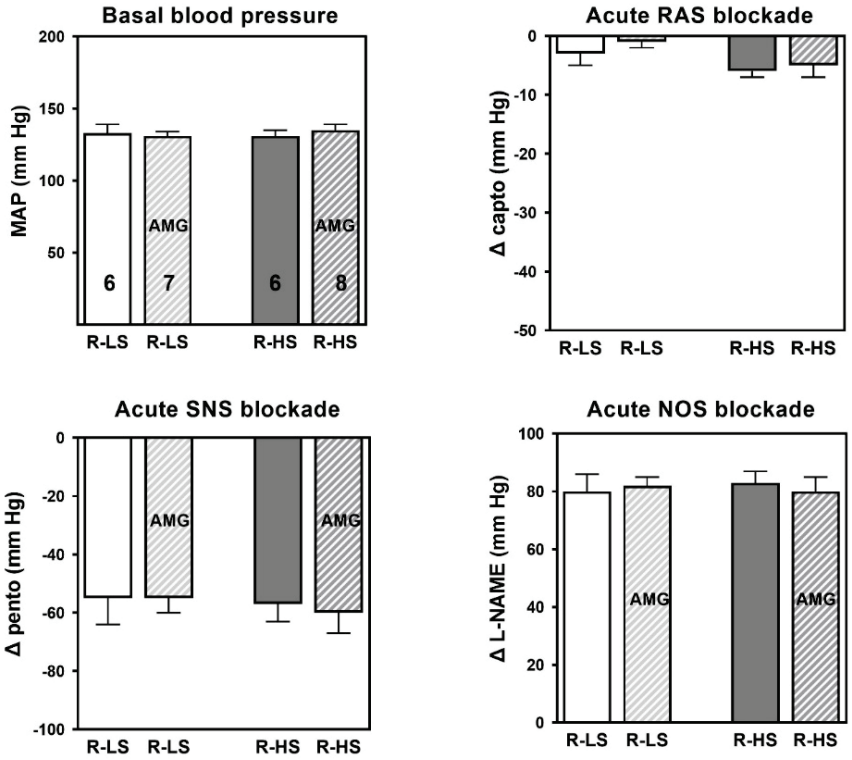

Fig. 2. Blood pressure effects of chronic peroral (po, 50 mg/kg/day) or intracerebroventricular (icv, 2 mg/kg/day) administration of aminoguanidine to male salt-resistant Dahl rats fed a low-salt (LS, 0.3 % NaCl) or a high-salt diet (HS, 4 % NaCl). MAP – basal mean arterial pressure, RAS blockade – MAP change after acute captopril injection (10 mg/kg iv), SNS blockade – MAP change after acute pentolinium injection (5 mg/kg iv), NOS blockade – MAP change after acute L-NAME injection (30 mg/kg iv). Data are means ± SEM, number of animals are given at the bottom of the columns.

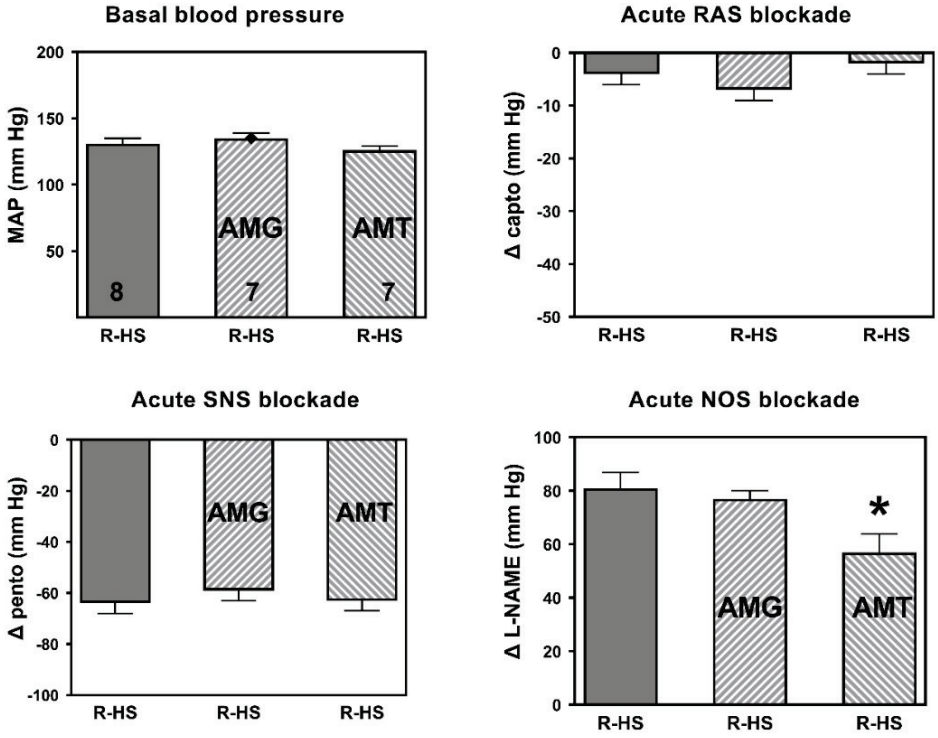

Fig. 3. Blood pressure effects of chronic intracerebroventricular administration of aminoguanidine (AMG, 2 mg/kg/day) or (2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine) (AMT, 0.5 mg/kg/day) to male salt-resistant Dahl rats fed a high-salt diet (HS, 4 % NaCl). MAP – basal mean arterial pressure, RAS blockade – MAP change after acute captopril injection (10 mg/kg iv), SNS blockade – MAP change after acute pentolinium injection (5 mg/kg iv), NOS blockade – MAP change after acute L-NAME injection (30 mg/kg iv). Data are means ± SEM, number of animals are given at the bottom of the columns. * significantly different (p<0.05) from the controls.

Conflict of Interest

There is no conflict of interest.

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