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Original Communication

Blood pressure and vascular function modification in young spontaneously hypertensive rats treated with nifedipine

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ABSTRACT

The aim of this study was to investigate the cardiovascular effects of long-term treatment with nifedipine in two distinct stages of early ontogenesis in spontaneously hypertensive rats (SHR). Four- and eight-week-old SHR and age-matched normotensive Wistar rats were treated with nifedipine (50 mg/kg/day) for four weeks. Systolic blood pressure was measured weekly by the tail-cuff technique. At the end of the treatment the animals were sacrificed and rings of their mesenteric arteries were suspended in organ baths and connected to a force-displacement transducer for measuring their reactivity under isometric condition. Neurogenic contractile responses were elicited by electrical stimulation of perivascular adrenergic nerves. We found that treatment with nifedipine prevented the elevation of blood pressure and decreased the relative heart weight values in SHR of both age groups. No changes in these parameters due to nifedipine were detected in Wistar rats. Adrenergic contractions in mesenteric arteries were decreased in nifedipine-treated SHR, particularly at 12th week; in contrast, arteries from 12-week-old Wistar rats treated with nifedipine became more sensitive to endo- and exogenous noradrenaline and responded with enhanced contractions. We conclude that nifedipine administration prevented the rise of blood pressure similarly in both stages of spontaneous hypertension development; however, it decreased the sympathoadrenergic vasoconstriction more effectively in 12-week-old SHR in which the initial blood pressure was already partially elevated.

KEYWORDS: spontaneously hypertensive rat, nifedipine, conduit arteries, adrenergic contraction

INTRODUCTION

Essential hypertension is a typical multifactorial disease in which the interactions between susceptibility genes and environmental factors are intensively studied [1]. Several animal models with genetic predisposition to hypertension are used for this purpose. The most used is the model of spontaneously hypertensive rats (SHR) as the pathogenesis of hypertension in these animals has several common traits with human essential hypertension [2, 3]. One of the most prominent factors contributing to pathological elevation of blood pressure in the substantial group of hypertensive patients as well as in SHR is the increased activity of the sympathetic nervous system. It may result from either elevated sympathetic drive from brain centres or increase in synaptically released neuro-transmitters in the periphery, and its effect could be potentiated by exaggerated neuromediator signalization in the target organs, *e.g.*, in vascular smooth muscle. Noradrenaline, the main neuro-transmitter of the sympathetic nervous system, increases vascular smooth muscle tension through several simultaneously acting intracellular pathways, many of them leading to the stabilization of open state of voltage-dependent calcium channels (VDCC) whereby they induce the tonic part of noradrenergic contraction [4]. Paulis *et al.* [5] pointed out the relationship between the increased sympathetic

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vasoconstriction and the abnormal function of L-type voltage-dependent calcium channels (L-VDCC) in arteries from SHR. They experimentally demonstrated the enhancement of nifedipine-sensitive component of noradrenergic contraction in this rat strain. The described effect was also found in other experimental models like salt hypertension in Dahl rats or nitric oxide (NO)-deficient hypertension [6] which are characterized by enhancement of sympathetic system as well [5, 7]. This indicates that the increased participation of L-VDCC in the maintenance of high blood pressure might not be exclusively associated with genetic abnormalities of these channels but it may be related to their abnormal activation by enhanced sympathetic tone [5].

In SHR, the initiation of hypertension is quite well defined; in the period between 4th and 10th week of life, the rapid elevation of blood pressure together with the increase of peripheral resistance and progressive hypertrophy in cardiovascular system are observed. Simultaneously, the maturation processes in cardiovascular sympathetic neuro-transmission occurs at this stage. Many authors suggest that a proper pharmacological treatment applied to SHR during this developmental period could significantly reduce the pathological rise in blood pressure and eliminate the pressure-induced or humorally mediated structural alterations in their cardiovascular system. This might attenuate the severity of hypertension and its accompanying complications in adult SHR. It is assumed that in older animals with established hypertensive disease, it is rather difficult to induce the regression of structural alterations in the heart and the vessel system that have already developed as a consequence of long-term influence of increased blood pressure [3].

Because of the confirmed relationship between abnormal function of VDCC and the enhanced sympathetic responses in cardiovascular system during the development of hypertension in SHR, we suggested that the blockade of these channels in the prehypertensive or early-hypertensive stages may be particularly beneficial in the context mentioned above. In this study we investigated the effects of four-week-lasting treatment with L-VDCC blocker on the cardiovascular system of young SHR to evaluate the possibility of prevention of its impairments leading to hypertensive state. The L-VDCC blocker used, nifedipine, was administered to these rats during the period between 4th and 8th week (early phase of rapid blood pressure elevation) and between 8th and 12th week (late phase of rapid blood pressure elevation) of their life. We analyzed the influence of this treatment on blood pressure elevation, relative heart weight (as a measure of myocardial hypertrophy), and adrenergic contractions of isolated superior mesenteric arteries, and compared its effectiveness in the two selected developmental stages in SHR.

MATERIALS AND METHODS

Experimental animals

The animal protocols used in this study were performed in accordance with the 'Guide for the Care and Use of Laboratory Animals' published by the National Institutes of Health, and approved by the Animal Health and Welfare Division of the State Veterinary and Food Administration of the Slovak Republic. All rats were housed at 22-24 °C on a 12:12-h dark-light cycle (06.00-18.00 h lights on) and maintained on a standard laboratory rat chow and tap water *ad libitum*.

From 4th week and from 8th week of age, male spontaneously hypertensive rats (SHR) and normotensive Wistar rats were treated with nifedipine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at 50 mg/kg/day (administered in chow) for the period of four weeks. Untreated rats of both strains served as controls for each respective age group. Systolic blood pressure was measured weekly in conscious rats by the non-invasive tail-cuff method. At the end of the treatment, rats were sacrificed under CO_2 anesthesia, their heart weight to body weight ratios were determined and superior mesenteric arteries were removed and prepared for isometric tension recording.

Functional studies on isolated mesenteric arteries

Functional studies were performed on isolated superior mesenteric arteries. The arteries were cut into rings (3.0-3.5 mm in width) and suspended in 20 ml organ baths filled with oxygenated (95% O_2 + 5% CO_2) modified Krebs solution maintained at 37 °C. The Krebs solution was prepared in the following composition (in mmol/l): NaCl 118, KCl 5,

$CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, and $CaNa_2$.EDTA 0.03. The arterial rings were set up for isometric tension recording using a force-displacement transducer Sanborn FT 10 (Sanborn, Baltimore, USA). The preparations were equilibrated under a resting tension of 10 mN for 60-90 min, and the Krebs solution was changed every 15 min.

Adrenergic contractions in endothelium-intact mesenteric arteries were determined as the responses to cumulatively applied exogenous noradrenaline (increasing concentrations were applied in a cumulative manner) or as the neurogenic responses elicited by electrical stimulation of periarterial sympathetic nerves. The arterial rings were stimulated by two parallel platinum plate electrodes placed on either side of the preparation and connected to an electrostimulator ST-3 (Hungary). Frequency-response curves to electrical stimuli were obtained using square pulses of 0.5 ms in duration, at supramaximal voltage (> 30 V), applied at 1-32 Hz, for a period of 20 s. In our previous unpublished observations we found that the contractions of rat mesenteric arteries elicited by electrical stimulation (using the described parameters of stimulation) are blocked by phentolamine or tetrodotoxin, indicating that they are induced mainly by nerve-released noradrenaline. In mesenteric arteries, contractions to 10^{-1} mol/l KCl were also determined.

Data analysis

The results are presented as means ± SEM. The absolute values of the contractions of mesenteric arteries to adrenergic stimuli are expressed in mN and normalized to the length of the respective ring preparations. Sensitivity to noradrenaline is expressed as pD_2 value, where $pD_2 = -\log EC_{50}$; EC_{50} being the molar concentration of the agonist that produces 50% of the maximal effect.

Statistical evaluation was carried out by using one-way analysis of variance (ANOVA). The results were considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

In this study the alterations in some cardiovascular parameters and their susceptibility to amelioration due to pharmacological intervention were assessed during the early ontogenesis in SHR. Between the age of 4 and 12 weeks, rapid increase of blood pressure was observed in SHR, with the values being significantly higher at 8th and even more at 12th week compared to that in age-matched normotensive Wistar rats (Figure 1). Concurrently, the heart weight to body weight ratio was increased in SHR (4.25 ± 0.16 at 8th week and 3.93 ± 0.10 at 12th week of life) compared to Wistar rats (3.00 ± 0.04 at 8th week and 2.81 ± 0.03 at 12th week of life) ($P < 0.001$ for SHR *vs.* Wistar rats in both age-groups), indicating myocardial hypertrophy

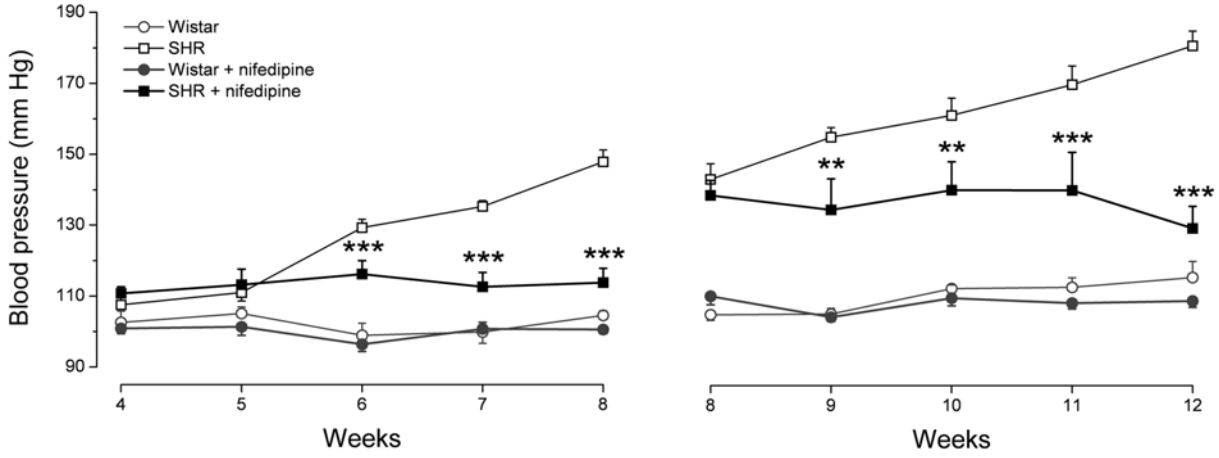

Figure 1. Effect of 4-week-lasting nifedipine treatment on blood pressure in two developmental stages of spontaneously hypertensive rats (SHR) and normotensive Wistar rats. Values represent mean ± SEM of 9-10 rats. $^{**}P < 0.01$, $^{***}P < 0.001$ control *vs.* nifedipine-treated SHR.

due to hypertension. The presented results are in accordance with the findings of most authors who demonstrated that at 4th week the level of blood pressure in SHR does not significantly differ from the values found in age-matched normotensive rat strains [8-10]; however, at around 5th week of age, it begins to rise rapidly, and the severity of cardiovascular impairment accentuates together with hypertension development in SHR.

In this study it was detected that the differences between SHR and Wistar rats in their mesenteric arterial responses to exogenous noradrenaline as well as in neurogenic contractions enhance with age and with blood pressure increase in SHR. At 8th week the maximum contractions to noradrenaline in mesenteric arteries from Wistar rats were not significantly different from those in SHR (2.60 ± 0.36 mN/mm in Wistar rats *vs.* 2.98 ± 0.18 mN/mm in SHR); however, at 12th week of age, the maximum noradrenaline contractions in SHR were significantly increased compared to those in Wistar rats (3.24 ± 0.27 mN/mm in Wistar rats *vs.* 4.23 ± 0.10 mN/mm in SHR; $P < 0.05$). The differences are obvious also in the dose-response curves seen in the figure 2 (A and B).

As the neurogenic contractions represent the responses to endogenous noradrenaline released as a neurotransmitter from electrically excited

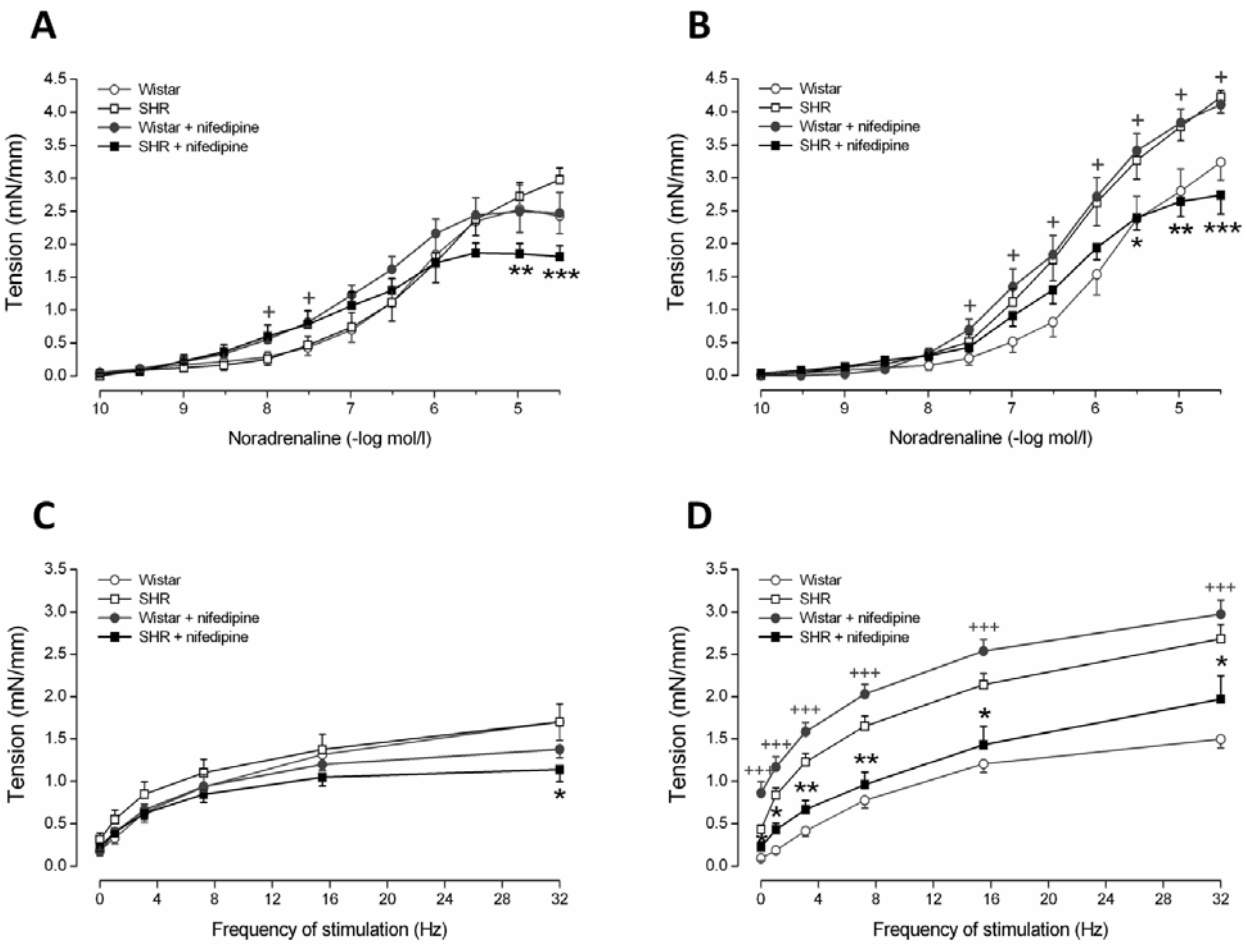

Figure 2. Effect of 4-week-lasting nifedipine treatment on concentration-dependent contractile responses to exogenous noradrenaline (A, B), and frequency-dependent contractile responses to electrical stimulation of perivascular nerves (C, D) in superior mesenteric artery from spontaneously hypertensive rats (SHR) and normotensive Wistar rats at 8th week (A, C) and 12th week (B, D) of age. Values represent mean ± SEM of 9-10 rats. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ control *vs.* nifedipine-treated SHR; $^{+}P < 0.05$, $^{+++}P < 0.001$ control *vs.* nifedipine-treated Wistar rats.

sympathetic nerve terminals within the arterial wall, it is possible to presume that the enhancement in these contractions in 12-week-old SHR *vs.* Wistar rats (Figure 2D) is at least partially due to the augmented arterial responses to noradrenaline itself; however, the presynaptic (nerve) mechanisms might also participate. Proportionally, the differences in mesenteric arterial contractions between 12-week-old SHR and Wistar rats are more pronounced in their frequency-dependent neurogenic responses (Figure 2D) compared to the dose-dependent exogenous noradrenaline contractions (Figure 2B) indicating that the increased sympathetic tone (or higher density of innervation [11]) plays an important role in these responses. One can speculate that the higher sympathetic drive (tone) in arteries during the development in SHR could induce the abnormal opening/expression of VDCC in their smooth muscle (as indicated by Paulis *et al.* [5]) and this part of the response becomes dominant in developing the contractile alterations of arterial system. Such idea might also be supported by the finding of the increased mesenteric arterial contraction to 100 mmol/l KCl in 12-week-old SHR (3.00 ± 0.25 mN/mm) compared to age-matched Wistar rats (2.30 ± 0.13 mN/mm) ($P < 0.05$), but not at 8th week of age (1.90 ± 0.25 mN/mm in Wistar rats *vs.* 2.34 ± 0.35 mN/mm in SHR), similar to the noradrenaline response. The contraction to 100 mmol/l KCl is mostly caused by non-specific (high potassium-induced) depolarization of smooth muscle cell membranes and subsequent activation of VDCC; therefore, the presented results indicate that the calcium current through these channels is excessive in SHR at 12th week of life, *i.e.*, in the age when the sympathoadrenergic contractions in their mesenteric arteries are evidently exaggerated (compared to age-matched normotensive Wistar rats). At this point it might be concluded that in SHR the ontogenetic period between 4th and 12th week (the phase of the rapid increase in their blood pressure) is critically important when considering the development of most of the arterial abnormalities.

Therefore, the effects of four-week-lasting treatment with nifedipine (calcium channel blocker) on the cardiovascular system of young SHR were investigated to evaluate the possibility of prevention of its abnormalities leading to hypertensive state. From the presented results it is evident that nifedipine treatment abolished the blood pressure increment during the particular periods of its administration in these rats (Figure 1). Correspondingly, the relative heart weight was smaller in nifedipine-treated SHR (3.85 ± 0.06 at 8th week and 3.53 ± 0.06 at 12th week of life, after 4-week-lasting nifedipine treatment) compared to untreated age-matched SHR ($P < 0.05$) (see the values above). It might be proposed that inhibition of blood pressure overload was the main mechanism by which nifedipine prevented the increase in heart mass. Several authors confirmed that calcium channel blockers are effective to prevent or regress cardiac hypertrophy [12, 13]. It seems that more specific pathways of inhibiting the myocardial hypertrophy could also be engaged in nifedipine effect; Zou *et al.* [13] demonstrated that suppression of calcineurin activity in the heart might be involved in this process. Moreover, morphological and functional changes were detected in aorta of adult SHR after one week of treatment with nifedipine [14]. These authors found that such treatment attenuates the abnormal aortic wall thickness, cross-sectional area and media-to-lumen ratio in SHR. The possibility of restriction on arterial hypertensive remodelling might also elucidate the reduction of noradrenergic contractions in nifedipine-treated SHR observed in this study, as discussed below. It is well-known that calcium influx into the smooth muscle cells represents one of the key signals for proliferation [15, 16]; therefore, inhibition of this process could prevent the abnormal (hypertension–induced) growth of the medial layer in arteries from SHR, leading to weaker contractile responses to adrenergic stimuli after nifedipine administration to these rats.

It is interesting that in nifedipine-treated SHR at 8th week of age, mesenteric arteries were more sensitive to exogenous noradrenaline (pD2 6.23 ± 0.16 in control SHR *vs.* 7.20 ± 0.28 in nifedipine-treated SHR; $P < 0.05$) although the contractions at high noradrenaline concentrations were markedly reduced due to nifedipine treatment, compared to untreated animals (Figure 2A). At 12th week, however, the sensitivity was not altered (pD2 6.27 ± 0.18 in control SHR *vs.* 6.57 ± 0.23 in nifedipine-treated SHR), but the absolute values of contractile responses at high noradrenaline doses were

decreased (Figure 2B). One can speculate that in younger SHR in which the alterations in VDCC function were not significantly manifested yet (see the above presented arterial contractions to depolarisation by high extracellular potassium), blockade of these channels with nifedipine might stimulate their expression/activity and slightly potentiate the sensitivity of the calcium current in mesenteric arteries of 8-week-old SHR; however, at high contractile stimulus (high noradrenaline doses) the reduction in arterial smooth muscle mass became manifested in developing smaller contractile responses, when compared to untreated SHR. In the later ontogenetic period (*i.e.*, between 8^{th} and 12^{th} week of age) when the calcium current through VDCC in arterial smooth muscle cells is already aberrant due to long-term exaggerated sympathetic stimulation, the inhibition of this current could not lead to its further compensatory increase; however, the reduction of contractile response at high noradrenaline doses was more pronounced because of the assumed decrease in medial layer thickness.

Similar character of modulation of arterial contractions due to nifedipine treatment in SHR was seen in neurogenic responses which were induced by endogenous noradrenaline, released after electrical stimulation selective for periarterial sympathetic nerves (Figure 2 (C and D)). It seems that the reduction in neurogenic contractions directly imitates the decrease in exogenous noradrenaline responses in nifedipine-treated SHR, and thus this decrease involves predominantly the postsynaptic processes at the arterial smooth muscle. However, the presynaptic (neurogenic) mechanisms might also be involved in the observed nifedipine-produced decrease in adrenergic responses. Several authors indicated that the calcium channel blockers (including dihydropyridines) might inhibit the neurogenic contractions also by suppressing the calcium-dependent release of endogenous noradrenaline from sympathetic nerve terminals in the vessel wall [17, 18]. This effect could consist in their non-specific interaction with the calcium channels located in the sympathetic nerves, although they represent a different category of VDCC compared to that located in the vascular smooth muscle cells. The modulation of sympathoadrenergic functions by dihydropyridines was demonstrated in *in vivo* experiments on anesthetized rats; it was shown that the application of nifedipine into the brain ventricles, or directly into the brainstem, significantly reduced the mean arterial pressure, heart rate, and the sympathetic nerve tone in peripheral organs of SHR [19-21]. Paulis *et al.* [5] found that previous sympathetic ganglion blockade by pentolinium in SHR almost eliminated the hemodynamic responses to acute application of nifedipine. These findings clearly indicate that nifedipine might also have the central sympathoinhibitory effects. The results of this study showing the decreased neurogenic contractions in mesenteric arteries from chronic nifedipine-treated SHR may thus also reflect the influence of nifedipine on sympathetic nerve system; the long-term inhibition of sympathetic efferentation could lead to reduction in noradrenaline synthesis and storage in nerve terminals within the arterial wall.

Different results were obtained in normotensive Wistar rats treated with nifedipine. Administration of this calcium channel blocker to both age-groups of these animals did not induce any significant change in their blood pressure (Figure 1) and in relative heart weight (3.10 ± 0.06 at 8^{th} week and 2.91 ± 0.05 at 12^{th} week of life, after 4-week-lasting nifedipine treatment). Interestingly, from the literature it seems that in normotensive rats nifedipine can induce the decline in blood pressure during acute [5] or short-term-lasting experiments (1 week of administration [14]); however, as indicated in the results of this study as well as in the findings of other investigators [22, 23], after longer period of nifedipine administration such decreasing effect is most likely eliminated by some compensatory mechanisms which are able to restore blood pressure to its initial values. One of such mechanisms could be the increase in arterial tone due to enhanced sensitivity of the sympathoadrenergic system, as seen from the presented results (Figure 2). In Wistar rat mesenteric arteries the increase in noradrenergic contractions was detected after 4-week-lasting nifedipine administration. In eight-week-old nifedipine-treated individuals the sensitivity of mesenteric arteries to exogenous noradrenaline was elevated (pD2 6.43 ± 0.13 in control Wistar rats *vs.* 7.05 ± 0.19 in nifedipine-treated Wistar rats; $P < 0.05$) (Figure 2A). In twelve-week-old Wistar rats administered with

nifedipine, the change in mesenteric arterial sensitivity to exogenous noradrenaline was not observed (pD2 6.03 ± 0.16 in control Wistar rats *vs.* 6.43 ± 0.17 in nifedipine-treated Wistar rats); however, the tension developed in response to exogenous as well as endogenous noradrenaline was markedly increased (Figure 2 (B and D)). The augmented activity/expression of VDCC in vascular smooth muscle cells as a compensatory response to long-term blockade of these channels may elucidate such observations, taking into consideration that calcium is one of the key ions important for cellular function and signalization.

CONCLUSION

The results of this study show that four-week-lasting blockade of VDCC with nifedipine in young SHR can ameliorate some functional and structural aspects of their cardiovascular system and delay the onset of hypertension development. It might be supposed that this effect could lead to the improved cardiovascular function in adulthood and subsequently to better survival of such individuals. However, it seems that the influence of nifedipine on rat cardiovascular system was dependent on the initial state of VDCC in vascular smooth muscle cells which was related to the level of activity of arterial sympathoadrenergic system. In the condition of their exaggerated activity or expression, the inhibitory effect of nifedipine on arterial contractile responses to adrenergic stimulation was manifested; however, in normal conditions (*e.g.*, in normotensive rats) its administration led to hypersensitivity to contractile stimuli, possibly due to compensatory increase in VDCC calcium current.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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