

# Involvement of BK<sub>Ca</sub> and K<sub>V</sub> Potassium Channels in cAMP-Induced Vasodilatation: Their Insufficient Function in Genetic Hypertension

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## Summary

Spontaneously hypertensive rats (SHR) are characterized by enhanced sympathetic vasoconstriction, whereas their vasodilator mechanisms are relatively attenuated compared to their high BP. The objective of our in vivo study was to evaluate whether the impaired function of BK<sub>Ca</sub> and/or K<sub>V</sub> channels is responsible for abnormal cAMP-induced vasodilatation in genetic hypertension. Using conscious SHR and normotensive WKY rats we have shown that under the basal conditions cAMP overproduction elicited by the infusion of  $\beta$ -adrenoceptor agonist (isoprenaline) caused a more pronounced decrease of baseline blood pressure (BP) in SHR compared to WKY rats. Isoprenaline infusion prevented BP rises induced by acute NO synthase blockade in both strains and it also completely abolished the fully developed BP response to NO synthase blockade. These cAMP-induced vasodilator effects were diminished by the inhibition of either BK<sub>Ca</sub> or K<sub>V</sub> channels in SHR but simultaneous blockade of both K<sup>+</sup> channel types was necessary in WKY rats. Under basal conditions, the vasodilator action of both K<sup>+</sup> channels was enhanced in SHR compared to WKY rats. However, the overall contribution of K<sup>+</sup> channels to cAMP-induced vasodilator mechanisms is insufficient in genetic hypertension since a concurrent activation of both K<sup>+</sup> channels by cAMP overproduction is necessary for the prevention of BP rise elicited by acute NO/cGMP deficiency in SHR. This might be caused by less effective activation of these K<sup>+</sup> channels by cAMP in SHR. In conclusion, K<sup>+</sup> channels seem to have higher activity in SHR, but their vasodilator action cannot match sufficiently the augmented vasoconstriction in this hypertensive strain.

## Key words

Isoprenaline • Cyclic AMP • Potassium channels • Genetic hypertension • Calcium channels

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## Introduction

Increased vascular tone and high blood pressure (BP) in spontaneously hypertensive rats (SHR) are generally attributed to the increased activity of sympathetic nervous system (SNS) (Head 1989, De Champlain 1990). Moreover, SHR are also characterized by relatively attenuated vasodilator systems since their overall activity failed to compensate for the augmented sympathetic vasoconstriction (Behuliak *et al.* 2011). Our previous work has shown that enhanced activity of SNS contributes to the maintenance of hypertension in SHR through G<sub>i</sub>-protein/cAMP-coupled pathway resulting in the increased calcium influx through L-type of voltage-dependent calcium channels (L-VDCC) (Pintérová *et al.* 2010). These channels play a major role in excitation-contraction coupling in vascular smooth muscle (Sonkusare *et al.* 2006). The importance of the cAMP pathway in the control of vascular resistance and BP in SHR has also been reported by Berg *et al.* (2009), showing stronger inhibitory effects of G<sub>i</sub>-proteins on adenylyl cyclase activity in hypertensive animals. However, in vascular smooth muscle cells, the activity of adenylyl cyclase/cAMP pathway is also regulated by the stimulation of  $\beta$ -adrenoceptors coupled to stimulatory G-proteins (G<sub>s</sub>). An attenuated  $\beta$ -adrenergic-mediated relaxation has been reported in vascular preparations from SHR when compared to those from normotensive rats (Arribas *et al.* 1994, Mallem *et al.* 2005) indicating

the importance of cAMP pathway in the maintenance of elevated BP in hypertension.

Many authors have reported the ability of cyclic nucleotides (both cAMP and cGMP) to modulate L-VDCC channel activity (Liu H *et al.* 1997, Taguchi *et al.* 1997, Ruiz-Velasco *et al.* 1998, Xiong and Sperelakis 2007). Tewari and Simard (1997) have found that nitric oxide (NO) reduces L-VDCC opening through cGMP-dependent mechanism. Several other studies (Liu H *et al.* 1997, Taguchi *et al.* 1997, Ruiz-Velasco *et al.* 1998) have confirmed the inhibitory effects of cGMP/PKG pathway on these calcium channels. Cyclic AMP signaling pathway plays a key role in vasorelaxation triggered by adenylyl cyclase-coupled receptors such as  $\beta$ -adrenoceptors (Orlov *et al.* 1996). It has been shown that under the physiological conditions a moderate rise of intracellular cAMP leads to a small enhancement of inward current through L-VDCC, whereas higher levels of intracellular cAMP lead to its inhibition (Ishikawa *et al.* 1993).

One of the possible ways by which cAMP/PKA pathway inhibits calcium entry through L-VDCC could be the activation of large-conductance  $\text{Ca}^{2+}$ -dependent ( $\text{BK}_{\text{Ca}}$ ) (Sadoshima *et al.* 1988, Scornik *et al.* 1993) or voltage-activated ( $\text{K}_{\text{V}}$ ) potassium channels (Aiello *et al.* 1995, Aiello *et al.* 1998) leading to a hyperpolarization of cell membrane (Ousterhout and Sperelakis 1987). However, all the above-mentioned studies were performed *in vitro* using vascular smooth muscle cells from different vascular beds, none of them representing the resistance vessels responsible for BP maintenance. Both  $\text{BK}_{\text{Ca}}$  and  $\text{K}_{\text{V}}$  channels are the dominant types of potassium channels expressed by vascular smooth muscle cells contributing to a negative feedback during agonist-induced vasoconstriction (Jackson 2000). Although the altered function of these potassium channels seems to be involved in the pathogenetic mechanisms of hypertension (Nelson and Quayle 1995), there are no studies comparing the involvement of  $\text{BK}_{\text{Ca}}$  and  $\text{K}_{\text{V}}$  channels in cAMP-induced vasodilatation in normotensive and hypertensive animals.

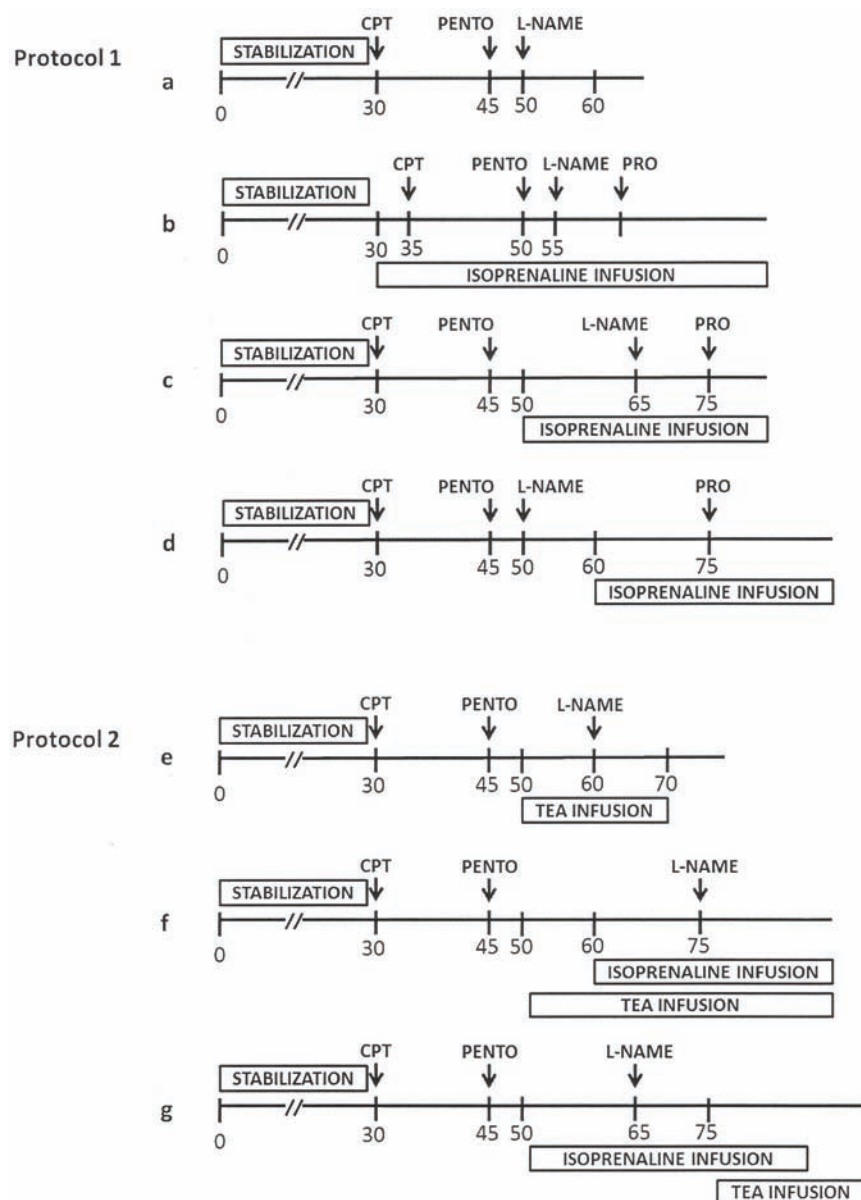
Thus, the aim of the present *in vivo* study was to evaluate the hypothesis that impaired function of  $\text{BK}_{\text{Ca}}$  and/or  $\text{K}_{\text{V}}$  channels might be one of the factors responsible for the abnormal cAMP-induced vasodilatation elicited by the infusion of  $\beta$ -adrenoceptor agonist (isoprenaline) in hypertensive animals. Regarding the *in vivo* studies, it should be kept in mind that the level of mean arterial BP is dependent on the extent of total

peripheral resistance, major determinant of which is vascular tone of small resistance arterioles. A central role in the regulation of the vascular tone is played by intracellular calcium ions, which primarily enter the cells through L-VDCC (Paulis *et al.* 2007). Potassium channels, the activity of which controls membrane potential, are one of the important regulators of L-VDCC open state (Jackson 2000). Therefore  $\text{K}^{+}$  channel activity is linked to calcium entry, vascular tone and BP. Working with this assumption, we considered that BP changes reflect the state of existing L-VDCC, which can be modulated by many vasoactive agonists including norepinephrine and nitric oxide (Lewis *et al.* 2005, Pintérová *et al.* 2010). *In vivo* approach, based upon the study of conscious rats, allowed us to evaluate the role of these systems at the level of resistance arterioles and to avoid the problems with tissue specificity and recording conditions, which complicate the *in vitro* studies. However, this approach also brings a number of disadvantages. The *in vivo* involvement of  $\text{K}^{+}$  channels in isoprenaline-induced vasodilatation had to be studied under the conditions of blocked sympathetic neurotransmission to eliminate the baroreflex influence on actual vasoconstriction. However, BP level reached after ganglionic blockade is very low, thus inappropriate for the evaluation of vasodilator effects of isoprenaline and their modulation by potassium channel blockers. Therefore, we have preferred an indirect method to determine the role of  $\text{BK}_{\text{Ca}}$  and  $\text{K}_{\text{V}}$  channels in cAMP-induced vasodilatation. Using  $\text{BK}_{\text{Ca}}$  and/or  $\text{K}_{\text{V}}$  channel blockers, we evaluated the extent by which these inhibitors eliminated the cAMP-induced prevention of BP rise following acute NO depletion. In fact, we have earlier demonstrated that the enhanced stimulation of adenylyl cyclase elicited by chronic  $\text{G}_i$ -protein inactivation with pertussis toxin, which caused a closure of L-VDCC, attenuated BP responses to acute NO synthase inhibition (Pintérová *et al.* 2010).

## Materials and Methods

### Animals

All experiments were carried out in conscious 12-week-old males of spontaneously hypertensive (SHR) rats and their normotensive controls Wistar-Kyoto rats (WKY) (from our own colony based upon breeding pairs from Charles River) housed under the standard conditions (temperature  $23 \pm 1$  °C, 12-h light-dark cycle, tap water and ST-1 pellet diet *ad libitum*). One day prior to the



**Fig. 1.** Design of studies used to evaluate the role of BK<sub>Ca</sub> and K<sub>V</sub> channels in cAMP-induced vasodilatation. Protocol 1: Control group was subjected to the sequential blockade of renin-angiotensin system by captopril (CPT 10 mg/kg i.v.), sympathetic nervous system by pentolinium (PENTO, 5 mg/kg i.v.) and NO-synthase by L-NAME (30 mg/kg i.v.) (a). The influence of cAMP overproduction was assessed by isoprenaline infusion (6 µg/kg/h i.v.), which started after the stabilization period (b), before (c) or after L-NAME administration (d). Propranolol (PRO) was used to test whether the effect of isoprenaline is caused by β-adrenoceptor stimulation. Protocol 2: To establish the role of BK<sub>Ca</sub> and K<sub>V</sub> channels in the effects of isoprenaline, the infusion of tetraethyl-ammonium (TEA, 100 mg/kg/h i.v.) or 4-aminopyridine (4-AP, 4 mg/kg/h i.v.) was used in control animals (e) as well as in animals subjected to isoprenaline infusion as described above (f, g). The horizontal lines represent time axis (in minutes).

experiment, polyethylene catheters were inserted into the left carotid artery (for BP measurement) and left jugular vein (for drug application), and exteriorized in the interscapular region under light ether anesthesia. BP of conscious animals (kept in small plexiglass cages) was monitored and recorded by PowerLab system (ADInstruments Ltd, Bella Vista, NSW, Australia) between 8 and 12 h a.m. to reduce circadian variations in BP levels. Each of the experimental protocol was performed after 30 min period of BP stabilization. To eliminate the influence of renin-angiotensin system (RAS) and to avoid the activation of baroreflex during the BP measurement, the experiments were performed under the conditions when RAS was blocked by angiotensin converting enzyme inhibitor (captopril, 10 mg/kg b.w.) and 15 min later ganglionic blocker (pentolinium, 5 mg/kg b.w.) was injected to eliminate the activity of

endogenous SNS (Fig. 1a). This study was approved by the *Ethical Committee of the Institute of Physiology AS CR* and performed according to *European Convention on Animal Protection and Guidelines on Research Animal Use*.

#### *Effect of isoprenaline-induced cAMP overproduction on blood pressure responses to nitric oxide synthase blockade with L-NAME (Protocol 1)*

Since the use of forskolin or phosphodiesterase-resistant analogues of cAMP is not suitable for the *in vivo* experiments, we induced the cAMP overproduction by β-adrenoceptor stimulation with isoprenaline (6 µg/kg/h i.v.). As seen in Figure 1, the infusion of isoprenaline was started after stabilization period (Fig. 1b), before (Fig. 1c) or after the NO synthase blockade with L-NAME (30 mg/kg i.v.) (Fig. 1d). To test whether isoprenaline

effects are mediated by the stimulation of  $\beta$ -adrenoceptors, the antagonist of  $\beta$ -adrenoceptors (propranolol, 1 mg/kg i.v.) was administered.

Furthermore, we have also focused our attention on the role of endogenous  $\beta$ -adrenoceptor stimulation. Therefore, we evaluated the role of this component in basal BP level by means of  $\beta$ -adrenoceptor antagonist (propranolol, 1 mg/kg i.v.).

#### *Effect of BK<sub>Ca</sub> and K<sub>V</sub> channel blockade on isoprenaline-induced changes of blood pressure responses to L-NAME (Protocol 2)*

The role of BK<sub>Ca</sub> and K<sub>V</sub> channels was determined using a continuous infusion of tetraethylammonium (TEA, 100 mg/kg/h i.v.) and 4-aminopyridine (4-AP, 4 mg/kg/h i.v.), respectively. To block only BK<sub>Ca</sub> channels, TEA was used in the final extracellular concentrations below 1 mM. To exclude the influence of TEA and 4-AP infusion *per se* on L-NAME-induced BP responses, the control animal groups were subjected to TEA or 4-AP infusion before L-NAME injection (Fig 1e). To evaluate the effect of these potassium channel inhibitors on isoprenaline-induced changes of BP responses to L-NAME, the infusions of these inhibitors were started before or after L-NAME injection (Figs 1f and 1g).

#### *Drugs*

All drugs were purchased from Sigma St. Louis, USA and dissolved in saline solution. Captopril, pentolinium and L-NAME were administered as an intravenous bolus in a volume of 1 ml/kg b.w. Isoprenaline, TEA and 4-AP were infused in a volume of 1 ml/kg/h.

#### *Statistics*

Results were expressed as means  $\pm$  SEM. The data were evaluated by one way analysis of variance and least significant difference *post-hoc* test. The groups were considered to differ significantly at  $P < 0.05$ .

## **Results**

#### *Effect of isoprenaline-induced cAMP overproduction on blood pressure responses to nitric oxide synthase blockade by L-NAME (Protocol 1)*

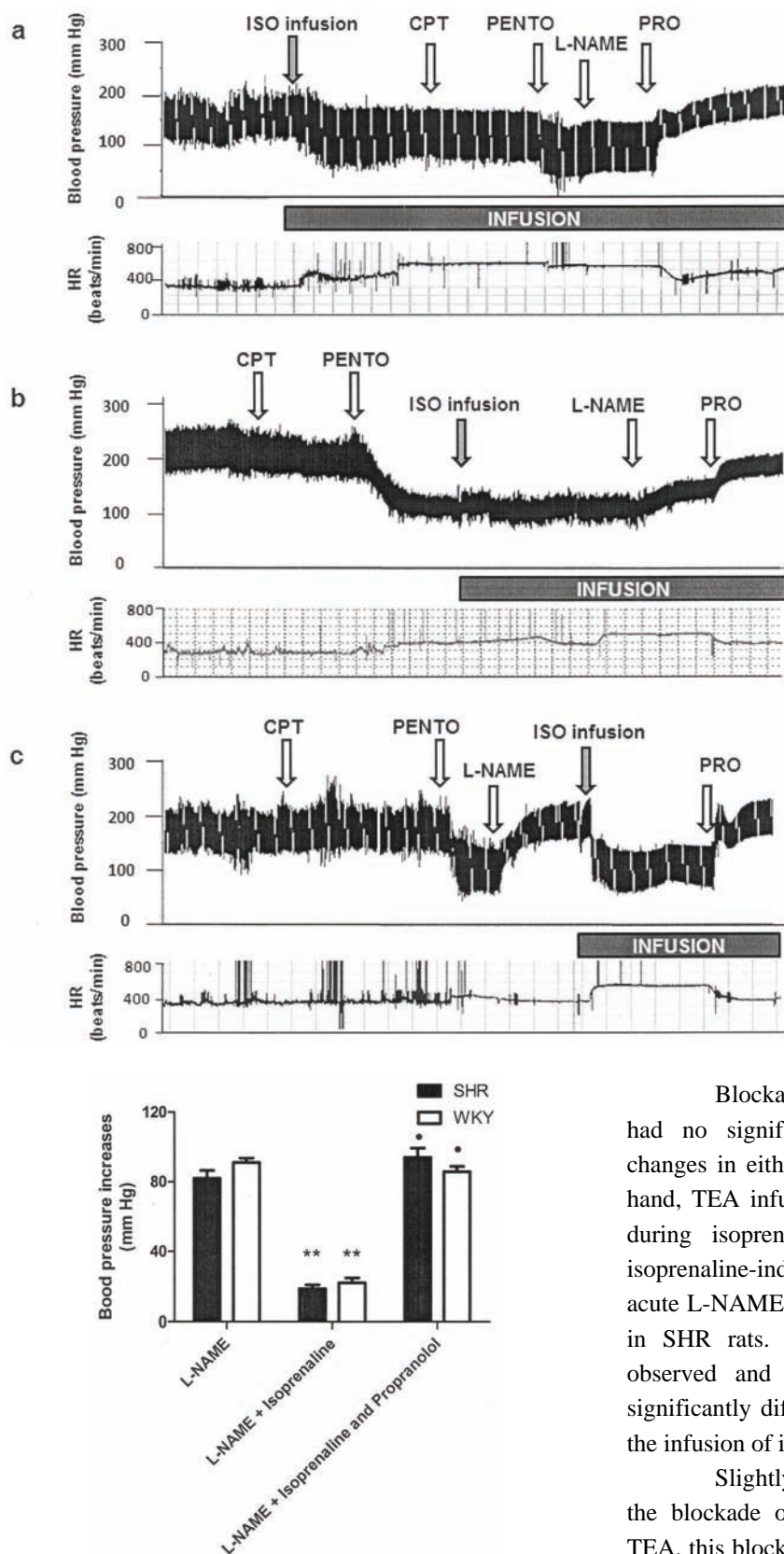
The basal values of mean arterial pressure were significantly elevated in SHR compared to WKY ( $176 \pm 5$  mm Hg and  $112 \pm 1$  mm Hg, respectively,  $n=18$ ,

$P < 0.01$ ). Isoprenaline infusion, which started just after the stabilization period (i.e. before the administration of any other substances), caused a significant decrease of baseline BP levels in both rat strains, which was much more pronounced in SHR compared to WKY rats ( $\Delta$  isoprenaline  $-54 \pm 7$  mm Hg in SHR and  $-9 \pm 3$  mm Hg in WKY,  $n=12$ ,  $P < 0.01$ ). Nevertheless, BP rises induced by subsequent acute inhibition of NO synthase with L-NAME were prevented by the overproduction of cAMP elicited by isoprenaline infusion. As evident from BP records in SHR rats, this was observed both when the infusion of isoprenaline started just after the stabilization period (Fig. 2A) and after the preceding blockade of renin-angiotensin and sympathetic nervous systems (Fig. 2B). Very similar depressing effects of isoprenaline infusion on BP responses to acute NO synthase blockade were also seen in normotensive WKY rats (Fig. 3). Moreover, isoprenaline infusion almost completely abolished the fully developed BP response to L-NAME (Fig. 2C). All isoprenaline effects were completely reversed by  $\beta$ -adrenoceptor blockade with propranolol injection while isoprenaline infusion continued (Fig. 2, Fig. 3).

It could be mentioned that the effect of propranolol on basal BP level (i.e. on  $\beta$ -adrenoceptors stimulated by endogenous catecholamines) was minimal in both rat strains ( $1.3 \pm 1.5$  mm Hg in SHR and  $3.5 \pm 1.1$  mm Hg in WKY). On the other hand, propranolol administration *per se* caused substantial heart rate decreases in both SHR and WKY ( $-40 \pm 15.8$  beats/min and  $-50 \pm 8.3$  beats/min, respectively).

#### *Effect of BK<sub>Ca</sub> and K<sub>V</sub> channel blockade on isoprenaline-induced changes of blood pressure responses to L-NAME (Protocol 2)*

This experiment was designed to determine the role of BK<sub>Ca</sub> and K<sub>V</sub> channels in isoprenaline-induced changes of BP responses to L-NAME. The values of basal mean arterial pressure (MAP) (Table 1) were similar to those observed in the previous protocol. Infusion of TEA (BK<sub>Ca</sub> blocker) or 4-AP (K<sub>V</sub> blocker), which started just after RAS inhibition and ganglionic blockade, induced significantly greater BP increases in SHR compared to WKY rats (Table 1). While TEA-induced BP responses were completely abolished by the following isoprenaline infusion in both rat strains, this infusion only partially reduced BP changes elicited by 4-AP (Table 1).



**Fig. 3.** Effect of isoprenaline infusion on BP responses to NO synthase blockade with L-NAME and elimination of this effect by propranolol,  $\beta$ -adrenoceptor blocker, in WKY and SHR rats subjected to a previous blockade of RAS by captopril and SNS by pentolinium. Values are means  $\pm$  SEM, \*\*  $P < 0.001$  vs. respective L-NAME group, \*  $P < 0.001$  vs. respective L-NAME + Isoprenaline group.  $n = 5-10$  in each group.

**Fig. 2.** Blood pressure and heart rate records in SHR rats. **(a)** After 30 min stabilization period, the animals were subjected to isoprenaline infusion (the onset of ISO infusion is indicated by dark arrow). This was followed by the blockade of renin-angiotensin system with captopril (CPT) and sympathetic nervous system with pentolinium (PENTO) to eliminate the influence of these two systems. Subsequently, the blocker of NO-synthase, L-NAME, was injected. **(b)** After the blockade of renin-angiotensin system with captopril (CPT) and sympathetic nervous system with pentolinium (PENTO), isoprenaline (ISO) infusion was started. This was followed by the administration of NO-synthase blocker, L-NAME. **(c)** Animals were subjected to isoprenaline infusion after previous blockade of renin-angiotensin system with captopril (CPT), sympathetic nervous system with pentolinium (PENTO) and NO-synthase with L-NAME. To test whether all isoprenaline effects were mediated by  $\beta$ -adrenoceptor-coupled pathway, the antagonist of  $\beta$ -adrenoceptors, propranolol (PRO, 1 mg/kg i.v.) was administered.

Blockade of  $BK_{Ca}$  channels with TEA infusion had no significant effect on L-NAME-induced BP changes in either WKY or SHR (Fig. 4a). On the other hand, TEA infusion restored BP responses to L-NAME during isoprenaline infusion and it diminished the isoprenaline-induced prevention of BP rise following acute L-NAME injection (Fig. 4a), but this was true only in SHR rats. In WKY, such a restoration was not observed and BP responses to L-NAME were not significantly different compared to those reached during the infusion of isoprenaline (Fig. 4a).

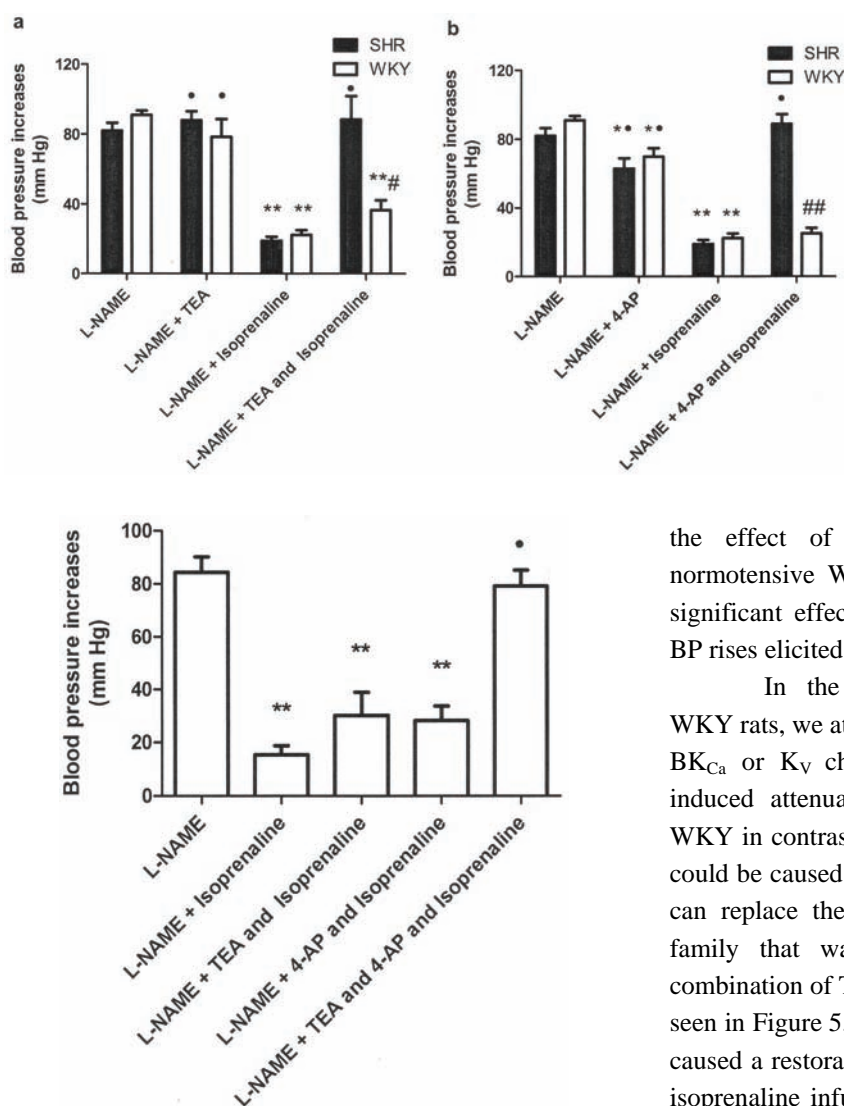
Slightly different effects were also elicited by the blockade of  $K_V$  channels with 4-AP. Contrary to TEA, this blockade of  $K_V$  channels slightly decreased BP responses to L-NAME in both rat strains (Fig. 4b). The infusion of 4-AP restored BP responses to L-NAME during isoprenaline infusion, abolishing thus the isoprenaline-induced prevention of BP rise to acute L-NAME injection (Fig. 4b). However, similarly to TEA,



**Table 1.** Basal values of mean arterial pressure (MAP) and MAP levels stabilized after ganglionic blockade with pentolinium (PENTO) in WKY and SHR rats, as well as MAP changes elicited by BK<sub>Ca</sub> and K<sub>V</sub> channel blockers ( $\Delta$  TEA and  $\Delta$  4-AP, respectively) under the conditions of ganglionic blockade and isoprenaline(ISO)-induced modifications of MAP responses to these K<sup>+</sup> channel blockers in both rat strains.

Rat strain	Basal MAP (mm Hg)	MAP after PENTO (mm Hg)	$\Delta$ TEA (mm Hg)	$\Delta$ 4-AP (mm Hg)	$\Delta$ TEA + ISO (mm Hg)	$\Delta$ 4-AP + ISO (mm Hg)
WKY	118 $\pm$ 1 <sup>#</sup>	51 $\pm$ 1 <sup>#</sup>	10 $\pm$ 2 <sup>#</sup>	21 $\pm$ 4 <sup>#</sup>	-14 $\pm$ 2	-8 $\pm$ 4*
SHR	178 $\pm$ 7	83 $\pm$ 5	22 $\pm$ 3	51 $\pm$ 7	-23 $\pm$ 4	-28 $\pm$ 7*

Data are means  $\pm$  SEM, n=8 in each strain. TEA – tetraethylammonium, 4-AP – 4-aminopyridine,  $\Delta$  TEA – MAP changes elicited by infusion of BK<sub>Ca</sub> channel blocker, TEA;  $\Delta$  4-AP – MAP changes elicited by infusion of K<sub>V</sub> channel blocker, 4-AP;  $\Delta$  TEA + ISO – isoprenaline-induced modification of TEA effects;  $\Delta$  4-AP + ISO – isoprenaline-induced modification of 4-AP effects. # P<0.05 vs. SHR, \* P<0.05 vs. the respective group before isoprenaline infusion.



**Fig. 5.** Effects of BK<sub>Ca</sub> channel blockade by tetraethylammonium (TEA), K<sub>V</sub> channel blockade by 4-aminopyridine (4-AP) and their combination (TEA + 4-AP) on isoprenaline-induced inhibition of BP responses to L-NAME in WKY rats subjected to a previous blockade of RAS by captopril and SNS by pentolinium. Values are means  $\pm$  SEM, \*\* P<0.001 vs. L-NAME group, • P<0.001 vs. L-NAME + Isoprenaline group. n=4-8 in each group.

**Fig. 4.** Influence of BK<sub>Ca</sub> channel blockade by tetraethylammonium (TEA) (a) and K<sub>V</sub> channel blockade by 4-aminopyridine (4-AP) (b) on BP responses to acute NO synthase blockade with L-NAME and the influence of these potassium channel blockers on isoprenaline-induced effect. The experiments were performed in WKY and SHR rats subjected to a previous blockade of RAS by captopril and SNS by pentolinium. Values are means  $\pm$  SEM, \* P<0.05, \*\* P<0.001 vs. respective L-NAME group, # P<0.05, ## P<0.001 vs. respective SHR, • P<0.001 vs. respective L-NAME + Isoprenaline group. n=5-10 in each group.

the effect of 4-AP was seen only in SHR. In normotensive WKY rats, the infusion of 4-AP had no significant effect on isoprenaline-induced prevention of BP rises elicited by L-NAME (Fig. 4b).

In the additional experiments performed in WKY rats, we attempted to elucidate why the blockade of BK<sub>Ca</sub> or K<sub>V</sub> channels had no effect on isoprenaline-induced attenuation of BP responses to L-NAME in WKY in contrast to SHR (Fig. 4). We supposed that this could be caused by the fact that one class of K<sup>+</sup> channels can replace the missing effect of another K<sup>+</sup> channel family that was inhibited. Therefore, we used the combination of TEA and 4-AP infusions in WKY rats. As seen in Figure 5, simultaneous infusion of TEA and 4-AP caused a restoration of BP responses to L-NAME during isoprenaline infusion. The BP responses restored by the combination of potassium channel blockers were not significantly different from the control L-NAME-induced BP increases (Fig. 5).

## Discussion

The hypothesis of this *in vivo* study was that altered function of BK<sub>Ca</sub> and/or K<sub>V</sub> channels might be one of the factors responsible for the abnormal  $\beta$ -adrenoceptor-mediated vasodilatation observed in SHR rats. Although we did not see the hypothetical impairment of  $\beta$ -adrenergic vasodilatation in SHR (Arribas *et al.* 1994, Mallem *et al.* 2005), we confirmed that a major part of the isoprenaline-induced vasodilatation is mediated by the activation of BK<sub>Ca</sub> and K<sub>V</sub> channels. Moreover, we have shown that while the inhibition of either class of K<sup>+</sup> channels led to the diminution of isoprenaline-induced BP effects in hypertensive rats, in normotensive WKY rats the absence of one class of K<sup>+</sup> channels could be compensated by the remaining K<sup>+</sup> channel family. This suggests the impaired function of these K<sup>+</sup> channel types in SHR.

The present study shows that cAMP overproduction elicited by isoprenaline-induced  $\beta$ -adrenoceptor stimulation decreased the level of basal BP. This effect was, however, substantially greater in hypertensive SHR compared to normotensive WKY rats ( $-54 \pm 7$  mm Hg vs.  $-9 \pm 3$  mm Hg). BP decreases elicited by isoprenaline infusion were always accompanied by maximal heart rate increases (Fig. 2) due to the stimulation of cardiac  $\beta$ -adrenoceptors. Despite the increase in cardiac output, isoprenaline caused BP lowering indicating a large isoprenaline-induced decrease in vascular resistance. We have shown a very similar BP reduction in our previous study (Pintérová *et al.* 2010) where the overproduction of cAMP was elicited by the pertussis toxin-induced inactivation of G<sub>i</sub> proteins, the effect being also greater in SHR than WKY rats ( $-58$  mm Hg vs.  $-15$  mm Hg). This indicates that the effect of increased cAMP levels on basal BP is not dependent on the cause of cAMP overproduction. The greater sensitivity of spontaneously hypertensive rats to vasodilator stimuli compared to normotensive WKY rats is in agreement with the *in vitro* studies showing an enhanced  $\beta$ -adrenoceptor-mediated relaxation of perfused hind limbs or isolated blood vessels from SHR (Deragon *et al.* 1978, Carvalho *et al.* 1987). However, Arribas *et al.* (1994) have observed that  $\beta$ -adrenoceptor-mediated relaxation of norepinephrine-precontracted arteries from SHR is decreased compared to those from WKY and they have ascribed this fact to impaired endothelial function. Since isoprenaline-elicited responses in their study were reduced by endothelial removal, they have suggested the

existence of endothelial  $\beta$ -adrenoceptors, which mediate vascular relaxation through NO release (Arribas *et al.* 1994). However, we have recently seen that vasodilatation elicited by beraprost, a stable analogue of prostacyclin acting directly on vascular smooth muscle cells through the elevation of cAMP (Coleman *et al.* 1994, Narumiya *et al.* 1999, Norel 2007), is also enhanced in SHR as compared to normotensive rats (Behuliak, unpublished data). Moreover, under the conditions of acute NO deficiency induced by L-NAME we have observed the augmentation of isoprenaline-induced BP reduction and there was no significant difference in isoprenaline-induced BP decreases between WKY and SHR (this study,  $-90 \pm 5$  mm Hg in WKY,  $-80 \pm 8$  mm Hg in SHR). Thus, our results question the idea of endothelial  $\beta$ -adrenoceptor-mediated vasorelaxation through the activation of NO pathway.

One of the possible explanations for a greater sensitivity of hypertensive rats to isoprenaline-induced vasodilatation could be the increased expression of  $\beta$ -adrenoceptors in SHR as compared to WKY (Oliver *et al.* 2009). This elevated  $\beta$ -adrenoceptor expression is, however, accompanied by the enormous increase in  $\alpha$ -adrenoceptor expression (Oliver *et al.* 2009), leading to the imbalance between sensitivity of vascular smooth muscle to vasoconstrictor and vasodilator adrenergic stimuli. When arteries from hypertensive animals are precontracted by high doses of norepinephrine targeting both  $\alpha$ - and  $\beta$ -adrenoceptors, the vasodilator effects of isoprenaline might be counteracted by increased  $\alpha$ -adrenergic vasoconstriction (Arribas *et al.* 1994). In contrast, in our *in vivo* study, where  $\alpha$ -adrenoceptors were exposed only to physiological concentrations of endogenous norepinephrine, the increased effects of isoprenaline-induced  $\beta$ -adrenoceptor stimulation need not to be masked by the hyperactivity of  $\alpha$ -adrenoceptors in SHR.

However, we can also speculate that the greater BP fall induced by isoprenaline in SHR could be related to a greater membrane depolarization of vascular smooth muscle compared with WKY rats (Harder *et al.* 1981, Stekiel *et al.* 1986). Stimulation of  $\beta$ -adrenoceptors by isoprenaline causes vascular smooth muscle hyperpolarization (Stekielski *et al.* 1993) which can be better seen under the conditions of depolarized membrane potential. This could also explain our above mentioned large isoprenaline-induced BP fall seen following NO synthase blockade with L-NAME. Since BP levels reached after L-NAME administration were very similar

in both rat strains, we can suppose the same changes for the membrane potential. Therefore it is not surprising that these BP falls were also very similar in both SHR and WKY rats.

On the other hand, the present results indicate that the role of endogenous  $\beta$ -adrenergic component in BP maintenance is negligible in both rat strains. Propranolol-induced  $\beta$ -adrenoceptor blockade caused only very small BP increases in both rat strains under the conditions of intact sympathetic nervous system. However, this might also be caused by propranolol-induced heart rate decreases, which could partially obscure propranolol effects on vascular levels.

The *in vivo* evaluation of the involvement of  $K^+$  channels in the isoprenaline-induced vasodilatation has to be done under the conditions of ganglionic blockade, because existing sympathetic vasoconstriction and its baroreflex modulation in conscious rats could obscure BP changes elicited by cyclic nucleotides and/or  $K^+$  channel activation. Since BP levels after such intervention are too low and hence not suitable for the study of isoprenaline-induced vasodilatation or its modulation by  $K^+$  channel blockers, we preferred to use an indirect method. Both cAMP and NO/cGMP dilate the arteries by the closure of L-VDCC channels (Xiong and Sperelakis 1995, Taguchi *et al.* 1997, Lewis *et al.* 2005). The inhibition of NO synthase results in the opening of L-VDCC and hence calcium influx, vasoconstriction and BP elevation (Pintérová *et al.* 2009). However, under the conditions of cAMP overproduction, the BP elevation induced by the inhibition of NO synthesis cannot become manifest due to the concurrent closure of L-VDCC by cAMP. Thus, vasodilator effects of cAMP can be indirectly measured as the ability of isoprenaline to prevent the BP rise elicited by the inhibition of NO-induced vasodilatation.

Under the conditions of ganglionic blockade, the increased formation of cAMP, which was induced by isoprenaline infusion, prevented BP rise elicited by L-NAME and abolished the already developed L-NAME-induced BP elevation. There were no significant differences between the two strains. This effect was mediated by the stimulation of  $\beta$ -adrenergic receptors, since it could be blocked by  $\beta$ -adrenoceptor antagonist, propranolol. Recently, we have seen a very similar attenuation of the L-NAME-induced BP rises when cAMP overproduction was induced by  $G_i$ -protein inactivation (Pintérová *et al.* 2010).

Furthermore, we estimated the involvement of  $K^+$  channels in isoprenaline-induced vasodilatation. Since

it has been reported that properties of both  $BK_{Ca}$  and  $K_V$  channels are altered in hypertension (Nelson and Quayle 1995, Cox 2002), we have evaluated the involvement of both potassium channel types in cAMP-mediated vasodilatation in normotensive and hypertensive rats. However, the use of conscious animals does not allow us to apply numerous toxic blockers of these  $K^+$  channels blockers commonly used for *in vitro* studies. Thus the role of  $K_V$  and  $BK_{Ca}$  channels in  $\beta$ -adrenoceptor-mediated vasodilatation was examined by means of TEA ( $BK_{Ca}$  channel blocker used in the concentration below 1 mM to block only this channel type) and 4-AP ( $K_V$  channel blocker). These two blockers were used to quantify the cAMP-induced attenuation of BP rise following acute NO depletion.

The blockade of  $BK_{Ca}$  or  $K_V$  channels significantly diminished the isoprenaline-induced prevention of BP rise following acute L-NAME injection in SHR suggesting that the activation of both potassium channel types is involved in the  $\beta$ -adrenoceptor-induced vasodilatation. This is in agreement with *in vitro* studies performed in cultured vascular smooth muscle cells demonstrating that both  $BK_{Ca}$  (Sadoshima *et al.* 1988, Scornik *et al.* 1993) and  $K_V$  channels (Aiello *et al.* 1995) are regulated by cAMP-dependent pathway. However, in contrast to SHR, the blockade of  $BK_{Ca}$  or  $K_V$  channels in WKY rats had no significant effect on isoprenaline-induced prevention of BP responses to L-NAME.

To elucidate why the blockade of  $BK_{Ca}$  or  $K_V$  channels had no effect on isoprenaline-induced attenuation of BP responses to L-NAME in WKY rats, we applied the combined blockade of  $BK_{Ca}$  and  $K_V$  channels. The simultaneous infusion of both  $K^+$  channel type inhibitors caused a restoration of BP responses to L-NAME during isoprenaline infusion, which was not significantly different from the control L-NAME-induced BP increases. Thus, we suggest that in normotensive WKY rats one class of  $K^+$  channels can replace the missing effect of another  $K^+$  channel family that was inhibited.

The increased expression of  $BK_{Ca}$  channels and augmented  $K^+$  current density was observed in SHR (Liu Y *et al.* 1995, (Liu Y *et al.* 1997). Moreover, Cox *et al.* (2001) have also shown the increased gene expression of prominent  $K_V$  channel  $\alpha$  and  $\beta$  subunits associated with a greater voltage-dependent  $K^+$  current density in systemic arteries from SHR compared to WKY. This is in accordance with our present results showing greater BP rises induced by the infusion of  $K_V$  channel blocker (4-



aminopyridine) in hypertensive rats (Table 1). These results, however, failed to confirm the hypothesis on the primary lack of K<sub>V</sub> channel action in the hypertensive animals (Cox 2002, Cox and Rusch 2002). We can suggest that the up-regulation of both K<sup>+</sup> channel types in hypertensive rats is a compensatory mechanism against the increased activity of vasoconstrictor systems (namely SNS).

In conclusions, the vasodilator action of both BK<sub>Ca</sub> and K<sub>V</sub> channels was found to be enhanced in SHR under basal conditions. Nevertheless, their overall contribution to BP lowering mechanisms seems to be insufficient in genetic hypertension because a concurrent activation of both classes of K<sup>+</sup> channels by cAMP overproduction is a necessary prerequisite for the prevention of BP rise elicited by acute NO/cGMP deficiency in SHR. In contrast, cAMP-induced activation of either BK<sub>Ca</sub> or K<sub>V</sub> channels is sufficient to prevent this BP rise in normotensive WKY rats. Most plausible explanations for this remarkable strain difference might be that 1) the “hyperpolarizing” function of K<sup>+</sup> channels in SHR is impaired by other factors partially depolarizing cell membrane in this rat strain or 2) cAMP-induced activation of these channels is less effective in hypertensive rats compared to normotensive ones. Further studies are required to clarify the potency of cAMP to activate particular classes of K<sup>+</sup> channels under the *in vivo* conditions and to determine their influence on membrane potential in resistance vessels of normotensive

and hypertensive animals.

Thus, our results indicate the impairment of cAMP-induced vasodilator function mediated by BK<sub>Ca</sub> and K<sub>V</sub> channels in resistance vessels of conscious rats with genetic hypertension.

### Conflict of Interest

There is no conflict of interest.

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### Abbreviations

4-AP – 4-aminopyridine, BK<sub>Ca</sub> – large-conductance calcium-activated potassium channel, BP – blood pressure, G<sub>i</sub>-protein – inhibitory heterotrimeric guanine nucleotide-binding protein, G<sub>s</sub>-protein – stimulatory heterotrimeric guanine nucleotide-binding protein, K<sub>V</sub> – voltage-dependent potassium channel, L-NAME – N<sup>o</sup>-nitro-L-arginine methyl ester, L-VDCC – L-type voltage-dependent calcium channels, MAP – mean arterial pressure, NO – nitric oxide, PKA – protein kinase A, PKG – protein kinase G, RAS – renin-angiotensin system, SHR – spontaneously hypertensive rat, SNS – sympathetic nervous system, TEA – tetraethylammonium ions, WKY – Wistar Kyoto rat.

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