*Physiol. Res. 58 (Suppl. 2): S43-S54, 2009*

**MINIREVIEW**

# Impaired Control of L-Type Voltage-Dependent Calcium Channels in Experimental Hypertension

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Received June 9, 2009
Accepted October 30, 2009

**Summary**

Blood pressure (BP) level results from the balance of vasoconstrictors (mainly sympathetic nervous system) and vasodilators (predominantly nitric oxide and endothelium-derived hyperpolarizing factor). Most of the forms of experimental hypertension are associated with sympathetic hyperactivity and endothelial dysfunction. It is evident that nitric oxide and norepinephrine are antagonists in the control of calcium influx through L-type voltage-dependent calcium channels (L-VDCC). Their effects on L-VDCC are mediated by cGMP and cAMP, respectively. Nevertheless, it remains to determine whether these cyclic nucleotides have direct effects on L-VDCC or they act through a modulation of calcium-activated $K^+$ and $Cl^-$ channels which influence membrane potential. Rats with genetic or salt hypertension are characterized by a relative (but not absolute) NO deficiency compared to the absolute enhancement of sympathetic vasoconstriction. This dysbalance of vasoconstrictor and vasodilator systems in hypertensive animals is reflected by greater calcium influx through L-VDCC susceptible to the inhibition by nifedipine. However, when the modulatory influence of cyclic nucleotides is largely attenuated by simultaneous ganglionic blockade and NO synthase inhibition, BP of spontaneously hypertensive rats remains still elevated compared to normotensive rats due to augmented nifedipine-sensitive BP component. It remains to determine why calcium influx through L-VDCC of hypertensive rats is augmented even in the absence of modulatory influence of major vasoactive systems (sympathetic nervous system, nitric oxide).

**Key words**

L-type voltage-dependent calcium channels • Calcium-activated $K^+$ and $Cl^-$ channels • Vasoactive systems • EDCF • Blood pressure control • Isolated arteries

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## Introduction

Hypertension and its target organ complications represent a great risk factor for cardiovascular health in developed societies. High blood pressure (or better said its surrogate increased systemic resistance) is caused by numerous factors ranging from altered activity of particular vasoactive systems over vascular smooth muscle (VSM) hyperreactivity up to the remodeling of resistance vessels. The balance of vasoconstrictor and vasodilator systems, which is modulated at both central and peripheral levels, is decisive for setting of vascular tone (Fig. 1). It is well known that $Ca^{2+}$ influx plays a key role in vascular contraction because lasting VSM contraction can hardly be elicited in the absence of extracellular calcium. This was also documented in our previous study (Paulis *et al.* 2007) in which norepinephrine (NE) caused only a transient phasic contraction of isolated artery incubated in $Ca^{2+}$-free medium, whereas sustained tonic contraction appeared

only when $Ca^{2+}$ was added into the incubation medium. A major part of this NE-induced tonic contraction could be abolished or prevented by nifedipine – a dihydropyridine antagonist of L-type voltage-dependent calcium channels (L-VDCC). Since tonic but not phasic contraction of NE-stimulated artery can be augmented by endothelium removal (Fig. 2) or inhibition of nitric oxide (NO) synthesis, it is evident that vasodilator effects of NO are mediated by inhibition of $Ca^{2+}$ influx through L-VDCC. Thus NE and NO appear to be the most prominent counterplayers in the control of nifedipine-sensitive $Ca^{2+}$ influx that is responsible for actual vascular tone.

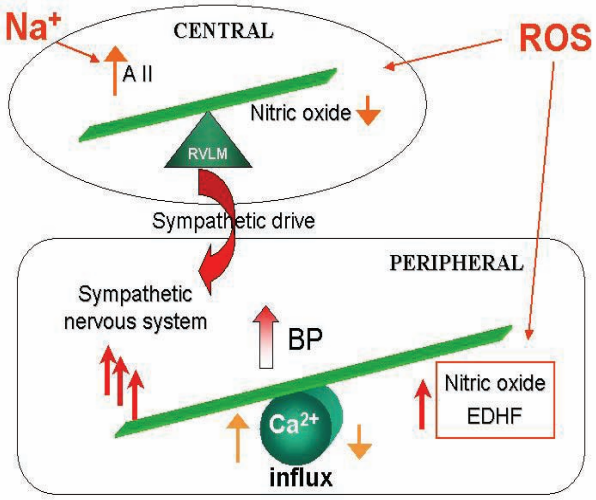


**Fig. 1.** The simplified scheme of major central and peripheral mechanisms regulating blood pressure through the control of sympathetic tone and calcium influx into smooth muscle of resistance vessels. A II – angiotensin II, ROS – reactive oxygen species, EDHF – endothelium-derived hyperpolarization factor, BP – blood pressure.

In most forms of experimental hypertension high blood pressure (BP) results from the dysbalance between sympathetic hyperactivity and vasodilator deficiency which is often relative rather than absolute. This could be documented in rats with genetic (hereditary hypertriglyceridemic (HTG) rats – Kuneš *et al.* 2002, spontaneously hypertensive (SHR) rats – Paulis *et al.* 2007, Hojná *et al.* 2007, Kuneš *et al.* 2008) or salt-dependent forms (Dahl rats – Zicha *et al.* 2001, Dobešová *et al.* 2002) of genetic hypertension. Somewhat different type of vasoconstrictor/vasodilator dysbalance was disclosed in rats with L-NAME-induced hypertension in which severe NO deficiency was accompanied by moderate enhancement of sympathetic and angiotensin-dependent vasoconstriction which are also involved in the maintenance of elevated BP (Pecháňová *et al.* 2004). Our further experiments indicated that missing NO is partially replaced by vasodilator effects of endothelium-derived hyperpolarizing factor (EDHF) in order to counterbalance partially augmented effects of above mentioned vasoconstrictor systems (Fig. 3).

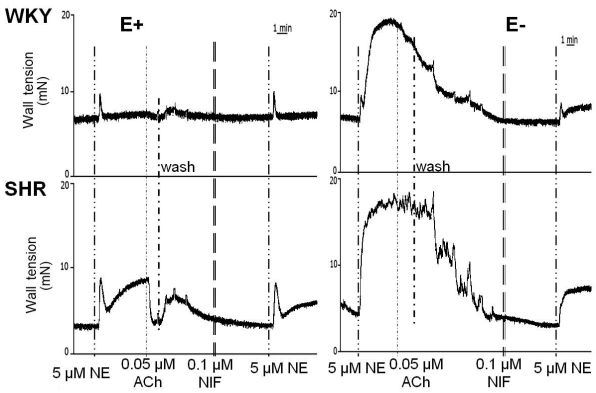


**Fig. 2.** Norepinephrine (NE)-induced contraction (mN) of femoral arteries isolated from WKY (upper panels) and SHR (lower panels) with (E+) or without endothelium (E–) prior and after the addition of nifedipine (NIF). The early transient phasic NE-induced contractions of arteries with intact endothelium reflect a mobilization of internal calcium stores, whereas tonic contractions are characteristic for deendothelized arteries. Tonic NE-induced contractions are based upon $Ca^{2+}$ influx mainly through voltage-dependent calcium channels susceptible to the inhibition by nifedipine. Note that moderate NE-induced tonic contractions can also be seen in endothelized SHR arteries.

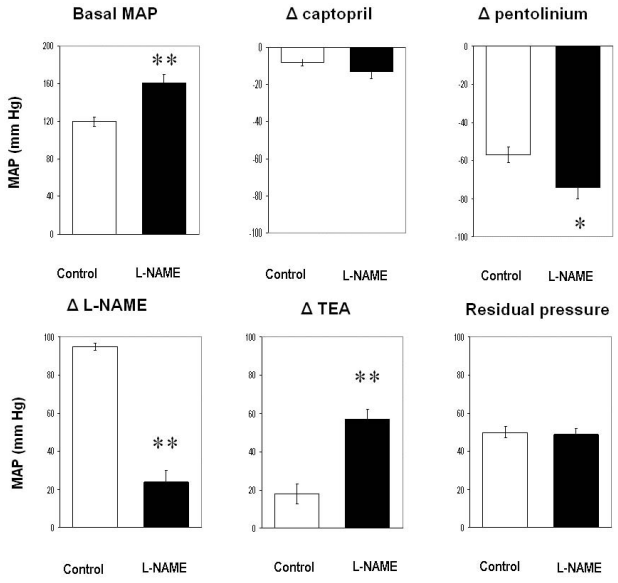


**Fig. 3.** Basal BP, BP changes elicited by the blockade of particular vasoactive systems and residual pressure (recorded after nitroprusside injection) in control and L-NAME-treated Wistar rats. Significant differences from untreated Wistar rats are indicated by *, ** ($p<0.05$, $p<0.01$). Data are means ± S.E.M. (n = 10-12). TEA – tetraethylammonium, L-NAME – $N^G$-nitro-L-arginine methyl ester.

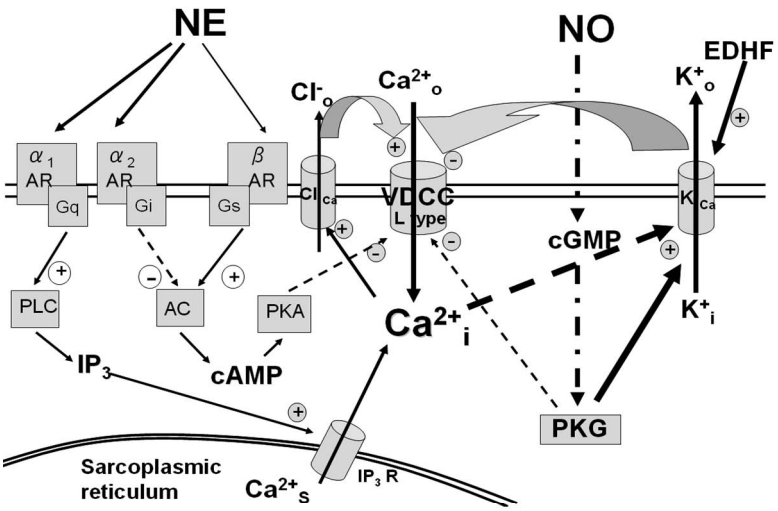


**Fig. 4.** The scheme of the influence of norepinephrine (NE) and nitric oxide (NO) on the control of $Ca^{2+}$ influx through voltage-dependent calcium channels of L type (VDCC L type) into vascular smooth muscle cells. Abbreviations: AR – adrenoceptors, Gq, Gi and Gs – particular G proteins, PLC – phospholipase C, AC – adenylate cyclase, PKA and PKG – protein kinases A and G, cAMP and cGMP – cyclic adenosine and guanosine monophosphates, $Cl_{Ca}$ and $K_{Ca}$ – calcium-activated chloride and potassium channels, EDHF – endothelium-derived hyperpolarization factor, $IP_3$ R – inositol triphosphate receptor.

Since increased sympathetic activity and/or decreased NO bioavailability tend to open L-VDCC (Fig. 4), it is not surprising that all so far examined forms of experimental hypertension displayed more pronounced BP reduction following the acute blockade of these calcium channels by nifedipine (Kuneš *et al.* 2004). As we have demonstrated in three different forms of experimental hypertension (SHR, Dahl, NO-deficient), the magnitude of nifedipine-induced BP reduction was proportional to initial (baseline) BP level (Kuneš *et al.* 2004). This linear relationship was present within the range of mean arterial pressure between 80 and 250 mm Hg under all experimental conditions so far investigated. This extraordinary strong biological phenomena can be disclosed in rats with developing or established hypertension as well as in rats subjected to various dietary or pharmacological antihypertensive interventions (Fig. 5). In addition, using isolated femoral arteries we succeeded to reveal an analogous relationship between maximal NE-induced wall tension and wall tension change following nifedipine addition to incubation medium (Paulis *et al*. 2007).

We have demonstrated in salt hypertensive Dahl rats that there is a steeper slope of the relationship between nifedipine-induced BP changes and basal BP compared to the relationship between BP changes induced by ganglionic blocker pentolinium and basal BP (Fig. 6). This indicates a progressive augmentation of $Ca^{2+}$ influx through L-VDCC with increasing blood pressure. It remains an open question whether greater $Ca^{2+}$ influx in hypertensive animals results mainly from enhanced sympathetic stimulation of their resistance vessels or is also a consequence of endothelial dysfunction involving relative NO deficiency.

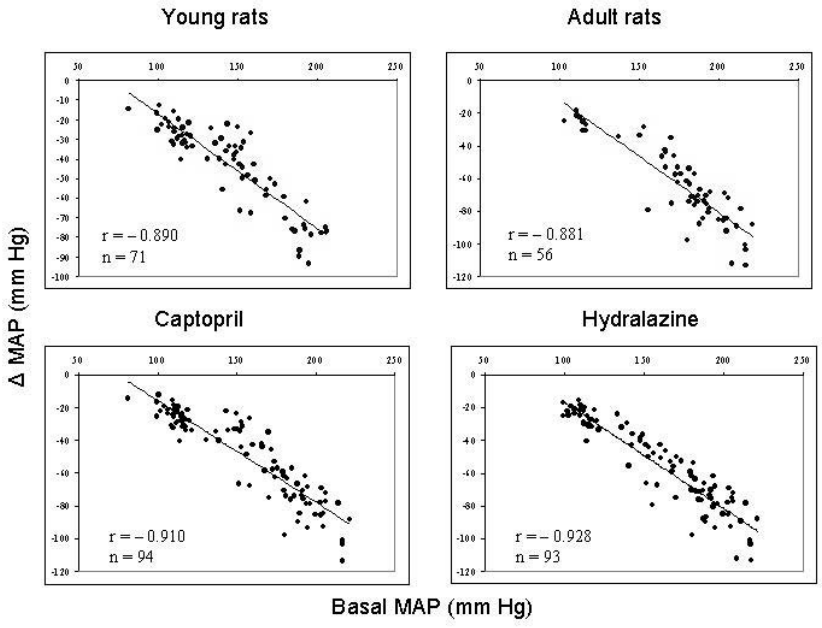


**Fig. 5.** The relationship of nifedipine-induced BP changes to basal BP in young and adult rats (upper panels) as well as in rats subjected either to active treatment with ACE inhibitor captopril or vasodilator hydralazine or studied after the withdrawal of these drugs (lower panels).

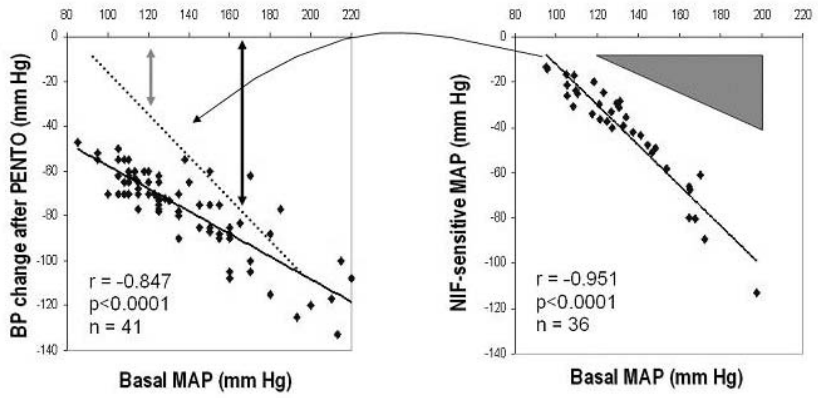


**Fig. 6.** The relationship of pentolinium-induced (left panel) and nifedipine-induced (right panel) BP changes to basal BP in Dahl rats. The dark triangle indicates the augmentation of endothelial dysfunction and nifedipine sensitivity with increasing blood pressure. The dotted line in the left panel corresponds to the regression line depicted in the right panel. Vertical arrows (left panel) depict the contribution of nifedipine-sensitive calcium influx to sympathetic BP component in normotensive and hypertensive animals.

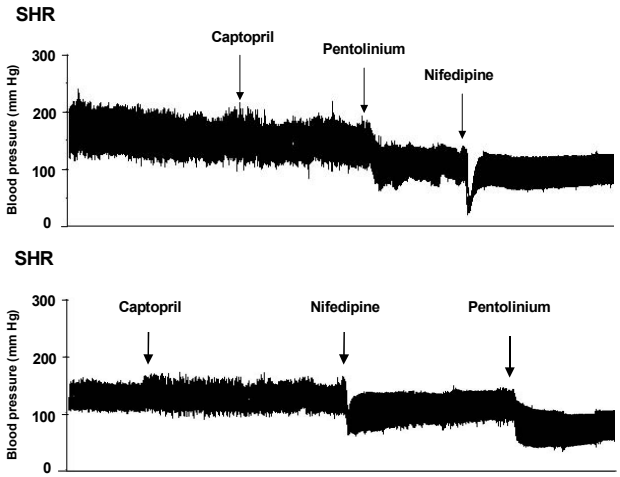


**Fig. 7.** Time course of BP changes elicited in SHR by the administration of nifedipine (0.4 mg/kg i.v.) after (upper panel) or before (lower panel) pentolinium injection (5 mg/kg i.v.).

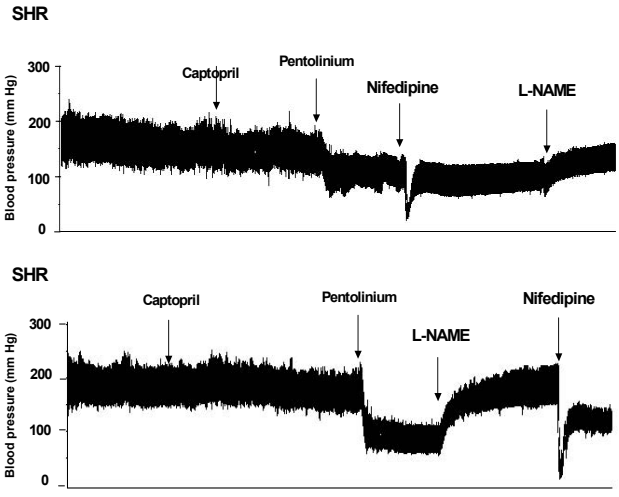


**Fig. 8.** Time course of BP changes elicited in SHR by the administration of nifedipine (0.4 mg/kg i.v.) before (upper panel) or after (lower panel) L-NAME injection (30 mg/kg i.v.).

## Vasoactive system dysbalance and augmented nifedipine-sensitive $Ca^{2+}$ influx

Our recent effort was focused to the elucidation of 1) the contribution of vasoconstrictor and vasodilator systems to enhanced nifedipine-sensitive $Ca^{2+}$ influx in hypertension, and 2) the mechanisms by which NE and NO can control the opening of L-VDCC.

First, we have demonstrated that nifedipine-sensitive BP component is a considerable part of sympathetic vasoconstriction represented by pentolinium-induced BP change (Fig. 7). It is evident that nifedipine has only minimal BP effect in pentolinium-pretreated SHR (–10±3 mm Hg), whereas pentolinium can still lower BP in nifedipine-pretreated rats (–49±7 mm Hg). Figure 8 shows that $Ca^{2+}$ influx through L-VDCC is modulated by endogenous NO which closes L-VDCC. Nifedipine considerably attenuates BP rise occurring after L-NAME injection (39 % of BP change observed prior to nifedipine injection to SHR) (Fig. 8, upper panel) and it also rapidly abolishes the already developed BP elevation elicited by acute L-NAME injection (BP decreased by 87 %) (Fig. 8, lower panel).

Thus enhanced NE stimulation or diminished NO availability can effectively raise blood pressure within a broad range through a modulation of nifedipine-sensitive $Ca^{2+}$ influx. It should be mentioned that vasodepressor role of NO can be partially replaced by EDHF in certain forms of hypertension characterized by pronounced NO deficiency (e.g. after chronic L-NAME administration). EDHF participation in these animals can be revealed by the administration of tetraethylammonium (TEA) which in concentrations up to 1 mM blocks $Ca^{2+}$-activated potassium channels ($BK_{Ca}$) (Fig. 9) (Zicha *et al.* 2006a).

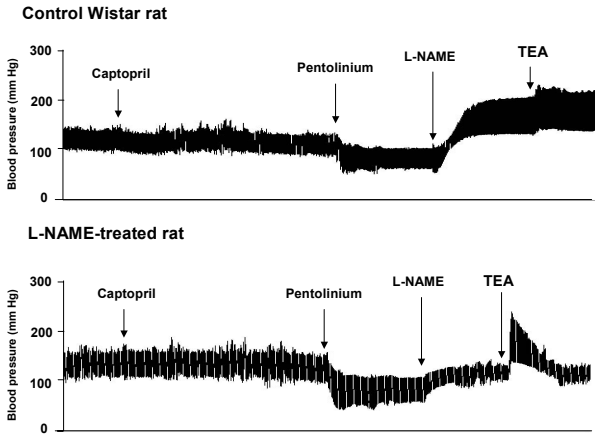


**Fig. 9.** Time course of BP changes elicited by tetraethylammonium (TEA, 15 mg/kg i.v.) in control (upper panel) and chronically L-NAME-treated Wistar rat (lower panel).

Nevertheless, our further experiments indicated that $Ca^{2+}$-activated potassium channels are controlled not only by EDHF but also by NO. BP elevation elicited by TEA injection in conscious captopril- and pentolinium-pretreated rats was considerably greater if TEA was administered prior to the inhibition of NO synthase (NOS) than following NOS inhibition (+54±6 vs. 86±3 mm Hg), although the combination of both drugs elicited similar BP rise irrespective of drug sequence (+104±5 vs. 98±3 mm Hg) (Dobešová *et al*., unpublished data). Similar relationship was also demonstrated in isolated femoral arteries (Lísková *et al*., unpublished data). Our results are in agreement with the proposal of complementary participation of NO and EDHF in vascular relaxation (Bauersachs *et al.* 1996, Fleming *et al.* 1996) when lowering of NO production enhances the influence of EDHF on $Ca^{2+}$-activated potassium channels in order to achieve sufficient membrane hyperpolarization. This replacement of vasodilator role of NO by EDHF is more pronounced after chronic than acute NOS inhibition (Gerová 1999, Desai *et al.* 2006). The importance of EDHF replacing the missing NO was further demonstrated in eNOS knockout mice (Waldron *et al.* 1999, Huang *et al.* 2000, Brandes *et al.* 2000, Scotland *et al.* 2005, Lidington *et al.* 2007).

## The role of inhibitory G proteins in the control of L-VDCC

We have paid a lot of attention to the possible mechanisms by which noradrenergic stimulation can cause nifedipine-sensitive tonic vascular contraction. A part of our effort was directed to the pathways involving inhibitory G ($G_i$) proteins. Figure 4 indicates that NE can bind to distinct alpha-adrenoceptors and activate $G_i$ protein pathway, leading thus to the attenuation of cAMP formation. We therefore used *in vivo* administration of pertussis toxin (PTX) for long-term $G_i$ protein inactivation (Lísková *et al.* 2007, Zemančíková *et al.* 2008, Čačányiová *et al.* 2008, Pintérová *et al*. 2006, 2007a,b, Zicha *et al.* 2006b). PTX pretreatment attenuated NE-induced contractile response of isolated arteries. In the presence of nifedipine there was no difference in *in vitro* contractile response to NE between femoral arteries isolated from intact or PTX-treated Wistar rats (Lísková *et al.* 2007). Moderate attenuation of contractile response to exogenous or endogenous NE was also observed in aorta or mesenteric artery of PTX-treated SHR (Zemančíková *et al.* 2008).

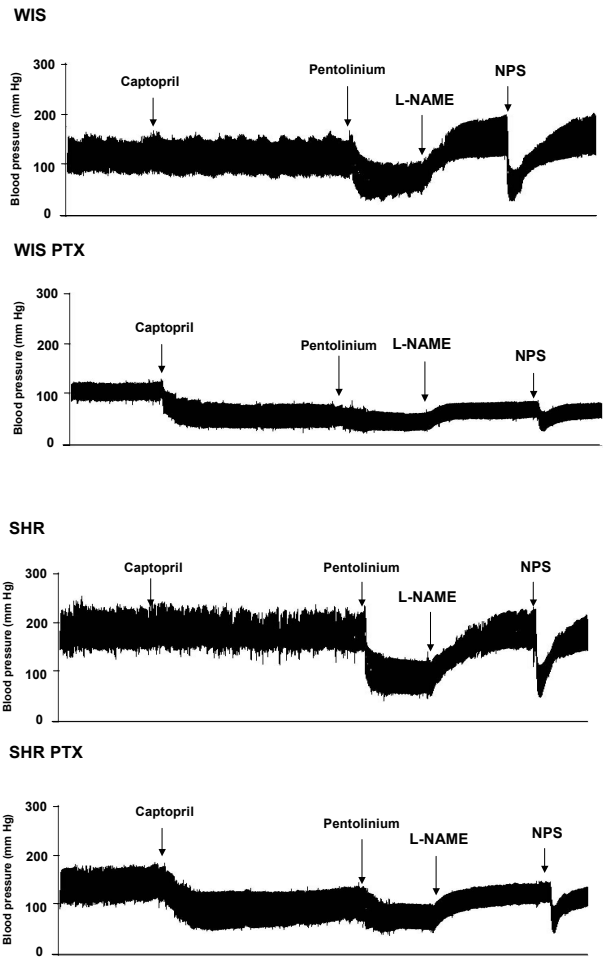


**Fig. 10.** Time course of BP changes induced by sequential blockade of renin-angiotensin system (captopril, 10 mg/kg i.v.), sympathetic nervous system (pentolinium, 5 mg/kg i.v.) and NO synthase (L-NAME, 30 mg/kg i.v.) in Wistar (WIS), PTX-treated Wistar, SHR and PTX-treated SHR animals. NPS – sodium nitroprusside.

**Table 1.** Blood pressure changes induced by acute administration of ganglionic blocker pentolinium (5 mg/kg i.v.) or ACE inhibitor captopril (10 mg/kg i.v.) to 14-week-old spontaneously hypertensive rats (SHR) and normotensive Wistar rats (WIS) before or three days after the treatment with pertussis toxin (PTX, 10 μg/kg i.v.).

| Drug | | BP change (mm Hg) | | BP change (mm Hg) |
|---|---|---|---|---|
| *Pentolinium* | SHR | –82.4±4.4 | WIS | –44.4±2.8 * |
| | SHR-PTX | –16.6±1.7 # | WIS-PTX | –14.4±2.2 # |
| *Captopril* | SHR | –3.5±2.2 | WIS | –5.9±2.4 |
| | SHR-PTX | –42.4±2.6 # | WIS-PTX | –36.0±4.6 # |

Data are means ± S.E.M. (n = 6-8). Significantly different ($p<0.05$): * from SHR, # from intact animals untreated with PTX.

It is evident from Figure 10 that in both SHR and Wistar rats PTX pretreatment considerably diminished BP reduction elicited by pentolinium injection (Table 1). The missing noradrenergic vasoconstriction was replaced by substantial activation of circulating angiotensin II as evidenced by a rapid pronounced BP fall after acute captopril administration (Fig. 10, Table 1) (Zicha *et al.* 2006b). Our further *in vivo* experiments indicated that chronic $G_i$ protein inactivation substantially attenuated BP response to exogenous NE, shifting the respective dose-response curve to the right by at least one order of magnitude. PTX-induced changes were similar to the effects of acute nifedipine administration (Pintérová *et al.* 2007c).

In these experiments we have also noticed that PTX pretreatment caused a considerable attenuation of L-NAME induced BP rise which was present especially in Wistar rats. The attenuation of BP response to acute NO deficiency in PTX-treated rats was further supported by their diminished BP lowering response to NO donor (sodium nitroprusside) administered during acute NOS inhibition (Fig. 10). The finding of attenuated BP response to L-NAME in PTX-pretreated rats suggested an interesting hypothesis according to which the enhanced cAMP production (due to inactivation of $G_i$ protein pathway) might diminish $Ca^{2+}$ influx elicited by the absence of cGMP following acute NOS inhibition. To evaluate this hypothesis we have performed some experiments on Wistar rats in which cAMP level was increased by isoprenaline infusion (β-adrenoceptor agonist) or decreased by propranolol administration (β-adrenoceptor antagonist). Figure 11 documents that increased cAMP formation following isoprenaline infusion almost abolished BP response to L-NAME and this BP response was normalized by propranolol injection, although isoprenaline infusion continued. It is also evident that β-adrenoceptor blockade not only permitted BP rise elicited by cGMP deficiency but also restored the sensitivity of BP of NO-deficient rats to sodium nitroprusside injection (Fig. 11). Thus cyclic nucleotides (both cAMP and cGMP) are involved in the control of $Ca^{2+}$ influx by inhibiting the permeability of L-VDCC for $Ca^{2+}$ ions (Pintérová *et al.* 2009a,b).

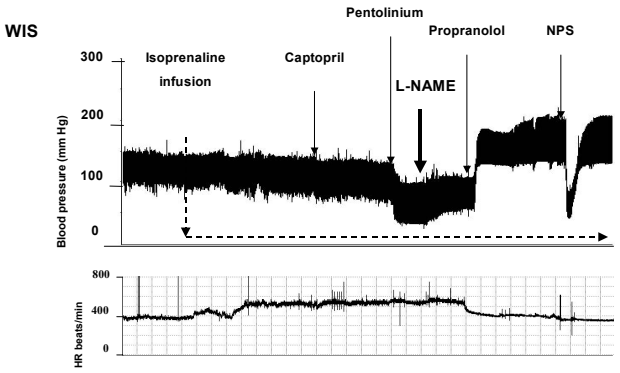


**Fig. 11.** The effect of β-adrenoceptor agonist isoprenaline infusion (100 ng/kg/min i.v.) on L-NAME-induced BP changes in Wistar rat and the restoration of L-NAME-induced BP rise by injection of β-adrenoceptor antagonist propranolol (1 mg/kg i.v.).

However, there must be additional mechanism(s) controlling the opening of L-VDCC in the absence of cyclic nucleotides. Such mechanism(s) might be important for the maintenance of high BP because SHR had elevated BP compared to Wistar rats even if they were subjected to acute combined blockade of RAS, SNS and NOS (MAP: 180±4 vs. 116±9 mm Hg, $p<0.001$), i.e. under the conditions when both cGMP and cAMP levels are expected to be low. This BP difference was almost entirely dependent upon $Ca^{2+}$ influx through L-VDCC because it was minimized by acute nifedipine administration (99±6 vs. 83±5 mm Hg, $p<0.10$) which lowered BP substantially more in SHR than in Wistar rats (–80±8 vs. –33±10 mm Hg, $p<0.001$). The mechanisms

underlying different L-VDCC opening in genetically hypertensive and normotensive rats remain to be investigated.

## The contribution of $\alpha_2$- and $\alpha_1$-adrenoceptors to NE-induced stimulation of $Ca^{2+}$ influx

Our attention was also focused on the role of particular α-adrenoceptors because we wanted to distinguish the contribution of $\alpha_2$- and $\alpha_1$-adrenoceptors to NE-stimulated $Ca^{2+}$ influx through L-VDCC (Fig. 4). Previous studies (van Meel *et al.* 1983, Kazda *et al.* 1985) indicated a greater impact of calcium antagonists on $\alpha_2$- than on $\alpha_1$-adrenergic vasoconstriction and this effects was even more pronounced in SHR compared to Wistar-Kyoto (WKY) rats. Our preliminary *in vitro* experiments carried out in femoral arteries isolated from SHR (Líšková *et al*., unpublished data) revealed major differences in the effects of L-VDCC closure (following nifedipine administration or PTX treatment) on the contraction elicited either by $\alpha_2$- or $\alpha_1$-adrenoceptor agonists (clonidine or phenylephrine) (Fig. 12).

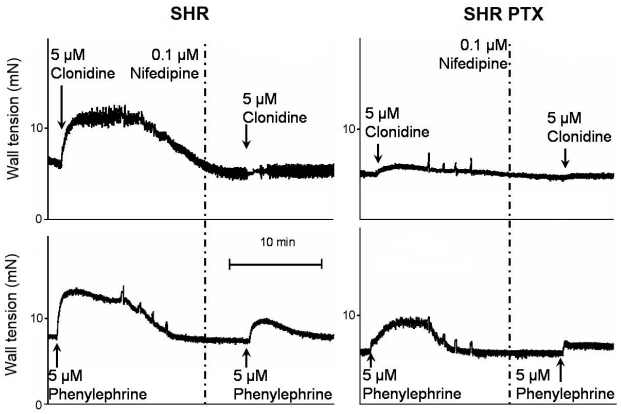


**Fig. 12.** The effect of nifedipine (NIF) on the contraction elicited by $\alpha_2$-adrenoceptor agonist clonidine (upper panels) or $\alpha_1$-adrenoceptor agonist phenylephrine (lower panels) in deendothelized femoral arteries isolated from control SHR (left panels) and SHR pretreated with pertussis toxin (20 μg/kg i.v. – 3 days before) (PTX SHR, right panels).

Clonidine-induced contraction was abolished not only by nifedipine presence in the incubation medium but also by PTX pretreatment (Fig. 12, upper panels). This indicates that $\alpha_2$-adrenergic vasoconstriction is entirely based upon $Ca^{2+}$ influx through L-VDCC which can be closed either by dihydropyridine antagonist nifedipine or following PTX-induced inactivation of Gi proteins through cAMP-mediated mechanisms (see above). On the other hand, phenylephrine-induced contraction was only attenuated by nifedipine or PTX, but the combination of these two interventions minimized contractile response elicited by phenylephrine (Fig. 12, lower panels). Although *in vivo* determined NE dose-response curves were very similar in SHR and WKY rats and nifedipine shifted these curves to the right in both rat strains (Pintérová *et al*. 2007c), there might be considerable differences in $\alpha_1$- and $\alpha_2$-adrenergic components of NE-induced BP responses. We therefore determined NE dose-response curves in the presence of $\alpha_1$- or $\alpha_2$-adrenoceptor antagonists (prazosin or yohimbine), which preserved selective $\alpha_2$- or $\alpha_1$-adrenergic components of BP response to NE (Behuliak *et al.* 2009).

Figure 13 shows that prazosin but not yohimbine lowered maximal BP response to NE in both strains suggesting a generally greater importance of $\alpha_1$- than $\alpha_2$-adrenergic vasoconstriction for BP response to NE. This is especially true in normotensive WKY rats, whereas the ratio between $\alpha_1$- and $\alpha_2$-adrenergic components of BP response to NE was reduced in SHR. In fact, the effect of yohimbine (rightward shift of NE dose-response curve) was more pronounced in SHR than in WKY rats, indicating that $\alpha_2$-adrenergic vasoconstriction might be enhanced in genetic hypertension. This is also in agreement with lower maximal BP response to NE seen in WKY pretreated with $\alpha_1$-adrenoceptor antagonist prazosin. It should also be noted that both $\alpha_1$- and $\alpha_2$-adrenoceptor antagonists reduced considerably the sensitivity of SHR to NE (increased $ED_{50}$), but these changes were absent in WKY rats (Fig. 13). The reasons for these strain-dependent changes in NE sensitivity remain to be elucidated.

Our further investigations revealed that in both rat strains acute nifedipine administration lowered the sensitivity to NE by one order of concentration without significant changes in maximal BP response to NE (Fig. 14, upper panels). Similar nifedipine effect was also observed when $\alpha_1$-adrenergic vasoconstriction was studied (in the presence of $\alpha_2$-adrenoceptor blocker yohimbine) (Fig. 14, lower panels). On the other hand, nifedipine lowered not only sensitivity to NE but also maximal BP response to NE (more in SHR than WKY) if we studied $\alpha_2$-adrenergic vasoconstriction (in the presence of $\alpha_1$-adrenoceptor blocker prazosin) (Fig. 14, middle panels). The above data confirm considerable importance of $\alpha_2$-adrenergic vasoconstriction in SHR as it was previously suggested by Kazda *et al.* (1985), Pettinger *et al.* (1982), Sanchez *et al.* (1986) and others.

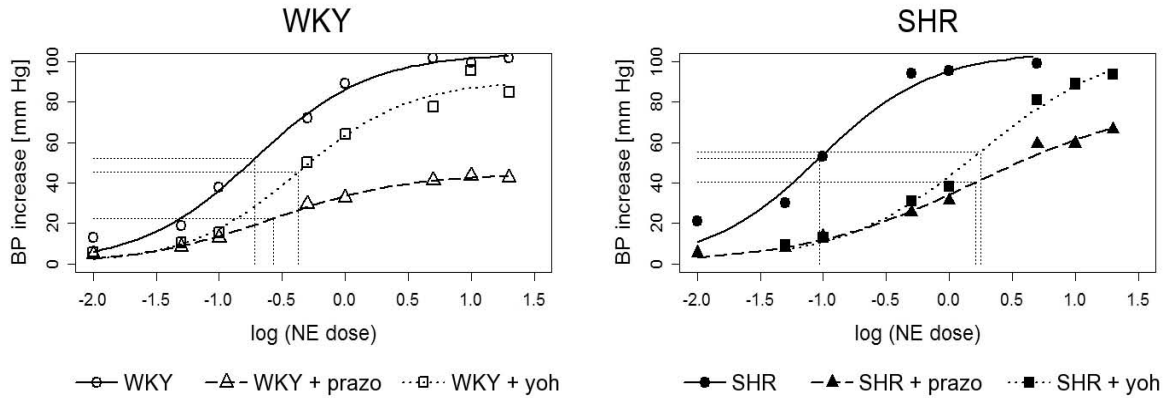


**Fig. 13.** The dose-response curves constructed from BP responses to NE recorded in conscious WKY (left panel) and SHR (right panel) rats pretreated with captopril (10 mg/kg i.v.) and pentolinium (5 mg/kg i.v.). NE dose-response curves were also determined in the presence of antagonists of either $\alpha_1$- or $\alpha_2$-adrenoceptors (prazosin or yohimbine, 1 mg/kg i.v. each). Thin dotted lines indicate half-maximal BP response (mm Hg) and $ED_{50}$ (log NE dose).

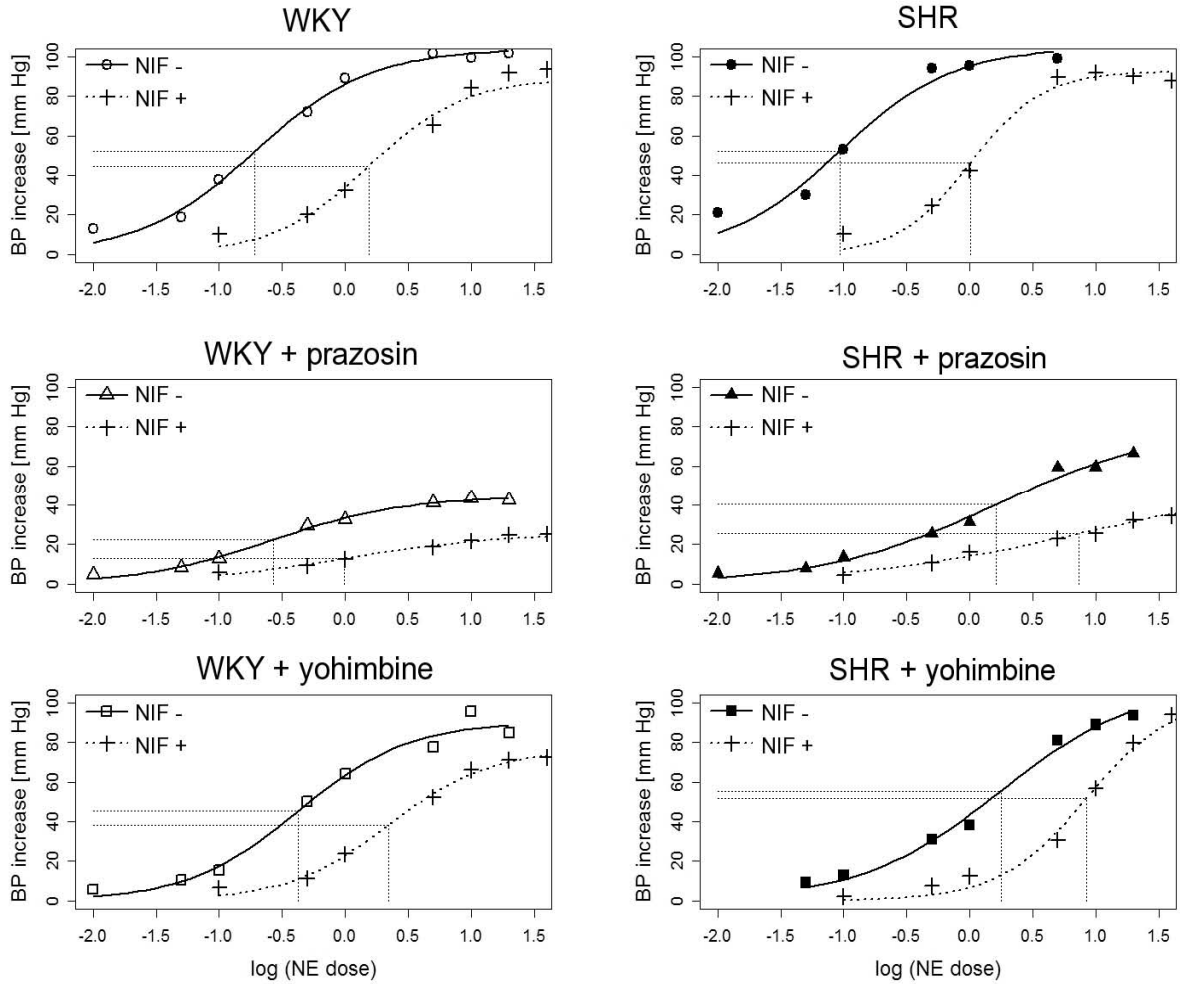


**Fig. 14.** The influence of acute nifedipine administration (0.4 mg/kg. i.v.) on NE dose-response curves measured in conscious WKY (left panels) and SHR (right panels) rats pretreated with captopril and pentolinium. NE dose-response curves were also determined in the presence of prazosin (middle panels) or yohimbine (lower panels) to evaluate $\alpha_2$- or $\alpha_1$-adrenergic components of BP response to NE.

## EDCF and $Ca^{2+}$-activated $Cl^-$ channels – open chapters in hypertension research

It remains an open question whether cyclic nucleotides influence L-VDCC directly or indirectly through the modulation of $Ca^{2+}$-activated $K^+$ and $Cl^-$ channels resulting in membrane potential changes (Fig. 4). Major augmentation of $Ca^{2+}$-activated $K^+$ and $Cl^-$ transport in arteries of rats with various forms of experimental hypertension was already described more than 30 years ago (Jones 1974). There is some evidence that NO and/or EDHF can enhance the activity of $Ca^{2+}$-activated $K^+$ channels leading thus to membrane hyperpolarization. On the other hand, there is only scarce information on the role and control of $Ca^{2+}$-activated $Cl^-$ channels the activation of which may lead to membrane depolarization and $Ca^{2+}$ influx through L-VDCC (Jackson 2000). Our recent observations point to a quite interesting relationship between NE-induced vasoconstriction and the so called endothelium-derived contracting factor (EDCF), which seems to be prostaglandin $H_2$ acting on thromboxane-prostanoid (TP) receptors of the vascular smooth muscle (Vanhoutte *et al.* 2005, Vanhoutte and Tang 2008). The role of EDCF in different forms of experimental hypertension is largely unknown, although its presence has been demonstrated in genetic hypertension of SHR (Lüscher and Vanjhoutte 1986, Rapoport and Williams 1996), salt hypertension of Dahl rats (Zhu *et al.* 1999, Zhou *et al.* 2001) and NO-deficient hypertension of L-NAME-treated rats (Zanchi *et al.* 1995, Paulis *et al.* 2008).

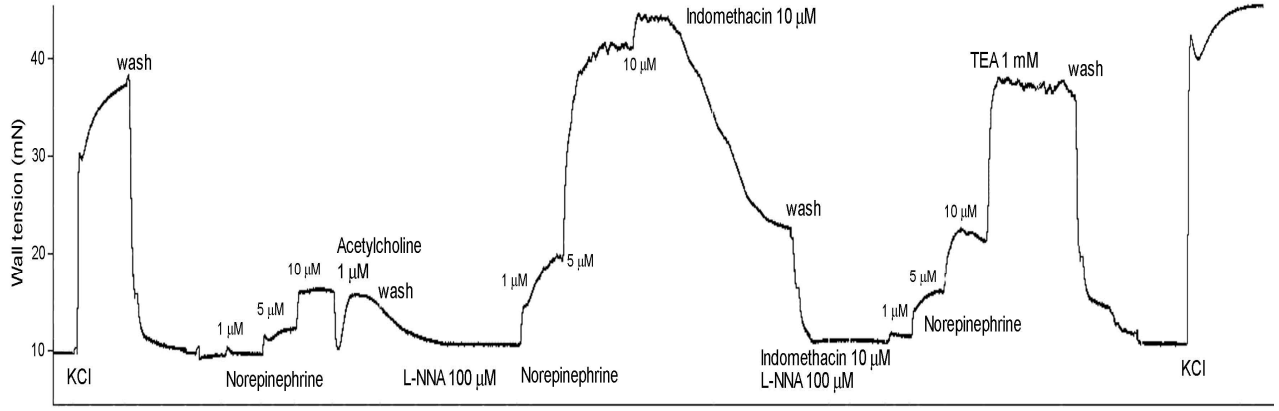


**Fig. 15.** NE dose-response determined in isolated femoral artery prior to and after L-$N^G$-nitro-L-arginine addition (L-NNA, 100 μmol/l). Note the therapeutic effect of indomethacin (10 μmol/l) when added to the already developed contraction as well as its preventive effect when applied prior to NE. The blockade of $Ca^{2+}$-activated $K^+$ channels by tetraethylammonium (TEA, 1 mmol/l) fully restored the augmented contractile response to NE (seen after NO synthase blockade by L-NNA).

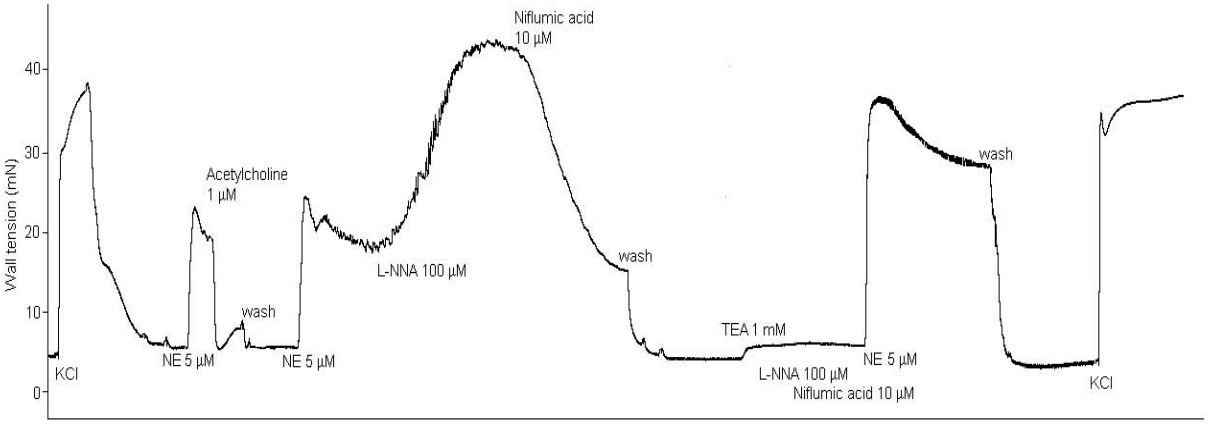


**Fig. 16.** The therapeutic and preventive effects of niflumic acid (10 μmol/l) on NE-induced contraction of femoral artery pretreated with L-NNA (100 μmol/l). Note that the addition of tetraethylammonium (TEA, 1 mmol/l) almost fully restored contractile response to NE attenuated by niflumic acid.

Figure 15 shows that under the conditions of NO deficiency a major part of NE-induced contraction of isolated femoral artery can be prevented by cyclooxygenase inhibitors (e.g. indomethacin). It seems that not only high doses of acetylcholine (Lüscher and Vanhoutte 1986, Koga *et al.* 1989, Watt and Thurston 1989) but also high NE doses augment the production of EDCF in rat conduit arteries. It is important to note that indomethacin does not only prevent NE-induced arterial contraction but can also rapidly abolish the already developed contraction (Fig. 15). The extent of this indomethacin-induced reduction of vascular wall tension is comparable to the effects of nifedipine. Similar effects on vascular wall tension of NE-precontracted artery can also be achieved by the addition of niflumic acid (Fig. 16). Niflumic acid is another cyclooxygenase inhibitor which was, however, reported to block $Ca^{2+}$-activated $Cl^-$ channels (Criddle *et al.* 1997, He and Tabrizchi 1997). Our recent data suggest that the relaxing effects of both indomethacin and niflumic acid result from the closure of L-VDCC which is parallel to the activation of $Ca^{2+}$-activated $K^+$ channels, because the blockade of these channels by TEA fully restores the contractile response to NE even in the presence of COX inhibitors (Fig. 16) (Líšková *et al.* 2009). Although we do not know whether the above mechanism is also pertinent to small resistance vessels, the extent of EDCF contribution to NE-induced arterial contraction as well as the possible relationship of EDCF to the control of L-VDCC make this research topic highly attractive.

## Conclusions

To elucidate the above abnormalities in hypertensive rats our future research should be focused on detailed mechanisms by which cyclic nucleotides modify the opening of L-VDCC, and to the role exerted by calcium-activated $K^+$ and $Cl^-$ channels in the control of membrane potential responsible for L-VDCC opening. Another important issue will be the role of endothelium-derived contracting factor in adrenergic vasoconstriction as well as the possible relation of this factor to the control of calcium-activated $Cl^-$ channels.

## Conflict of Interest

There is no conflict of interest.

## Acknowledgements

The experimental work described in this review has been partially supported by AV0Z 50110509, and research grants of GA CR 305/08/0139 and 305/09/0336, IGA AV CR IAA500110902 and 1M0510 (Ministry of Education of the Czech Republic).

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