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DIPLOMA THESIS

L-serine Induced Effects on Blood Pressure in Normotensive and Hypertensive Rats: The Influence of Anesthesia.

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Pronouncement

I declare that I have developed this thesis “L-serine Induced Effects on Blood Pressure in Normotensive and Hypertensive Rats: The Influence of Anesthesia.” independently, using only literature listed in references.

Prague, Signature

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Summary

Anesthetics cause profound alterations in respiratory and cardiovascular systems. Our experiments demonstrated that different anesthetics caused different changes in blood pressure regulating components. The role of particular BP regulating systems was disclosed by their selective inhibition - sympathetic nervous system blocked by pentolinium (peripheral ganglionic blockade), renin-angiotensin system by captopril (angiotensin converting enzyme blocker) and nitric oxide production by L-NAME (nitric oxide synthase blocker). Components of blood pressure regulating mechanisms in conscious normotensive Wistar rats and spontaneously hypertensive rats were compared with four different groups of anesthetized rats by pentobarbital, ketamine-xylazine, chloralose-urethane and isoflurane. Each anesthesia caused different hemodynamic changes. If hemodynamic conditions should be similar to conscious rats, the most suitable anesthetic is pentobarbital.

L-serine-induced effects represent endothelium-derived hyperpolarizing factor (EDHF)-mediated response, which is a type of endothelium-dependent regulation of vascular tone, independent of nitric oxide and prostacyclin production. Pronounced L-serine effects on blood pressure were shown in NO-deficient type of hypertension. Our study demonstrated its pronounced effects in Dahl rats with salt-induced hypertension. In conscious animals, the type of L-serine induced effects depends whether sympathetic nervous system, renin-angiotensin system or nitric oxide production are blocked.

Keywords: blood pressure, anesthesia, sympathetic nervous system, renin-angiotensin system, nitric oxide, L-serine, endothelium-derived hyperpolarizing factor**,** salt hypertension

Abstrakt

Anestetika výrazně ovlivňují respirační a kardiovaskulární soustavu. Naše pokusy ukázaly, že různá aneststetika působí na jednotlivé komponenty regulující krevní tlak odlišně. Úloha jednotlivých systémů regulujících krevní tlak byla určena pomocí selektivních inhibitorů - sympatický nervový systém byl zablokován pomocí pentolinia (periferní gangliová blokáda), renin-angiotenzinový systém pomocí captoprilu (inhibitor angiotenzin konvertujícího enzymu) a produkce oxidu dusnatého pomocí L-NAME (inhibice syntázy oxidu dusnatého). Jednotlivé komponenty regulující krevní tlak u normotenzních Wistar potkanů a spontánně hypertenzních potkanů byli porovnané se čtyřmi skupinami potkanů anestezovaných pentobarbitalem, ketaminem-xylazinem, chloralozou-uretanem a izofluranem. Každé z použitých anestetik způsobilo odlišné hemodynamické změny. Pro potřeby měření za podmínek co nejpodobnějších bdělému stavu, pentobarbitalová anestézie se jeví jako nejvhodnejší.

L-serinem-indukované účinky na krevní tlak představují odpověď zprostředkovanou endoteliálním hyperpolarizačním faktorem, což je na endotelu závislá regulace cévního tonu, která je nezávislá na produkci oxidu dusnatého a prostacyklinu. Výrazné účinky L-serinu byly prokázány u NO-deficientní hypertenze. Naše výsledky ukázaly jeho zvýšené účinky u Dahlových potkanů s vyvinutou solnou hypertenzí. U bdělých zvířat závisí účinky L-serinu na tom, zda je inhibován sympatický nervové systém, renin-angitenzinový systém nebo systém oxidu dusnatého.

Klíčová slova: krevní tlak, anestézie, sympatický nervový systém, renin-angiotenzinový systém, oxid dusnatý, L-serine, endotelialní hyperpolarizační faktor, solní hypertenze

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List of Abbreviations

ACE	angiotensin converting enzyme
BK_{Ca}	large conductance Ca^{2+}-activated K^+ channels
BP	blood pressure
cGMP	cyclic guanosine monophosphate
CNS	central nervous system
COX	cyclooxygenase
CU	chloralose-urethane
DR	Dahl salt-resistant rat
DS	Dahl salt-sensitive rat
EDHF	endothelium-derived hyperpolarizing factor
EDRF	endothelium-derived relaxing factor
EETs	epoxyeicosatrienoic acids
$GABA_A$	gamma-aminobutyric acid receptors
IK_{Ca}	intermediate conductance K^+ channels
ISO	isoflurane
K_{ATP}	ATP-sensitive K^+ channels
KX	ketamine-xylazine
L-NAME	L-N^G-Nitroarginine methyl ester
L-VDCC	L-type voltage-gated Ca^{2+} channels
NO	nitric oxide
NOS	nitric oxide synthase
NMDA	N-methyl-D-aspartate receptors
MLC	myosin light chain
P	pentobarbital
PGI_2	prostacyclin
RAS	renin-angiotensin system
SHR	spontaneously hypertensive rat
SK_{Ca}	small conductance K^+ channels
TRP	transient receptor channels
VSMC	vascular smooth muscle cells

1. Introduction

Arterial hypertension affects 20-30% of adult population in the developed societies and belongs to the main risk factors responsible for cardiovascular diseases. Elevated blood pressure usually occurs without any symptoms, but the complications are final result of hypertension - referred as end-organ damage. A considerable number of effective antihypertensive drugs are available, but the reliable blood pressure control is reached only in a small part of hypertensive patients. The reason of elevated blood pressure is clear only in 5-10% of patients, suffering mainly from the secondary hypertension with specific disorder of particular organs or blood vessels (the kidneys, the adrenal glands or aorta). Far more common is the primary or essential hypertension, a multifactorial disorder which evolves from the interactions of multiple genes with environmental factors. Vascular endothelium maintains homeostasis of the vascular wall and largely contributes to the blood pressure maintenance. Endothelium may also be influenced by multiple environmental and genetic factors. Its dysfunction is associated with a reduction of nitric oxide bioavailability, prostaglandin production and other vasodilator mediators. This remaining nitric oxide and prostaglandin independent vasodilatation has been partly attributed to the endothelium-derived hyperpolarizing factor (EDHF), the knowledge of it is being very limited so far and therefore concerns our study.

Anesthesia cause profound alterations in the respiratory and cardiovascular systems. The determination of anesthesia effects on distinct blood pressure regulating components (such as sympathetic nervous system, renin-angiotensin system and nitric oxide system) in rats compared to conscious rats is insufficiently explored. To facilitate the analysis of data obtained in anesthetized rats, our study deals with the comparison of four different anesthetics on blood pressure regulating components in rats.

2. Blood pressure control

Blood pressure (BP) is a function of vascular resistance and cardiac output, two variables controlled by the autonomic nervous system. Cardiac output is dependent on end-diastolic volume, myocardial contractility and heart rate, which are under sympathetic and parasympathetic control. Vascular resistance is mainly under sympathetic control (Guyenet, 2006). Autonomic nervous system maintains BP and buffers rapid changes in arterial pressure via baroreflex response. The changes of systemic BP are sensed by the arterial baroreceptors, located in carotid sinus and aortic arch. Baroreceptors respond to the change in stretch of the vascular wall and trigger reflex adjustments. The rise in BP causes a decrease of heart rate, cardiac contractility, vascular resistance and venous return. These changes are induced by sympathetic inhibition and parasympathetic activation. Conversely, decrease in BP increases sympathetic and decreases parasympathetic nerve activity to restore BP (Lanfranchi and Somers, 2002).

In the regulation of BP is also involved the renin-angiotensin system, endothelium-dependent mechanisms and myogenic response of resistance arteries. Cardiovascular homeostasis is dependent on the complex interaction between all above mentioned factors.

2.1 The sympathetic control

The autonomic nervous system links central nervous system (CNS) with visceral effectors. Sympathetic and parasympathetic arms of this system are made up of cholinergic neurons located within the CNS and innervate sympathetic ganglia, visceral organs and enteric ganglionic neural network. Cardiovascular targets (heart, blood vessels, kidneys and adrenal medulla) are controlled by primary noradrenergic neurons located in sympathetic ganglia. The activity of sympathetic nervous system has a dominant role in both short-term and long-term BP control (Guyenet, 2006). The output of arterial baroreceptors terminates within the nucleus tractus solitarii in caudal medulla, which is responsible for the short-term regulation of arterial pressure. The anteroventral region of the third ventricle of the hypothalamus was detected as a crucial region in the long-term regulation of sympathetic activity. This region is able to sense plasma concentrations of several hormones and sends the information to paraventricular nucleus of the hypothalamus. Neurons from this nucleus are connected to the sympathetic premotor neurons in the rostral ventrolateral medulla (Osborn, 2005). This part of medulla contains tonically active neurons that provide a major source of tonic excitatory drive to the preganglionic neurons in the spinal cord and control sympathetic nerve discharge (Dampney et al., 2002). These neurons show ongoing activity at rest and discharge in bursts that are highly synchronized with the arterial pulse and respiration (Guyenet, 2006).

Sympathetic regulation of vascular contractility is mediated via α- and β-adrenoreceptors. The activation of α-adrenoreceptors causes vasoconstriction, whereas β-adrenoreceptors mediate vasodilatation. Both subtypes occur presynaptically and postsynaptically. They are affected differentially by adrenaline coming from the blood or noradrenaline coming from sympathetic nerve terminals. Vascular tone results from the simultaneous activation of numerous receptors that are differentially influenced in various vascular areas. Potential role of some adrenoreceptor subtypes must be carefully considered (Guimarães and Moura, 2001).

2.2 The renin-angiotensin system

The renin-angiotensin system (RAS) activation is dependent on the synthesis and release of renin from juxtaglomerular cells of the renal afferent arteriole. In the blood renin cleaves angiotensinogen released from the liver to form angiotensin I. Angiotensin converting enzyme (ACE) hydrolyzes inactive angiotensin I into the biologically active angiotensin II. Angiotensin II induces vasoconstriction by interacting with angiotensin type 1 receptors located on vascular smooth muscle cells (Paul et al., 2006). Angiotensinogen is also synthesized in most parts of the brain and angiotensin II (considered as a neuropeptide) may influence BP regulation in different brain sites (McKinley et al., 2003). Peripheral angiotensin II has a major influence on the long-term regulation of sympathetic activity. Although it does not cross the blood-brain barrier, angiotensin II affects sympathetic nervous system through the circumventricular organs (Dampney et al., 2002). In addition to cleavage of angiotensin II ACE also metabolizes vasodilatator bradykinin, producing thus vasoconstriction by two different mechanisms (Figure 1).

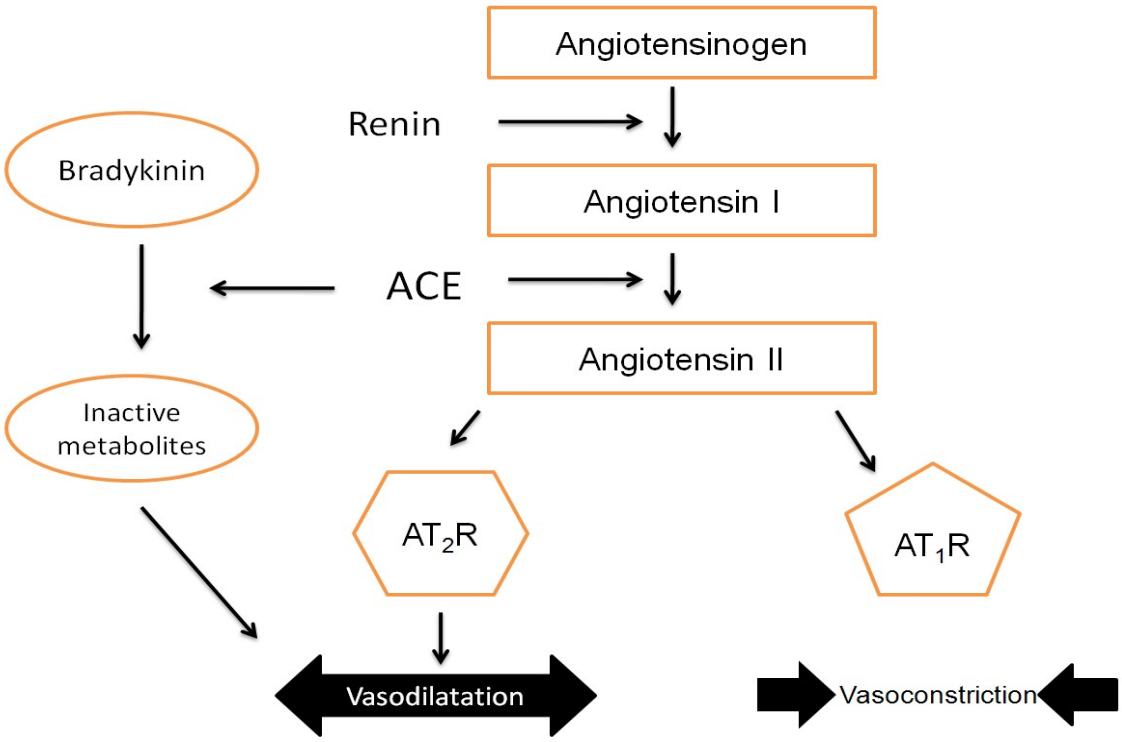

Figure 1. Schematic representation of the renin-angiotensin system. (adapted from Nguyen and Touyz, 2011).

Renin-angiotensin system integrates complex processes such as cardiovascular function, aldosterone secretion, renal tubular Na^+ reabsorption, thirst, smooth muscle hypertrophy and organ fibrosis to maintain BP and the perfusion of vital organs. Most of the vasoactive effects of angiotensin II occur via AT_1 receptors which are widely distributed in all organs (Nguyen and Touyz, 2011). Its effects depend on the cellular expression and activation of these receptors. Angiotensin II also binds to AT_2 receptors, which act in the opposite direction than AT_1 receptors (Mehta and Griendling, 2007).

2.3 Endothelium-dependent vasodilatation

The endothelium regulates vascular tone through the release of soluble mediators that relax vascular smooth muscle. Mechanisms responsible for endothelium-dependent smooth muscle relaxation are dependent on the increase of intracellular Ca^{2+} concentration in endothelial cells. This is generated either by local hormones such as acetylcholine, bradykinin, substance P, histamine or by shear stress. Endothelium releases factors such as nitric oxide, prostacyclin (PGI_2), epoxyeicosatrienoic acids (EETs), hydrogen peroxide, carbon monoxide or initiate endothelium-derived hyperpolarizing factor (EDHF) pathway (Edwards et al., 2010).

Endothelial nitric oxide (NO) is produced when L-arginine is transformed to L-citrulline through the catalysis by nitric oxide synthase (NOS) which requires Ca^{2+} for its activation. Endothelial NOS is bound to caveolin 1 in the caveolae (microdomains of the plasma membrane) and this interaction is regulated by Ca^{2+} binding calmodulin. Caveolin 1 inhibits NOS activity and this interaction is broken by increased Ca^{2+} so that NOS is activated for NO synthesis (Rath et al., 2009). NO diffuses from endothelial cells to vascular smooth muscle cells (VSMC). NO activates soluble guanylyl cyclase and produces cyclic guanosine monophosphate (cGMP) in VSMC. The accumulation of cGMP causes the activation of cGMP-dependent protein kinase, which decreases the sensitivity of contractile apparatus to Ca^{2+}, resulting in smooth muscle relaxation. Another possible pathway of NO-caused vasodilatation is NO-induced opening of K^+ channels, which may produce hyperpolarization of VSMC (Tare et al., 1990). The synthesis of NO by NOS is inhibited by L-arginine analogs N^G-monomethyl-L-arginine, N^G-nitro-L-arginine, L-NA methylester and dimethylarginine. Methylene blue or oxyhemoglobin are used to inhibit the activity of soluble guanylyl cyclase.

Prostacyclin (PGI_2) is a product of arachidonic acid metabolism by cyclooxygenase (COX) pathway. When PGI_2 is released from endothelium, it stimulates G_S-coupled IP receptors with subsequent activation of adenylyl cyclase and elevation of intracellular cAMP. Myocyte cAMP activates protein kinases, which activate K^+ channels, leading thus to vasodilatation (Edwards et al., 2010). Alternative pathway of arachidonic acid metabolism involves cytochrome P450 which generates EETs. EETs also act as vasodilatation agents through K^+ channels opening or transient receptor channels (TRP)

activation. Vanilloid TRP 4 receptor might be responsible for the vasodilatation induced by EETs (Campbell and Fleming, 2010).

2.3.1 Endothelium-derived hyperpolarizing factor

There are several definitions of the term endothelium-derived hyperpolarising factor (EDHF). One of them is that hyperpolarization attributed to EDHF is mimicked by certain K^+ channel agonists and it is not affected by the inhibitors of NOS or COX (Ozkor and Quyyumi, 2011). Another definition is that EDHF is a pathway which initiate vascular myocyte hyperpolarization and can be blocked by scyllatoxin or UCL 1684 (small conductance K^+ channels - SK_{Ca}) + TRAM 34 (intermediate conductance K^+ channels - IK_{Ca}). Experiments aimed on EDHF are performed after the inhibition of NO and PGI_2 production, because these agents can act similarly as EDHF (Edwards et al., 2010). Under the physiological conditions EDHF represents another vasodilatation mechanism, which functions as the back-up system for NO pathway. Altered EDHF function may be linked with various pathological conditions (Yang et al., 2007).

Before 1980 it was well known to pharmacologists that acetylcholine was vasodilatator *in vivo*, but it contracted isolated vascular preparations *in vitro*. This problem was solved by Furchgott and Zawadzki (1980) by careful preparation of arterial strips to preserve their delicate endothelial layer. These experiments showed that the relaxant effect was mediated by the release of an extracellular agent named endothelium-derived relaxing factor (EDRF). Seven years later, EDRF was characterized as NO (Ignarro et al, 1987). Bolton et al. (1984) demonstrated that carbachol (non-selective cholinergic agonist, more stable than acetylcholine) caused direct contraction of the myocytes, but also endothelium-dependent relaxation and myocyte hyperpolarization. It was assumed that this hypothetical factor was the same as the described EDRF, but Kuriyama and Suzuki (1978) have shown that glyceryl trinitrate relaxed blood vessels without generating myocyte hyperpolarization. Later Chen et al. (1988) showed that endothelium-dependent hyperpolarization following the exposure to acetylcholine was unaffected by indomethacin or by the blockade of NO pathway.

The term EDHF was introduced in 1987 to describe hyperpolarizations not associated with NO or prostacyclin pathway. At that time, it was assumed that EDHF opens myocyte K^+ channels. Pharmacological breakthrough was that EDHF-mediated

myocyte hyperpolarizations were unaffected by either apamin (SK_{Ca} blocker) or charybdotoxin (assumed BK_{Ca} blocker) alone but they were totally abolished by a mixture of these two toxins. Iberiotoxin, more selective BK_{Ca} blocker, could not substitute for charybdotoxin (Zygmunt and Högestätt, 1996). It was recognized that charybdotoxin could not only block BK_{Ca} but also IK_{Ca} (Garcia et al., 1995). Edwards and Weston (1998) showed that SK_{Ca} and IK_{Ca} channels were located on endothelium and were activated by acetylcholine. They proposed that K^+ increase in myoendothelial space may result in the activation of Na^+/K^+-ATPase and inward rectifying K^+ channels, which leads to the hyperpolarization of smooth muscle cells. Another possibility was suggested that gap junctional communication is responsible for this hyperpolarization (Griffith et al., 2004).

At the beginning, it was assumed that EDHF action is exerted by some entity or a "factor". Nowadays, it seems that no factor is necessary and the investigators rather use the term "endothelium-dependent hyperpolarization" (Garland et al., 2011). The classical EDHF pathway is initiated by endothelial cell hyperpolarization following the activation of SK_{Ca} and IK_{Ca} channels. Hyperpolarization of vascular smooth muscle cells is achieved through myoendothelial gap junctions or by the increase of K^+ concentration in myoendothelial space and subsequent activation of Na^+/K^+-ATPase and inward rectifier potassium channels (K_{IR}) (Figure 2). There are also other endothelium-dependent pathways involving smooth muscle cell hyperpolarization, such as NO, PGI_2, EETs, hydrogen peroxide or carbon monoxide. However, for these pathways the hyperpolarization of endothelial cells is not a necessary prerequisite for smooth muscle relaxation (Edwards et al., 2010).

EDHF response is induced either by acetylcholine (Chen et al., 1988) or by the activation of IK_{Ca} and SK_{Ca} channels by their agonists (Ozkor and Quyyumi, 2011). One of supposed agonists is the non-essential amino acid L-serine. Mishra et al. (2008) were the first who reported its direct cardiovascular effects *in vitro* and *in vivo*. L-serine evoked endothelium-dependent vasodilatation, which was abolished by apamin and TRAM 34, ouabain, barium chloride and KCl-induced depolarization. They also reported the *in vivo* effect because they observed a rapid reversible dose-dependent fall of mean arterial pressure, which was inhibited by apamin and charybdotoxin.

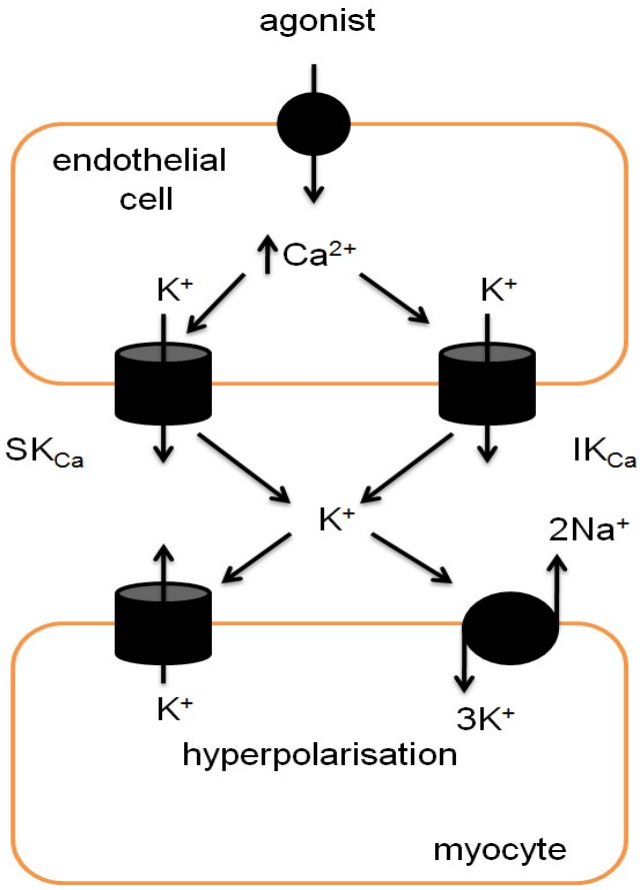

Figure 2. Schematic representation of EDHF pathway, showing K^+ efflux in endothelial cell from SK_{Ca} and IK_{Ca} channels and activation of myocyte K_{IR} and Na^+/K^+ATPases. This mechanism is considered as classical EDHF pathway which is blocked by combination of scyllatoxin and TRAM 34 (Adapted from Edwards et al., 2010)

2.4 Smooth muscle mechanisms of vascular contraction

Smooth muscle contraction is proportional to membrane potential. Therefore its resting value is important for BP control and the change of the resting value may contribute to chronic elevation of BP. Contraction of vascular smooth muscle cells (VSMC) is caused by the elevation in cytosolic Ca^{2+} concentration mainly due to increased Ca^{2+} influx through voltage-dependent Ca^{2+} channels. In the most vascular smooth muscle cells are dominant dyhydropyridine-sensitive L-type voltage-gated Ca^{2+} channels (L-VDCC). They are modulated by vasoconstrictors which open them and by vasodilators which close them. These channels are inhibited by the increase in intracellular Ca^{2+} or by the activation of cGMP-dependent protein kinase (Jackson, 2000). Thus membrane potential controls intracellular Ca^{2+} concentration and simultaneously intracellular calcium concentration affects mechanisms determining membrane potential. Intracellular Ca^{2+} creates a positive feedback loop by the initiation of depolarization via nonselective cationic transient receptor potential channels and this loop is balanced with a negative feedback associated with Ca^{2+}-activated potassium channels (Nilsson, 1998). K^+ channels mediate hyperpolarizing K^+ efflux and their opening results in the diffusion of K^+ out of the cells. Resulting membrane hyperpolarization closes L-type Ca^{2+} channels and causes vasodilatation. On the other hand, closure of K^+ channels causes depolarization and vasoconstriction. Four different classes of K^+ channels are expressed by vascular cells. Inward rectifier K^+ channels conduct K^+ into cells during membrane potential negative to K^+ equilibrium potential and their outward K^+ flow is limited. ATP-sensitive K^+ channels (K_{ATP}) close with the increase in intracellular ATP and may play a role in both processes - vasodilatation and vasoconstriction. Large conductance Ca^{2+}-activated K^+ channels and voltage-gated K^+ channels, activated by membrane depolarization, plays negative feedback role during vasoconstriction (Jackson, 2000). Phosphorylation of the regulatory myosin light chain (MLC) is a major molecular engine of VSMC contraction and is necessary for cross-bridge cycling and generation of contractile forces. Vascular tone is a function of dynamic balance between phosphorylation and dephosphorylation of regulatory MLC by the opposite activities of MLC kinase and MLC phosphatase. Vasoconstrictors act through G-protein coupled receptors and activates MLC kinase via Ca^{2+}/calmodulin dependent signaling pathway. However, they may also act through the inhibition of MLC phosphatase, this way is called Ca^{2+} sensitization pathway (Akata, 2007a,b).

2.5 Experimental models of hypertension

Arterial hypertension affects approximately 20-30% of adult population in developed countries and belongs to main risk factors responsible for the development of cardiovascular diseases. In order to understand the pathophysiology of arterial hypertension many animal models with induced or genetic forms of experimental hypertension have been developed. Animal models share many features common to human hypertension and allow a pathophysiological analysis of the factors responsible for the development of arterial hypertension (Dornas and Silva, 2011). Existence of multiple systems substituting each other in the control of BP, and the difficult dissociation of primary events from the consequences of elevated BP leave still many unanswered questions in hypertension research.

A widely used model of genetic hypertension is the inbred spontaneously hypertensive rat (SHR) and its normotensive control Wistar-Kyoto rat (Okamoto and Aoki, 1963). SHR rats are characterized by mean arterial pressure of about 180-200 mmHg as compared to 115-130 mmHg in normotensive rats. High BP of SHR is maintained by sympathetic nervous system activity. Due to the polygenic nature of genetic hypertension the exact causes of hypertension development in SHR are not known enough (Zicha & Kunes, 1999).

Another genetic model is Dahl salt-sensitive rat (DS) (Dahl et al., 1962). When Dahl SS rats are fed high-salt diet, they become hypertensive, indicating that this is a genetic model of salt hypertension. Transgenic hypertension models can be generated by overexpression of a specific gene. A representative of this type of hypertension is the TGR (mREN2) 27 transgenic rat (Mullins et al., 1990).

The borderline hypertensive rat, which is a genetic model of hypertension elicited by environmental stimuli (salt, stress, fat), is produced as the first filial generation of SHR and WKY rats, possessing genetic information from both parents (Dornas and Silva, 2011).

Experimental models of induced hypertension are achieved by chronic blockade of NO synthesis, renal ischemia, angiotensin II infusion, environmental factors, stress, denervation of sinoaortic baroreceptors etc. Chronic NO-deficient hypertension is achieved by NO synthase inhibition by L-NAME and the pathogenesis of this model also involves central neurvouss mechanisms and renin-angiotensin system changes. Angiotensin

hypertension is induced by chronic infusion of low doses of this vasoconstricting peptide (Bader, 2010).

Renovascular hypertension is induced by renal artery stenosis, when ischemic kidney secrets renin, which leads to BP elevation. Renovascular type of hypertension may be induced either as two kidney, one-clip hypertension (2K, 1C) in which one renal artery is constricted while the contralateral kidney is left intact,or as one kidney, one-clip hypertension (1K, 1C) when one renal artery is constricted and the contralateral kidney is removed. Two-kidney, two-clip hypertension can be produced by the constriction of both renal arteries or aorta above renal arteries (Dornas and Silva, 2011).

2.6 Anesthetics and their effects on cardiovascular system in rat

General anesthesia is defined as the loss of pain perception and the loss of reflex and spontaneous muscle activity. To be properly anesthetized, the animal must be immobile and unaware of painful stimuli (Brunson 1997). The anesthetics cause unconsciousness by blocking the brain ability to integrate information. They work through ion channels that regulate synaptic transmission and hyperpolarize neurons by increasing their inhibition or by decreasing their excitation. Similar changes are also observed during non-rapid eye movement sleep (Ries and Puil 1999). Among these channels are gamma-aminobutyric acid receptors ($GABA_A$), N-methyl-D-aspartate receptors (NMDA), acetylcholine receptors, glycine receptors, cyclic nucleotide-gated channels or K^+ channels (for the targets of particular anesthetics see Table 1). Another similarity between natural sleep and anesthetic-induced loss of consciousness is the inhibition of thalamus leading to a similar pattern of cortical inhibition (Franks 2008). Complex posterior brain areas comprising the lateral temporo-parieto-occipital junction and perhaps a mesial cortical core are most likely the final targets for anesthetic action (Alkire et al., 2008).

Hypnotic drugs induce a state resembling deep sleep. Different general anesthetic agents can provide similar levels of hypnosis, but the degree of analgesia achieved can vary widely. General anesthesia requires both loss of consciousness and loss of pain sensation. If no analgesic component is provided, even intense pain will not be perceived under deep anesthesia, but this may result in increased post-operative pain (Flecknell 2009). Some drugs are used in combination because of insufficient anesthesia depth or anesthesia quality.

Tissue concentrations of injectable anesthetics are difficult to measure. Absorption, distribution or elimination of drugs injected intravenously, intramuscularly and intraperitonealy result in different anesthetic depth. This may cause differences in experimental conditions, which are not desirable. On the other hand, volatile anesthetics are delivered continuously via lungs and alveolar anesthetic concentrations closely approximate their arterial blood concentrations. The delivery of volatile anesthetic can be regulated on a continual basis to ensure that all animals are at similar anesthetic depth. It makes the inhalation anesthetics more adequate for anesthesia in the current research (Brunson, 1997).

	Pentobarbital	Ketamine	Chloralose	Isoflurane
$GABA_A$	+	+	+	+
NMDA	0	-	0	-
K^+ channels	-	-	-	-
Glycine	0	+	+	+
nAchR	-	-	-	-

Table 1. Anesthetics neuronal targets. $GABA_A$ - gamma-aminobutyric acid receptor, NMDA - Nmethyl-D-aspartate receptor, nAchR – nicotinc acetylcholine receptor. Adapted from Alkire et al., 2008; (+) - potentiation; (-) - inhibition.

2.6.1 Barbiturates

Barbiturates may be classified as hypnotic sedatives, reflecting their dose-dependent ability to produce either sedation or a deeper hypnotic state (Fish, 1997). Most used barbiturates are short-acting pentobarbital, or ultrashort-acting thiopental, inactin and methohexital. From a chemical point of view, barbiturates are closed-chain ureic compounds, derived from barbituric acid (López-Muñoz et al., 2005). Pentobarbital has been most widely used laboratory animal anesthetic, but like other barbiturates it has very poor analgesic activity and is probably best used to provide hypnosis rather than anesthesia (Flecknell 2009). Pentobarbital was reported to lower BP and heart rate (Saha et al., 2007) and to decrease the activity of the sympathetic nervous system (Maignan et al., 2000). On the other hand, thiopental appears to affect less cardiovascular system and its anesthesia seems to be more stable (Brammer et al., 1993). Barbiturates generally act as central nervous depressant. They potentiate actions of the agonists of $GABA_A$ receptors and glycine receptors. They also inhibit potassium channels and NMDA, AMPA, nicotinic, acetylcholine or serotonin receptors (Oakley et al., 2012; Alkire et al., 2008).

2.6.2 Disociative anesthetics

Ketamine, tiletamine, cyclohexamines and phencyclidine have been characterized as dissociative anesthetics (Soma 1983). “Dissociative” refers to apparent dissociation of

the patient consciousness from its environment. Ketamine is NMDA receptor antagonist and is actuallythe most widely used injectable anesthetic in animals, due to its wide margin of safety and compatibility of other drugs (Fish, 1997). Because ketamine is short-acting, produces variable analgesia, causes muscle rigidity and high doses of ketamine are needed for surgical anesthesia, it may be used with other drugs augmenting anesthesia such as xylazine (Flecknell 2009). Xylazine, which is classified as α_2-adrenoreceptor agonist (Hsu, 1981), leads to the inhibition of sympathetic nerve activity, induces antinociception, sedation and muscle relaxation (Fish, 1997). Stimulatory effect of ketamine on the cardiovascular system do not offset the depressant effect of xylazine, and their combination results in lowered BP (Fish, 1997; Saha et al., 2007). Ketamine probably blocks K_V channels (Kim et al., 2007) and also interacts with others channels required for the induction of anesthesia (Alkire et al., 2008).

2.6.3 Hypnotics

Hypnotics are drugs producing a dose-dependent sedation, hypnosis (sleep), anesthesia eventually coma. The mostly used nonbarbiturate hypnotics are chloral hydrate, α-chloralose, urethane, etomidate or propofol. Chloralose, which is a product of glucose with anhydrous chloral, produces hypnosis of long duration with poor analgesia. To achieve deep anesthesia it may be administered together with urethane (ethyl ester of carbamic acid) possesing analgesic properties (Fish, 1997). Combination of chloralose and urethane does not produce any changes in heart rate and peripheral resistance, but significantly lowers BP due to cardiac output decrease (Smith and Hutchins 1980; Bertera et al., 2009). In addition to the effects of hypnotics on ion channels involved in the induction of anesthesia (Alkire et al., 2008), urethane inhibits cardiovascular responses that are mediated by peripheral and central α_2-adrenoreceptors (Armstrong et al,. 1982).

2.6.4 Volatile anesthetics

First inhalation anesthetics used were dimethyl ether and chloroform. Because of flammability of dimethyl ether and toxicity of chloroform the search for new inhalation anesthetics was needed. The addition of fluorides and bromides to gas anesthetic

molecules resulted in the development of new inhalation anesthetics such as halothane, which has the disadvantage of depressed ventilation and circulatory functions, arrythmias and hepatic necrosis (Jones, 1990). Later, methoxyflurane and its four derivates – enflurane, isoflurane, desflurane and sevoflurane were accepted as inhalation anesthetics (Brunson, 1997). Isoflurane become a popular anesthetic for animals due to a very rapid induction or recovery from anesthesia. Another reason is that its depth of anesthesia can be easily altered (Flecknell, 1997). The main advantage of using isoflurane is that it undergoes less biotransformation than any other agent and is almost completely eliminated in the exhaled air (Eger, 1981). Cardiovascular effects of volatile anesthetics such as halothane, enflurane, isoflurane and sevoflurane are similar. They sustain myocardial contractility, minimally increase central venous pressure and lower BP by decreasing vascular resistance in nearly all tissues, particularly in the muscle (Conzen et al., 1992; Eger, 1981; Seyde et al., 1987). Isoflurane increases heart rate and decreases left ventricular work and myocardial oxygen consumption (Eger, 1981). The inhibitory effects of these substances on vascular contraction may be caused by the inhibition of voltage-gated Ca^{2+} influx (Akata et al., 2007) or by the activation of K_{Ca} and K_{ATP} channels producing vascular smooth muscle hyperpolarization (Yamazaki et al., 1998; Stekiel et al., 2001).

3. Aims of study

It is well known that anesthesia causes many cardiovascular changes compared to conscious state. Participation of BP regulating components (sympathetic nervous system, renin-angiotensin system and nitric oxide system) may be disclosed by the selective inhibition of their pressor effects. To specify the changes in participation of these systems in BP control between anesthetized and conscious animals, the first aim of my work was to determine the participation of these systems under commonly used types of anesthesia. Since the participation of these systems in normotensive and hypertensive animals is different, my second aim was to evaluate if anesthesia may have different influence in hypertensive animals.

- **To determine the influence of anesthesia on BP and the participation of principal vasoactive systems.**
- **To evaluate anestesia influence in spontaneously hypertensive rats.**

In previous studies L-serine evoked BP fall which was pronounced in NO-deficient hypertension. L-serine-induced BP fall represents EDHF response which is endothelium-dependent regulation of vascular tone by NO- and prostacyclin-independent pathway. This pathway may be a perspective mechanism of BP control in hypertension. My third aim was to confirm previous experiments (Mishra et al., 2008) carried out in anesthesia and to determine L-serine-induced BP effects in conscious salt hypertensive animals.

- **To evaluate L-serine-induced blood pressure fall in conscious and anesthetized rats.**

- **To determine the difference between normotensive and hypertensive salt-sensitive Dahl rats.**

4. Methods

4.1. Animals

The experiments determining the influence of anesthesia on BP control mechanisms were carried out in 20-week-old male Wistar rats (WIS) fed with ST1 rat chow containing 1% NaCl. As genetic model of established hypertension served 20-week old male spontaneously hypertensive rats (SHR) fed also with ST1 rat chow.

Experiments concerning with L-serine-induced effects were performed on male Dahl salt-sensitive rats (DS) and male Dahl salt-resistant rats (DR). Salt hypertension was elicited by feeding of DS rats with 5% NaCl diet for 5 weeks from the age of 12 weeks (DS-HS). As controls without developed hypertension served Dahl salt-sensitive rats fed with 0.3% NaCl diet (DS-LS) and also normotensive Dahl salt-resistant rats, fed with high-salt diet (DR-HS) or low-salt diet (DR-LS). DR-HS rats did not develop hypertension when compared to DR-LS rats.

All groups had a free access to water and food *ad libitum*. Animals were housed under standard laboratory conditions (23 ± 1° C temperature; 12 h light-dark cycle). Procedures and experimental protocols were approved by the Ethical Comittee of the Institute of Physiology AS CR, and conformed to the European Convention on Animal Protection and Guidelines on Research Animal Use.

4.2 Blood pressure measurement

In experiments with conscious rats, they were cannulated under isoflurane anesthesia 24 hours before. On the other hand, the effects of particular anesthesia (pentobarbital, ketamine-xylazine, isoflurane or chloralose-urethane) were studied in animals cannulated one hour before the experiment under studied anesthesia. Carotid artery was cannulated with polyethylene catheter (PE 50) and connected to the measurement device - PowerLab system (ADI Instruments Ltd, Bella Vista NSW, Australia). Jugular vein, cannulated with polyethylene catheter (PE 10), was used for the application of drugs.

Cannules were filled with heparin to avoid their obstruction caused by blood clotting, tunneled under the skin and exteriorized in the interscapular region. Blood pressure experiments were performed between 8:00 and 12:00 to reduce circadian variations. Four animals were monitored simultaneously.

4.3 Drugs

All drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA). All doses were given as an intravenous bolus in a volume of 1 $ml.kg^{-1}$ body weight. Indomethacin was dissolved in 160 mM Na_2CO_3, and all the other drugs were dissolved in saline solution (0.9 % NaCl). Some additional group of rats were injected with Na_2CO_3 solution to ensure that this vehicle did not produce any BP changes.

4.4 Anesthesia

Injectable anesthetics were administered in a volume of 1 $ml.kg^{-1}$ body weight. Doses of anesthetics were chosen according to Flecknell (2009). Pentobarbital was injected intraperitonealy in a concentration of 60 $mg.kg^{-1}$. A combination of ketamine and xylazine was injected intramuscularly in a concentration 75 $mg.kg^{-1}$and 5 $mg.kg^{-1}$ respectively. The combination of urethane and chloralose was injected intraperitoneally in a concentration of 500 $mg.kg^{-1}$and 100 $mg.kg^{-1}$, respectively. Inhalation anesthetic – 2% isoflurane (ISO) was administered through a vaporizer. Anesthetized rats were kept under heating lamp and their temperature was continually checked, the variation in body temperature during the experiment did not exceed ± 2°C.

4.5 Experimental protocols

4.5.1 Protocol 1 - Blockade of the RAS, SNS and NO system

After the stabilization of BP (30 minutes), baseline values were recorded. A sequential blockade of the principal pressor systems was performed according to a modified protocol of Minami et al. (1995) as described by Zicha et al. (2001). Initially, intravenous bolus of captopril (10 $mg.kg^{-1}$ body weight) was injected to block angiotensin-converting enzyme. Ten minutes later, sympathetic nervous system was inhibited by ganglionic blocker pentolinium - nicotinic acetylcholine receptor antagonist (5 $mg.kg^{-1}$ body weight). After BP stabilization in about 5 minutes, the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 30 $mg.kg^{-1}$ body weight) was given and BP was monitored for the next 20 minutes (Figure 3). After BP stabilization sodium nitroprusside – NO donor (SNP, 20 $\mu g.kg^{-1}$) was injected to determine minimal (residual) BP. Used doses of drugs elicited maximal BP effects (Zicha et al., 2001).

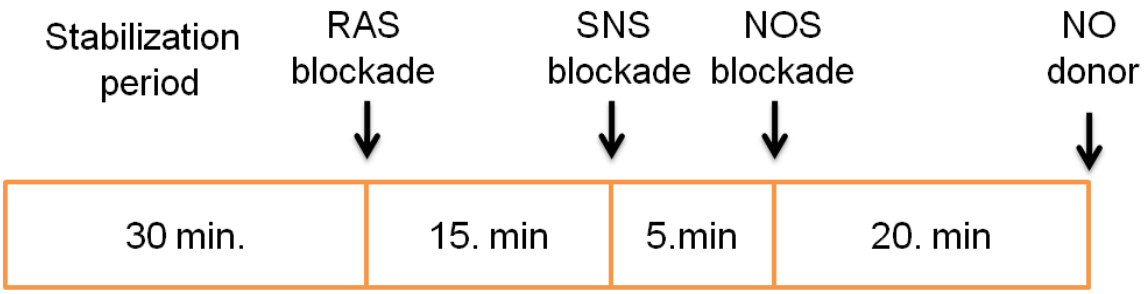

Figure 3. Time course of Protocol 1. Renin-angiotensin system (RAS) blockade by captopril; sympathetic nervous sytem (SNS) blockade by pentolinium; nitric oxide synthase (NOS) blockade by L-NAME.

4.5.2 Protocol 2 - L-serine administration after COX and NOS inhibition

After the stabilization of BP (30 minutes), baseline values were recorded and L-serine was injected in increasing concentrations 0.5 - 2 mmol.kg^{-1}. Thereafter, NOS blockade by L-NAME was performed and L-serine administration was repeated.

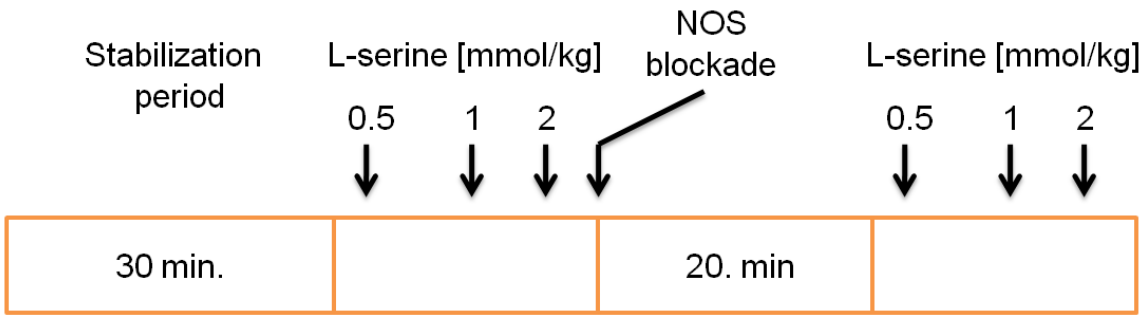

Figure 4. Time course of Protocol 2. L-serine doses in rising concentration 0.5-2 mmol.kg^{-1};before and after nitric oxide synthase (NOS) blockade.

In additional experiments cyclooxygenase (COX) was inhibited by indomethacin (bolus 10 mg.kg^{-1} followed by infusion 60 mg.hod^{-1}.kg^{-1}). Thereafter, NOS blockade by L-NAME was added to induce a combined NOS and COX inhibition which eliminated the respective vasodilatator systems (NO, PGI_2). L-serine in increasing concentration was administered after each blockade. Finally, sodium nitroprusside – NO donor (SNP, 20 µg.kg^{-1}) was injected to determine residual BP. SNP-induced hypotensive response was compared to maximal L-serine (2 mmol.kg^{-1}) BP fall.

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4.6 Statistical analysis

The data were expressed as mean ± SEM. The effects of the drugs were analyzed as absolute changes in BP in mmHg. Student`s t-test was performed for a two-group comparison. For a multiple group analysis one-way analysis of variance and *post-hoc* Fisher's LSD test was performed. The data were considered significant when the P values were <0.05.

5. Results

5.1 Influence of different anesthetics on blood pressure control

5.1.1 Blood pressure effects of RAS, SNS and NO system inhibition and influence of anesthesia in normotensive rat

The contribution of renin-angiotensin system, sympathetic nervous system and NO production to BP maintenance was measured by the procedures described in Protocol 1 (Chapter 4.5.1). Conscious Wistar rats were compared with four different groups of animals anesthetized by pentobarbital (P), ketamine-xylazine (KX), chloralose-urethane (CU) or isoflurane (ISO). System participation in BP control was revealed by BP changes, which were elicited by the use of specific inhibitors of vasoconstriction and vasodilatation systems. The contribution of these systems was expressed by the absolute value of BP change.

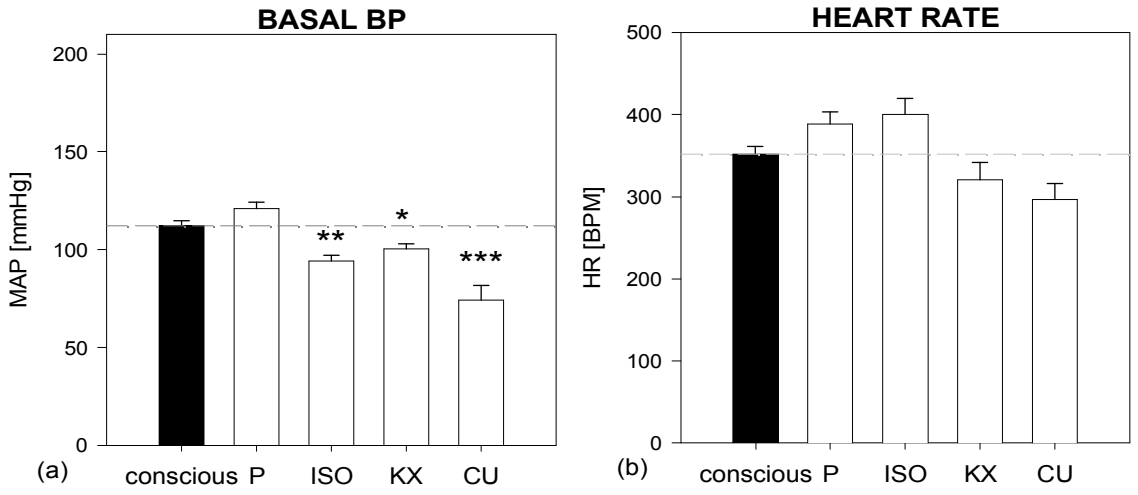

Figure 5. (a) Mean arterial pressure (MAP) and (b) heart rate (HR) in **Wistar rats** anesthetized by pentobarbital P, ISO isoflurane, KX - ketamine xylazine, CU - chloralose-urethane; * P < 0.05; ** P < 0.01; *** P < 0.001; compared with conscious rats (*post-hoc* Fisher's LSD test); n=31.

Mean arterial pressure (MAP) of conscious Wistar rats was 112 ± 2 mmHg. Basal BP did not change after pentobarbital anesthesia (121 ± 3 mmHg), but ketamine-xylazine (100 ± 2 mmHg), chloralose-urethane (75 ± 7 mmHg) and isoflurane (94 ± 3 mmHg) caused significant BP lowering. Heart rate in all anesthetized groups did not differ significantly from the conscious values (Figure 5).

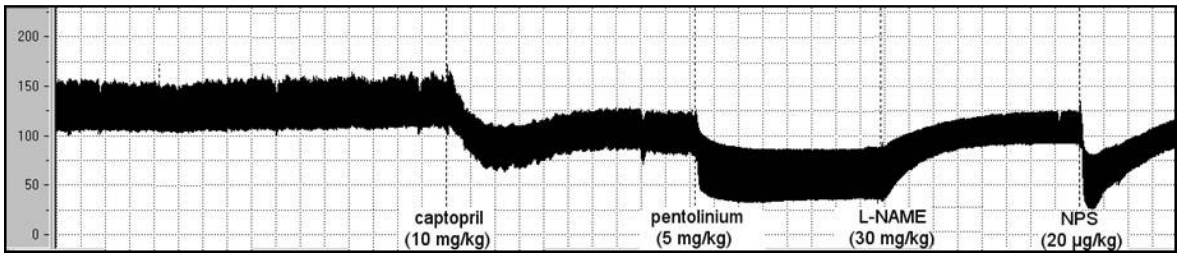

Figure 6. Protocol 1. Captopril (10mg/kg) – blockade of ACE; Pentolinium (5mg/kg) – blockade of sympathetic nervous system; L-NAME (30mg/kg) – blockade of nitric oxide synthase.

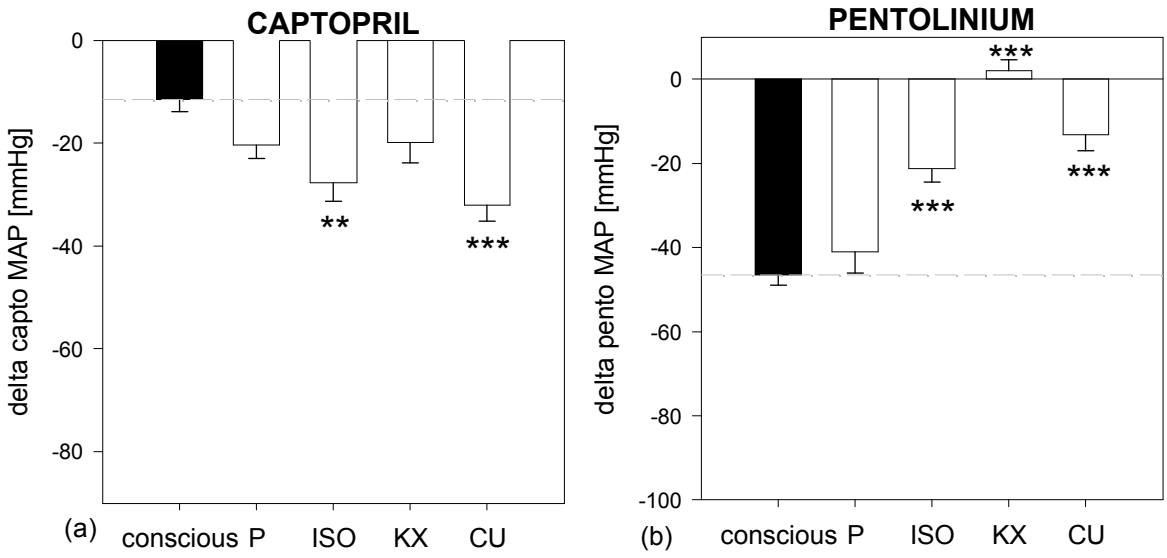

Figure 7. MAP response of **Wistar rats** to (a) to renin-angiotensin system blockade by captopril; (b) to sympathetic nervous system blockade by pentolinium P - pentobarbital, ISO - isoflurane, KX - ketamine xylazine, CU - chloralose-urethane;* P < 0.05; ** P < 0.01; *** P < 0.001; compared with conscious rats (*post-hoc* Fisher's LSD test); n=31.

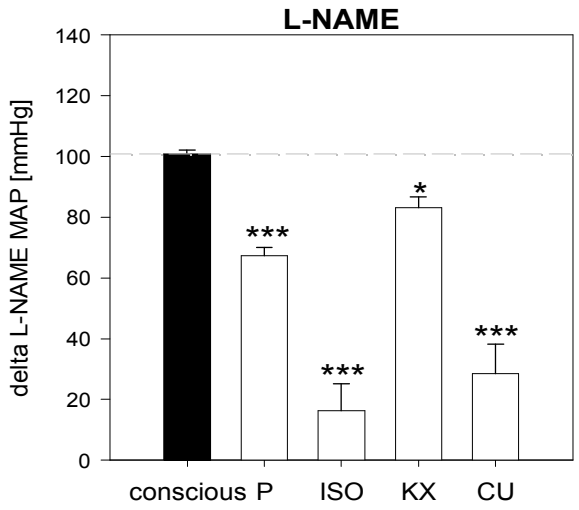

Figure 8. MAP response of **Wistar rats** to NOS blockade by L-NAME; P - pentobarbital, ISO - isoflurane, KX - ketamine xylazine, CU - chloralose-urethane;* P < 0.05; ** P < 0.01; *** P < 0.001; compared with conscious rats (*post-hoc* Fisher's LSD test); n=31.

Renin-angiotensin system contribution to the BP maintenance (captopril-induced BP change) in conscious rats was 11 ± 2 mmHg. This contribution was non-significantly increased by pentobarbital or ketamine-xylazine anesthesia (both 20 ± 4 mmHg). Significant augmentation of BP response to captopril compared to conscious group was caused only by chloralose-urethane (32 ± 3 mmHg) and isoflurane (27 ± 4 mmHg) anesthesia (Figure 7.a).

The contribution of sympathetic nervous system to BP maintenance (pentolinium-induced BP change) in conscious rats (47 ± 2 mmHg) was similar to pentobarbital anesthesia (41 ± 5 mmHg). Under chloralose-urethane and isoflurane anesthesia its participation was significantly attenuated (13 ± 4 mmHg and 21 ± 3 mmHg) and ketamine-xylazine anesthesia totally abolished sympathetic nervous system participation (Figure 7.b).

BP response to NOS inhibition was attenuated in all anesthetized groups as compared with conscious animals (100 ± 1 mmHg) – less under pentobarbital (67 ± 3 mmHg) and ketamine-xylazine (83 ± 3 mmHg) anesthesia than under isoflurane (16 ± 9 mmHg) and chloralose-urethane (28 ± 10 mmHg) anesthesia (Figure 8).

5.1.2 Blood pressure effects of RAS, SNS and NO system inhibition and influence of anesthesia in spontaneously hypertensive rat

The contribution of renin-angiotensin system, sympathetic nervous system and NO production to BP maintenance was measured by the procedures described in Protocol 1 (Chapter 4.5.1). Conscious SHR rats were compared with four different groups of animals anesthetized by pentobarbital (P), ketamine-xylazine (KX), chloralose-urethane (CU) or isoflurane (ISO). MAP of conscious SHR rats was 190 ± 3 mmHg. Different types of anesthesia influenced BOP of SHR in am similar manner as in normotensive rats, but anesthesia-induced BP changes were more pronounced in SHR than in Wistar rats. Basal BP did not change after pentobarbital anesthesia (193 ± 3 mmHg), but ketamine-xylazine (112 ± 2 mmHg), chloralose-urethane (129 ± 7 mmHg) and isoflurane (138 ± 11 mmHg) caused significant BP lowering.

BP changes elicited in SHR by the blockade of particular vasoconstrictor and vasodilator systems were qualitatively similar as those observed in normotensive Wistar rats measured under equivalent conditions, but the magnitude of BP responses was usually augmented due to the elevation of basal BP in SHR (Figure 9). The most obvious differences were a major contribution of renin-angiotensin system to BP maintenance in CU-anesthetized SHR and an abnormally high BP response to L-NAME in KX-anesthetized SHR (Figure 9).

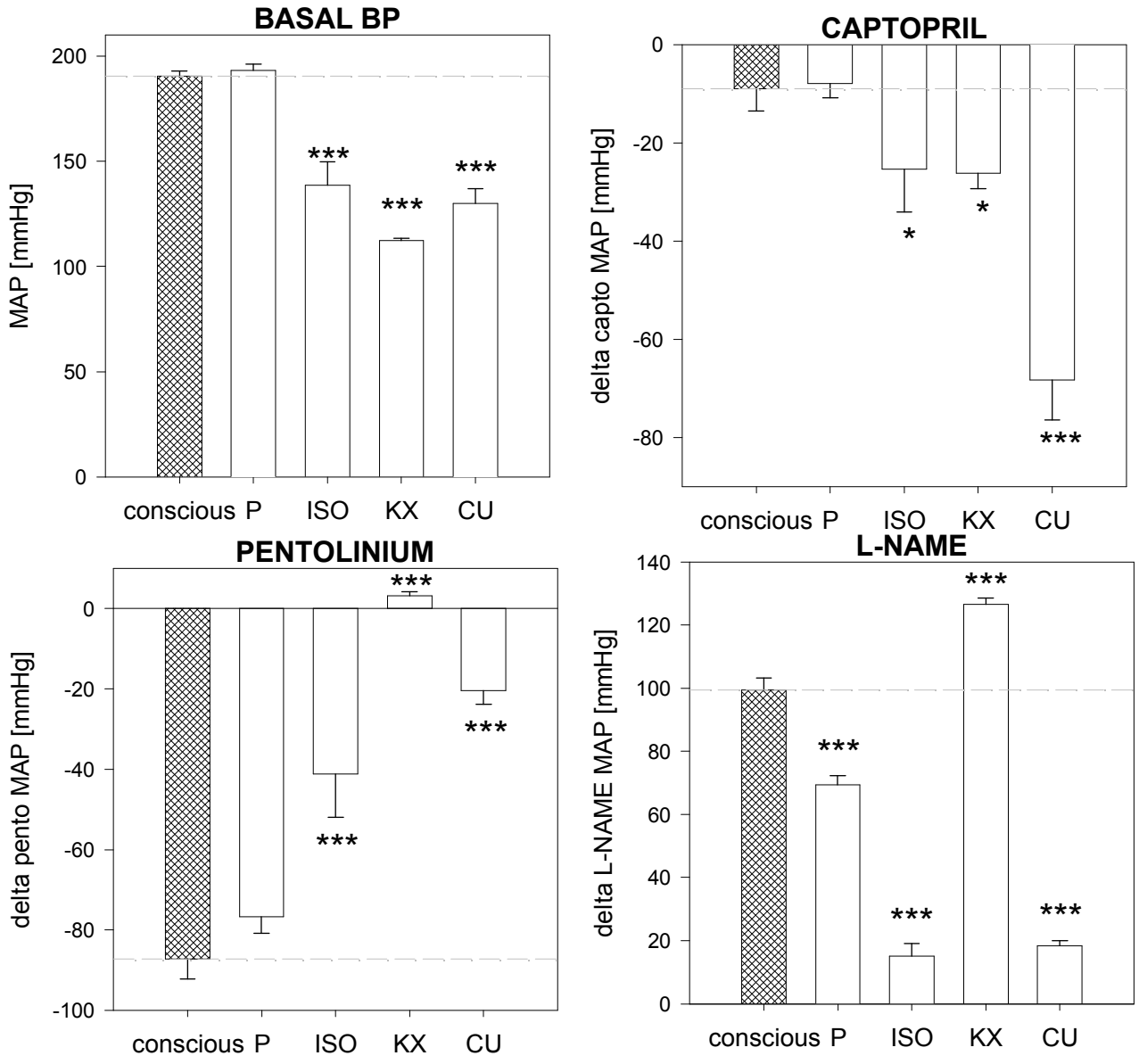

Figure 9. MAP response of **SHR** to the blockade of BP regulating mechanisms by pentolinium (PENTO), captopril (CPT) and L-NAME under different anesthesia * P < 0.05; ** P < 0.01; *** P < 0.001; compared with conscious rats (*post-hoc* Fisher's LSD test); n=32.

5.1.3 Blood pressure effects of RAS, SNS and NO system inhibition and influence of anesthesia in Dahl rats

The contribution of renin-angiotensin system, sympathetic nervous system and NO production to BP maintenance was measured by the procedures described in Protocol 1 (Chapter 4.5.1). Conscious DS rats were compared with four different groups of animals anesthetized by pentobarbital (P), ketamine-xylazine (KX), chloralose-urethane (CU) or isoflurane (ISO). MAP of conscious DS-HS rats was 178 ± 6 mmHg and DS-LS 153 ± 5 mmHg. The use of pentobarbital and ketamine-xylazine anesthesia lowered BP, RAS contribution to BP maintenance (captopril-induced BP change) was pronounced in ketamine-xylazine when compared to pentobarbital and SNS contribution was abolished in ketamine-xylazine and pronounced in pentobarbital anesthesia. NO contribution to BP regulation was slightly enhanced in DS-HS rats compared to DS-LS rats anesthetized by pentobarbital.

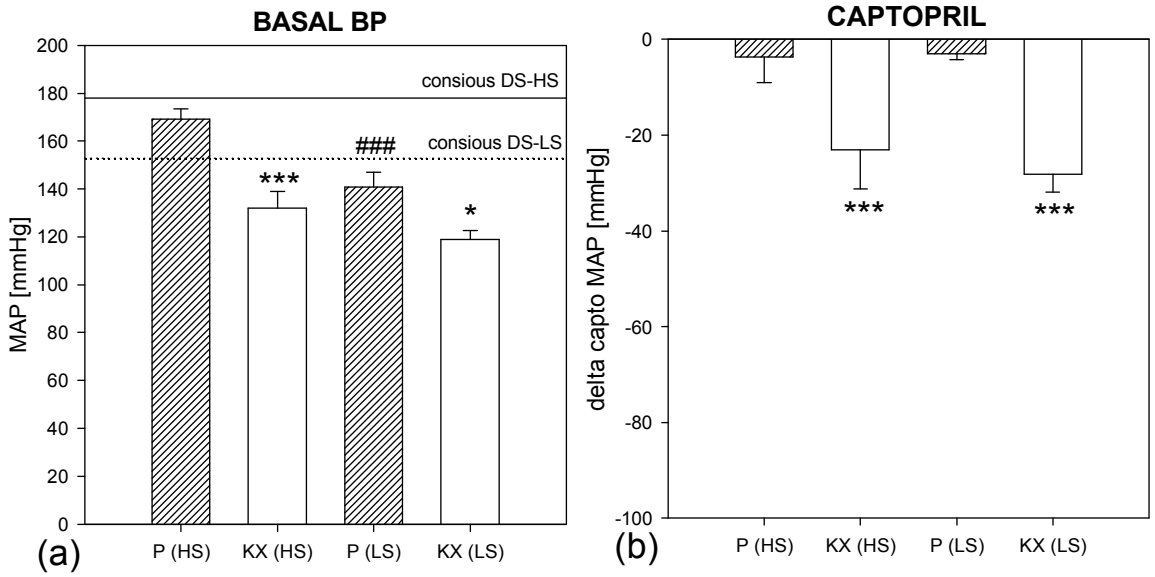

Figure 10. MAP of DS-HS and DS-LS rats under pentobarbital (P) and ketamine-xylazine (KX) anesthesia (a) and response to renin-angiotensin system blockade by captopril (b) * P < 0.05; ** P < 0.01; *** P < 0.001; compared between P and KX anesthesia; # P < 0.05; ## P < 0.01; ### P < 0.001 compared between HS and LS diet (*post-hoc* Fisher's LSD test); n=13.

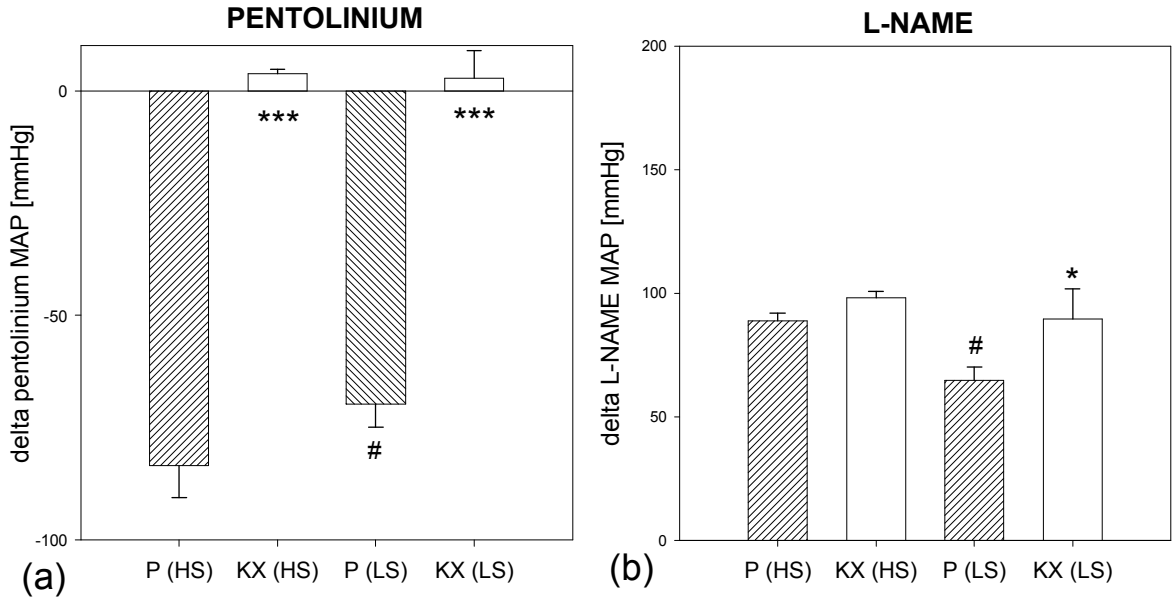

Figure 11. BP change after SNS blockade by pentolinium (a) and NO system blockade by L-NAME(b) in DS-HS and DS-LS rats under pentobarbital (P) and ketamine-xylazine (KX) anesthesia and response to renin-angiotensin system blockade by captopril; * P < 0.05; ** P < 0.01; *** P < 0.001; compared between P and KX anesthesia; # P < 0.05; ## P < 0.01; ### P < 0.001 compared between HS and LS diet (*post-hoc* Fisher's LSD test); n=13.

5.2 Blood pressure effects of L-serine administration

5.2.1 L-serine-induced MAP fall before and after NO system inhibition

L-serine administration evoked a dose-dependent BP fall in anesthetized Dahl rats (Figure 12). L-serine evoked only a slight dose-dependent BP fall under pentobarbital anesthesia before NOS blockade, but after this blockade L-serine induced a pronounced BP fall. Under ketamine-xylazine anesthesia L-serine evoked pronounced BP fall both before and after NOS blockade (Figure 12). Dahl salt-sensitive rats on high-salt diet were characterized by the elevated BP compared to salt-sensitive rats on low salt diet (Table 2). Elevated BP was not associated with any significant change of L-serine-induced dose-dependent BP fall.

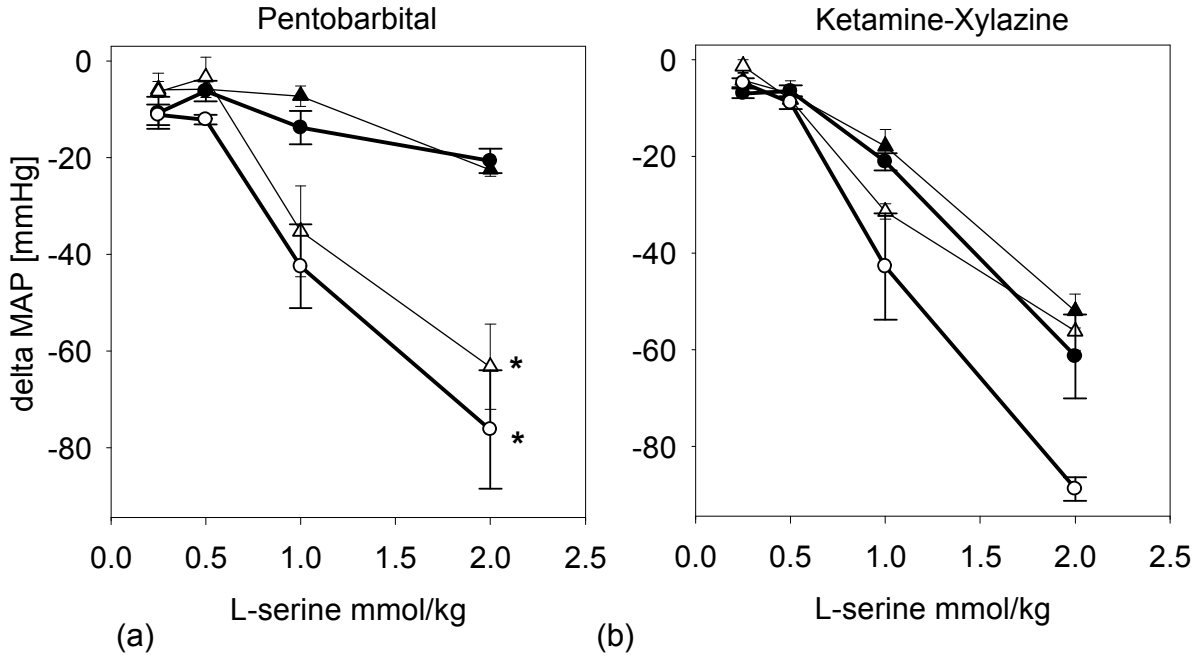

Figure 12. The dose-response curves to L-serine administration in Dahl rats under pentobarbital (a) and ketamine-xylazine (b) anesthesia. DS-LS rats before (▲) and after L-NAME administration (Δ) are compared with DS-HS rats before (●) and after L-NAME administration (○); n=25; * P < 0.001; compared with rats before L-NAME administration.

strain	MAP – pentobarbital [mmHg]	MAP - ketamine-xylazine [mmHg]
DS-HS	176 ± 12	142 ± 5
DS-LS	97 ± 9	115 ± 6

Table 2. Basal mean arterial pressure (MAP) under pentobarbital and ketamine-xylazine anesthesia in Dahl salt-sensitive rats on high-salt diet (DS-HS) and low-salt diet (DS-LS); n=25.

5.2.2 L-serine administration after consecutive COX and NO system inhibition

In pentobarbital-anesthetized Dahl rats fed a low-salt diet (DS-LS) the blockade of the cyclooxygenase (COX) by indomethacin increased BP while L-serine in the dose of 2 mmol.kg^{-1} caused a pronounced BP fall (Figure 13). When compared these BP changes with the response to L-serine administration in intact rats (Figure 12), this BP fall was augmented. Combination of indomethacin with L-NAME caused further BP increase and further augmentation of L-serine-induced BP fall. Maximal L-serine dose (2 mmol. kg^{-1}) elicited BP fall which was about 50% of that caused by NO donor – sodium nitroprusside (NPS).

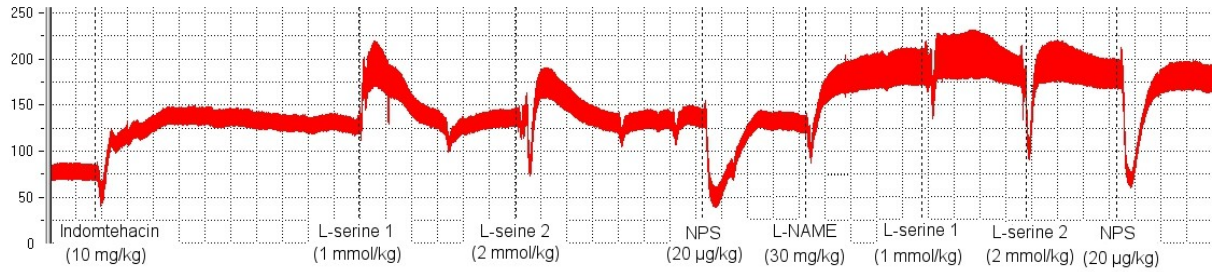

Figure 13. BP recording showing the effects of L-serine (LS, 1 and 2 mmol.kg^{-1}) or nitroprusside (NPS, 20 µg.kg^{-1}) administration before and after NOS blockade by L-NAME in SS_LS rats; n=10.

5.2.3 L-serine effects in conscious Dahl rats

All previous experiments with L-serine were performed in anesthetized Dahl rats, because L-serine failed to cause a dose-dependent BP fall in conscious intact animals (Figure 15). However, after NOS blockade by L-NAME the administration of L-serine permitted the occurrence of vasodilating changes even in conscious rats. The complete blockade of NOS, renin-angiotensin system and sympathetic nervous system enabled L-serine to cause a significant dose-dependent BP fall in all experimental groups (Figure 14). This BP fall was more pronounced in salt-sensitive strain, irrespective of the diet and BP because there was no significant difference between salt hypertensive DS-HS and normotensive DS-LS rats (Table 3). This was true for both the last dose of L-serine (2 mmol/kg) and subsequent NPS administration used to unveil maximal BP fall (Figure 14).

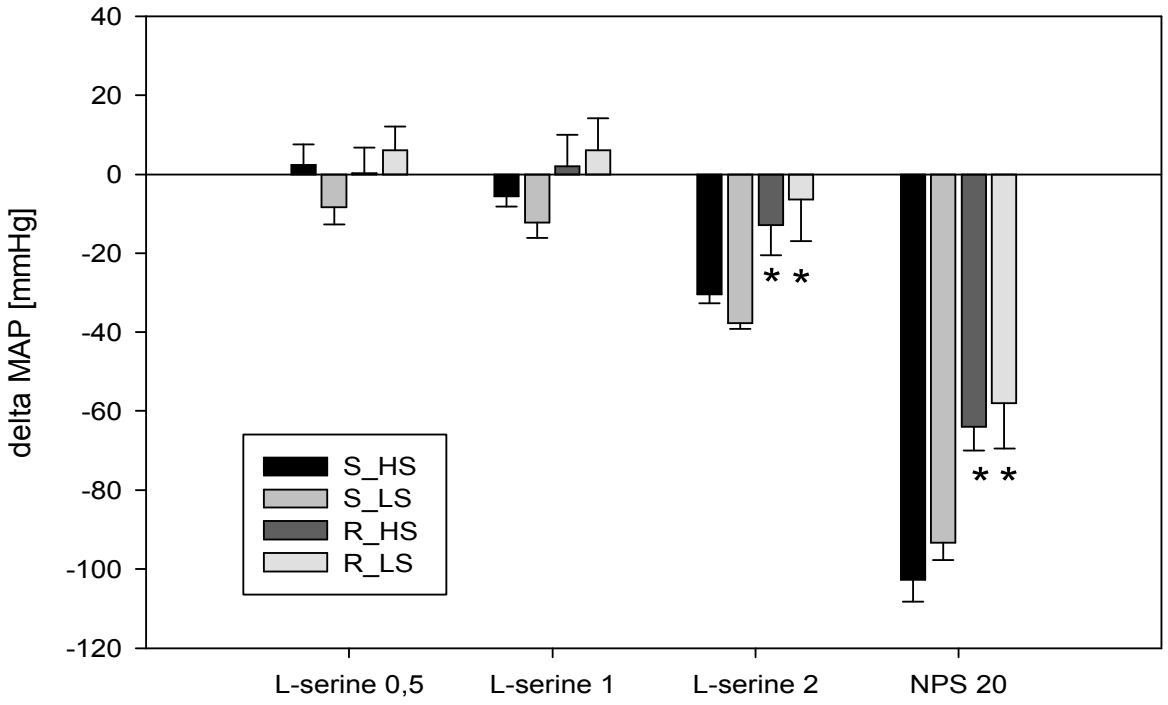

Figure 14. MAP response to consecutive administration of increasing L-serine doses (determined after the complete blockade of all three vasoactive systems) as compared to MAP changes induced by sodium nitroprusside (NPS). * P < 0.001; compared with rats on the same diet; n=17.

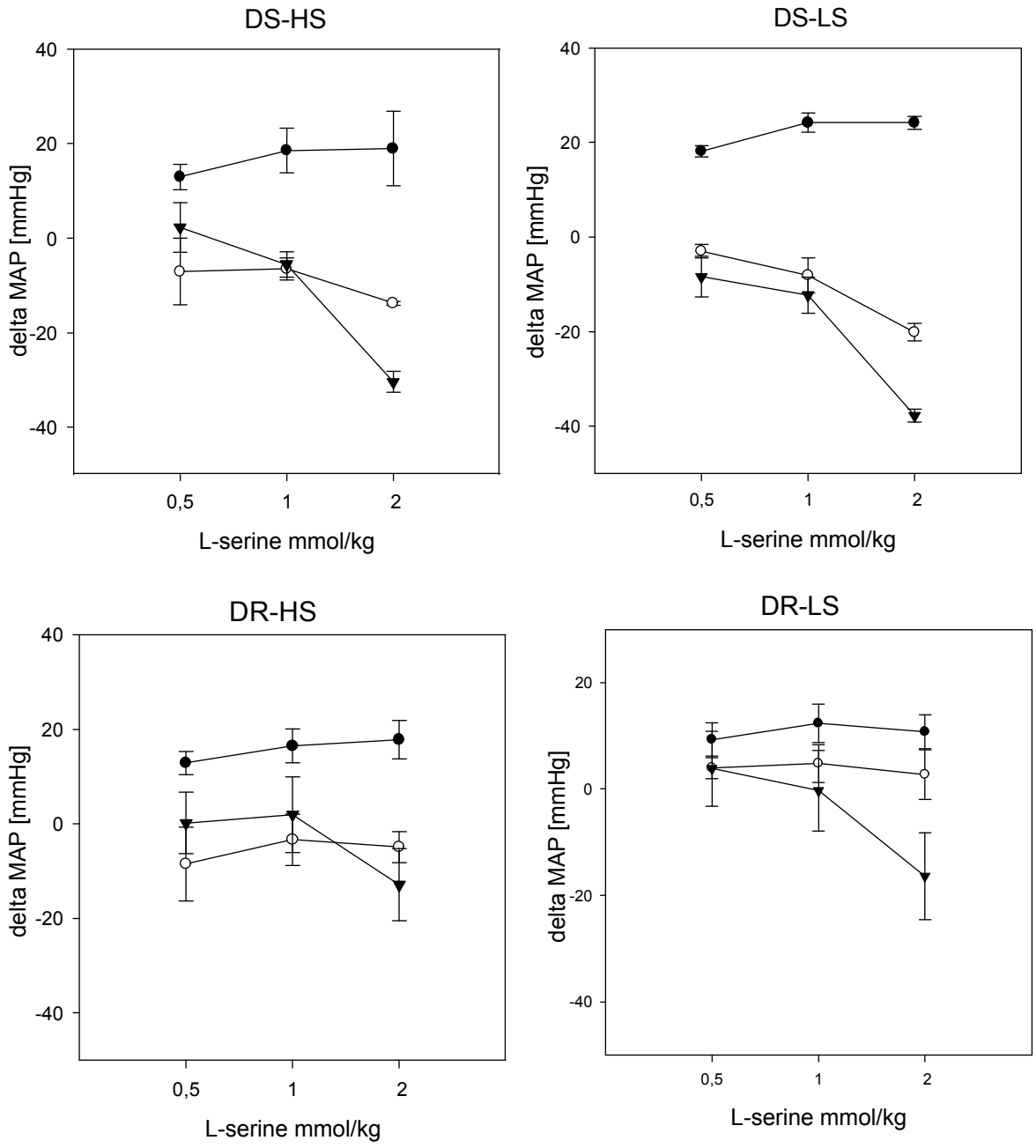

Figure 15. The dose-response curves to L-serine in conscious Dahl animals before any blockade, after NOS blockade by L-NAME and after the complete blockade (NOS, sympathetic nervous system and renin-angiotensin system) in Dahl salt-sensitive (DS) and Dahl salt-resistant (DR) rats fed either high-salt (HS) or low-salt (LS) diets; n=17.

Strain	MAP - basal [mmHg]	MAP - L-NAME [mmHg]	MAP - complete [mmHg]	MAP - NPS [mmHg]
DS-HS	178	213	175	72
DS-LS	153	190	155	63
DR-HS	129	154	116	52
DR-LS	114	133	105	47

Table 3. Mean arterial pressure (MAP) of conscious Dahl rats after the stabilization period (basal), after the nitric oxide synthase blockade (L-NAME), after the blockades of renin-angiotensin system and sympathetic nervous system (complete) and residual BP after the administration of nitric oxide donor (NPS), n=17.

6. Discussion

6.1 Different anesthesia and blood pressure control

Anesthetics cause profound alterations in respiratory and cardiovascular systems. In our study I intended to reveal the role of particular systems of BP maintenance in conscious and anesthetized rats. This was achieved by a consecutive blockade of the renin-angiotensin system, sympathetic nervous system and NO synthase. The contribution of these mechanisms to BP regulation was represented by the absolute value of BP changes seen after the each system blockade. The participation of these main BP regulating systems was compared between anesthetized and conscious rats. Each anesthesia caused different hemodynamic changes regarding to conscious state and the summary of their effects is depicted in Table 3.

	MAP	RAS	SNS	NO production
Pentobarbital	=	=	=	↓
Isoflurane	↓	↑	↓	↓
Ketamine-xylazine	↓	=	↓	↓
Chloralose-urethane	↓	↑	↓	↓

Table 4. MAP changes in Wistar rats caused by pentobarbital, isoflurane, ketamine-xylazine and chloralose-urethane anesthesia and the influence of anesthesia on BP regulating systems revealed by their inhibition.

Pentobarbital anesthesia caused less hemodynamic changes than the other anesthetics, because basal BP, sympathetic nervous system and renin-angiotensin system BP components were not changed compared to conscious animals. The main pressor system regulating BP under pentobarbital anesthesia remained sympathetic nervous system

similar to conscious animals. However, pentobarbital is due to its poor analgesic activity no longer recommended as the anesthetic agent. The unsuppressed sympathetic control may be the reason for its poor analgesic activity. The only system in which this anesthesia differed from conscious animals was NO availability. This difference may be caused by anesthesia-induced activation of other vasodilatator systems distinct from nitric oxide, which may contribute to the attenuation of nitric oxide-dependent mechanism (Toda et al., 2007).

The combination of ketamine-xylazine totally abolished sympathetic BP control component. This effect is probably caused by xylazine, which is an agonist of the α_2-adrenergic receptors, which lowers central sympathetic outflow (Hsu, 1981; Guimarães and Moura, 2001). A complete peripheral sympathetic blockade results in significantly lowered BP. It is probable that this mechanism is responsible, because use of ketamine alone has cardiovascular stimulating effects (Flacknell, 2009). To confirm this fact we tried to anesthetize the rats by ketamine alone, but due to its poor anesthetic and analgesic effect in rat (Flecknell, 2009), the animals were not anesthetized enough for surgical procedure and measurement of BP.

The inhalation anesthetic isoflurane caused the attenuation of all measured pressor or dilator systems involved in BP maintenance. This attenuation may be caused by the hyperpolarization of vascular smooth muscle cells (Stekiel et al., 2001) and/or by the inhibition of Ca^{2+} influx through voltage-gated Ca^{2+} channels (Akata et al., 2007).

Chloralose-urethane caused a decrease in BP by the suppression of all BP regulating systems. According to Smith and Hutchins (1980) heart rate and peripheral resistance were not significantly changed but BP, cardiac index and stroke volume were reduced. I observed that mean arterial pressure was depressed and all BP regulating systems were attenuated. The observed suppression of all cardiovascular systems under chloralose-urethane or isoflurane anesthesia should be considered in all studies using these anesthetics when the obtained cardiovascular data are translated to conscious animals.

Anesthetics influenced BP regulating mechanisms similarly in spontaneously hypertensive rats as in normotensive Wistar rats. Mean arterial pressure was attenuated in all used anesthesia. Compared to Wistar rats ketamine-xylazine elicited greater BP fall in SHR, which corresponds with elevated activity of sympathetic nervous system in SHR (Head, 1989). The magnitude of BP responses was augmented in size, due to the elevation of basal BP. BP response to SNS blockade by pentolinium was abolished under ketamine-xylazine and preserved under pentobarbital anesthesia (Figure 7). Blockade of RAS caused

similar BP changes with the exception of chloralose-urethane anesthesia, where captopril caused a pronounced BP fall. It is possible that CU anesthesia in SHR strain may potentiate renin-angiotensin system.

Pentobarbital and ketamine-xylazine anesthesia induced similar BP effects in Dahl salt sensitive rats. Mean arterial pressure was attenuated, sympathetic nervous system and renin-angiotensin system components were affected as in Wistar and spontaneously hypertensive rats.

6.2 Lowering of blood pressure by L-serine

L-serine action has already been investigated in NO-deficient type of hypertension *in vivo*. L-serine evoked a rapid, reversible, dose-dependent fall in mean arterial pressure, which was more pronounced in L-NAME-treated hypertensive rats than in the control rats. This acute BP lowering effect of L-serine was significantly inhibited by apamin and charybdotoxin pretreatment, i.e. by the combination that blocks Ca^{2+}-activated K^+ channels underlying the endothelium-derived hyperpolarizing factor (EDHF) response. The authors suggested that the opening of K^+ channels (IK_{Ca}, SK_{Ca}) may be responsible for vascular effects of L-serine and L-serine may serve as an inductor of EDHF-mediated vasodilatation (Mishra et al., 2008).

My experiments were designed to confirm the effects of L-serine in salt-induced model of experimental hypertension and to determine the influence of anesthesia. Mishra et al. (2008) used barbiturate anesthesia (thiopental) in their *in vivo* studies. In my study pentobarbital was used as barbiturate anesthesia and ketamine-xylazine as a different type of anesthesia. In animals with preserved NO synthesis the increasing doses of L-serine caused a more pronounced dose-dependent BP fall under ketamine-xylazine anesthesia as compared to pentobarbital anesthesia. After the acute blockade of NO synthesis by L-NAME, the differences in L-serine-induced BP falls between these two anesthetic states diminished (Figure 12). Thus L-serine-induced EDHF-mediated vasodilatation is more pronounced in the absence of NO synthesis. A pronounced BP effect of L-serine before NO synthesis blockade was found under ketamine-xylazine anesthesia which inhibited sympathetic nervous system. It means that the suppression of sympathetic nervous system is also an important factor for L-serine-induced BP effects (Figure 12).

Since NO and sympathetic nervous system participation in BP control is influenced by anesthesia, the administration of L-serine in conscious rats before and after the blockade of each system was also performed. L-serine did not produce any BP effect in intact conscious animals. Blockade of NO synthesis increased BP and enabled a manifestation of L-serine-induced BP effects. It is assumed that L-serine evokes EDHF response, which functions as a substitution for NO system (Ozkor and Quyyumi, 2011). Thus BP effect of L-serine after NO blockade confirms these assumptions. Consecutive blockades of sympathetic nervous system and renin-angiotensin system enhanced BP effect of L-serine, indicating that L-serine-induced BP effects were attenuated by the activity of these vasoconstrictor systems. This observation clarifies the pronounced L-serine-induced BP effect observed under ketamine-xylazine anesthesia before the blockade of NO synthesis.

As far as salt-induced hypertension in Dahl rats is concerned, L-serine produced more profound BP falls in salt-sensitive rats, indicating that EDHF response may serve as a part of antihypertensive mechanisms in this rat strain. According to studies in conscious rats (Figure 14), this mechanism was also augmented in normotensive salt-sensitive rats compared to salt-resistant animals. This suggests the increased importance of this vasodilator mechanism in salt-sensitive Dahl rats even without developed hypertension. It was shown in our laboratory that Dahl salt-sensitive rats are relatively deficient in NO system (Behuliak et al., 2011). Thus the increased importance of EDHF mechanism may be related to the relative NO deficiency in this rat strain

7. Conclusions

It is well known that anesthetized animals differ in their responses to experimental stimulations and the researcher using anesthetized animals should always be aware of particular anesthesia-induced changes, especially in the studies of physiological systems such as BP. There are not only the differences between experimental conditions in studies using conscious and anesthetized rat, but there are also major differences dependent on the type of anesthesia used. Our study shows major differences between four commonly used types of anesthesia in comparison with conscious animals that were disclosed on the basis of the inhibition of main BP regulating systems. The inhibition of particular system causes BP changes which represent the contribution of these systems in BP maintenance. When the experimental protocol is scheduled, the anesthesia should be selected according to its properties and/or effects. If hemodynamic conditions should be similar to conscious rats, the most suitable anesthetic is pentobarbital.

The second part of my study confirmed vasodilatator effects of L-serine, which are mediated by endothelium-derived hyperpolarizing factor (EDHF). They are evident in the absence of nitric oxide and they are augmented by the blockade of sympathetic nerve activity. In intact conscious animals L-serine did not produce any BP fall. The other important finding is that L-serine induced increased EDHF activity in salt-sensitive Dahl strain, which has a genetic predisposition to high BP. It is evident that abnormal EDHF response in Dahl salt-sensitive animals is not responsible for the development of salt hypertension. Just the opposite, EDHF response serves to augment vasodilatation systems contributing to the attenuation of elevated BP. Further studies are necessary to evaluate whether EDHF stimulation may be beneficial in hypertension treatment.

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