
REVIEW

The Abnormalities of Adrenomedullary Hormonal System in Genetic Hypertension: Their Contribution to Altered Regulation of Blood Pressure

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Summary

It is widely accepted that sympathetic nervous system plays a crucial role in the development of hypertension. On the other hand, the role of adrenal medulla (the adrenomedullary component of the sympathoadrenal system) in the development and maintenance of high blood pressure in man as well as in experimental models of hypertension is still controversial. Spontaneously hypertensive rats (SHR) are the most widely used animal model of human essential hypertension characterized by sympathetic hyperactivity. However, the persistence of moderately elevated blood pressure in SHR subjected to sympathectomy neonatally as well as the resistance of adult SHR to the treatment by sympatholytic drugs suggests that other factors (including enhanced activity of the adrenomedullary hormonal system) are involved in the pathogenesis of hypertension of SHR. This review describes abnormalities in adrenomedullary hormonal system of SHR rats starting with the hyperactivity of brain centers regulating sympathetic outflow, through the exaggerated activation of sympathoadrenal preganglionic neurons, to the local changes in chromaffin cells of adrenal medulla. All the above alterations might contribute to the enhanced release of epinephrine and/or norepinephrine from adrenal medulla. Special attention is paid to the alterations in the expression of genes involved in catecholamine biosynthesis, storage, release, reuptake, degradation and adrenergic receptors in chromaffin cells of SHR. The contribution of the adrenomedullary hormonal system to the development and maintenance of hypertension as well as its importance during stressful conditions is also discussed.

Key words

Adrenal gland • Chromaffin cells • Epinephrine • Blood pressure • Cardiovascular system • Stress

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Introduction

The sympathetic nervous system (SNS) in cooperation with other humoral and local factors is involved in the regulation of arterial blood pressure (BP) through the changes of regional vascular resistance and/or cardiac output. SNS contribution varies under particular circumstances, such as postural changes, physical exercise, stress, etc. It is widely accepted that SNS plays a crucial role in the development of human hypertension (Fisher and Paton 2012) and various forms of experimental hypertension (Mancia and Grassi 2014). On the other hand, the role of adrenal medulla (the adrenomedullary component of the sympathoadrenal system) in the development and maintenance of high blood pressure in man as well as in experimental models is still controversial (Floras 1992, Elam and Grassi 2000). Spontaneously hypertensive rats (SHR) are the mostly used animal model of human essential hypertension (Yagil and Yagil 2001). They develop hypertension

without any physiological, pharmacological or surgical intervention at the age of 5-12 weeks and their mean arterial pressure in adulthood achieves 160-180 mm Hg, which is in contrast to 110-130 mm Hg in adult normotensive controls of Wistar-Kyoto (WKY) strain (Judy and Farrell 1979, Behuliak *et al.* 2015). Numerous functional and structural abnormalities were described in SHR including abnormal neurohumoral regulation, vascular hypertrophy, impaired endothelium-dependent relaxation, renal dysfunction, etc. (Zicha and Kunes 1999, Pintérová *et al.* 2011). The sympathetic nervous system is considered to be involved in the pathogenesis of hypertension in SHR since the sympathetic activity rises dramatically in parallel with BP development (Judy and Farrell 1979). Moreover, the development of hypertension in SHR can be substantially attenuated by neonatal sympathectomy (destruction of sympathetic nervous system, e.g. by guanethidine administration) (Lee *et al.* 1987, Korner *et al.* 1993) but this intervention is markedly less efficient in reducing BP in adult SHR (Yamori *et al.* 1972, Ferrari *et al.* 1991, Vavřínová *et al.* 2019b). The persistence of moderately elevated BP in SHR subjected to neonatal sympathectomy as well as the resistance of adult SHR to guanethidine treatment suggests that other factors are involved in the pathogenesis of hypertension in SHR, including the enhanced activity of the adrenomedullary system (Borkowski 1991, Lee *et al.* 1991a, Lee *et al.* 1991b). Indeed, some papers reported increased plasma levels of epinephrine and its metabolite metanephrine in SHR compared to normotensive WKY rats (Vlachakis *et al.* 1980, Szemerédi *et al.* 1988, Vavřínová *et al.* 2019b). On the other hand, similar plasma levels of epinephrine and metanephrine were described by many other researchers (Szemerédi *et al.* 1988, Moura *et al.* 2005, Behuliak *et al.* 2018, Vavřínová *et al.* 2019a). These discrepancies can be partly explained by the influence of stress during particular experimental conditions since the plasma levels of epinephrine are increased more in SHR than in WKY rats following stress (McCarty *et al.* 1978, Kvetnansky *et al.* 1979). This review deals with numerous abnormalities in the adrenomedullary system described in SHR with established hypertension as well as before the development of high blood pressure (Kumai *et al.* 1994, Miranda-Ferreira *et al.* 2008, Vavřínová *et al.* 2019a). This approach might help to distinguish the abnormalities that are rather the consequences of high blood pressure from those that can play a decisive role in the pathophysiology of hypertension in SHR.

The functional organization of the adrenomedullary system

In general, the autonomic nervous system (sympathoadrenal and parasympathetic branch) is regulated by a complex central neural network (e.g. nucleus of the solitary tract, NTS; paraventricular nucleus of hypothalamus, PVN; rostral ventrolateral medulla, RVLM; nucleus ambiguus, NA etc.), which control the activity of particular efferent preganglionic neurons innervating either sympathetic postganglionic neurons, adrenal medulla or parasympathetic postganglionic neurons. The sympathetic and parasympathetic postganglionic neurons form synapses with target tissues (e.g. vascular smooth muscle cells, cardiac conduction system, etc.), whereas the chromaffin cells of adrenal medulla release catecholamines into the blood stream thus influencing the distant tissues in the organism. The regulation of sympathoadrenal and parasympathetic nervous system is complex and exerts reciprocal interactions of both systems at the level of PVN, RVLM, preganglionic neurons as well as at the level of postganglionic neurons and target tissues allowing the precise regulation of organ and tissue functions (Ondicova and Mravec 2010). Considering the adrenomedullary system, the evidence indicates that there are two separate populations of chromaffin cells releasing either epinephrine or norepinephrine, which are regulated by distinct neural pathways allowing the differential secretion according to the physiological demands of the organism (Flatmark 2000, de Diego *et al.* 2008). Thus, there is a high norepinephrine release by adrenal medulla during cold exposure (although the major part of norepinephrine during cold exposure is released by sympathetic nerve endings), whereas high epinephrine release was found during hypoglycemia (Khalil *et al.* 1986, Vollmer *et al.* 1992).

Transneuronal retrograde cell-body labeling technique demonstrated that there are at least 5 brain areas directly involved in the regulation of efferent sympathoadrenal preganglionic neurons: caudal raphe nuclei, ventromedial medulla, rostral ventrolateral medulla (RVLM), A5 cell group, and paraventricular nucleus of hypothalamus (PVN) (Strack *et al.* 1989). RVLM is one of the most important central regions involved in cardiovascular regulation, which integrates the information coming from various peripheral receptors (vestibular receptors, skeletal muscle receptors, nociceptors etc.), nucleus of the solitary tract (mediating

baroreceptor and chemoreceptor reflexes), PVN (involved in the regulation of body fluids, metabolism and temperature) and higher brain regions. RVLM is also a crucial structure for the baroreflex regulation which determines both sympathoadrenal and sympathoneural outflow (Guyenet 2006, Dampney 2016). Anterograde and retrograde tracing method demonstrated that neurons projecting to sympathoadrenal preganglionic neurons are concentrated in the more rostral part of the rat RVLM and less at the caudal level of the nucleus (Zagon and Smith 1993, Pyner and Coote 1998). In contrast, the neurons projecting to cervical preganglionic neurons are more dispersed from rostral to caudal levels of the RVLM nucleus (Pyner and Coote 1998). However, a double-virus transneuronal labeling technique revealed that some RVLM neurons are involved in the regulation of both sympathoneural and sympathoadrenal outflow, which probably work under certain circumstances where parallel sympathetic activation is desirable, such as during the fight-or-flight response (Jansen *et al.* 1995). Physiological studies in cats revealed the existence of the RVLM territories the stimulation of which could preferably activate particular sympathetic outflows regulating different functions of the organism, e.g. muscle vasoconstriction, visceral vasoconstriction and kidney function (McAllen and May 1994). However, no functional experiments differentiating RVLM areas involved in the regulation of either sympathoneural or sympathoadrenal outflows in the rat were published so far. In RVLM of SHR, immunohistochemical staining demonstrated increased basal number of Fos-positive immunoreactive neurons compared to WKY rats (Minson *et al.* 1996, Palmer and Printz 1999). On the other hand, the BP reduction after the administration of NO donor sodium nitroprusside increased a number of Fos-positive immunoreactive RVLM neurons in WKY rats but not in SHR (Minson *et al.* 1996). Similarly, a psychological stimulus (airpuff startle) caused lower activation of RVLM neurons in SHR than in WKY rats (2-fold or 4-fold increase, respectively) (Palmer and Printz 1999). A faster firing rate was described in RVLM and PVN neurons of SHR compared to WKY rats (Matsuura *et al.* 2002, Li *et al.* 2008, Stern *et al.* 2012) which is in line with the above mentioned increased basal Fos immunoreactivity. Moreover, the hyperpolarization of RVLM and PVN neurons (induced by the microinjection of a viral vector coding for human inward-rectifier Kir2.1-potassium channel) resulted in the reduction of sympathetic outflow and BP decrease in SHR (Gerald

et al. 2014, Gerald *et al.* 2016). Thus, it is clear that the hyperactivity of the brain centers regulating sympathetic outflow contribute to high BP in SHR but a more detailed information concerning the regulation of sympathoneural and sympathoadrenal outflows would be desirable.

The cell bodies of sympathoadrenal preganglionic neurons are located in the intermediolateral cell column between the first and the thirteenth thoracic segments (Zagon *et al.* 1993, Pyner and Coote 1994, Pyner and Coote 1998, Mueller *et al.* 2011). Sympathoadrenal preganglionic neurons occupy the lateral aspect of the intermediolateral cell column, whereas preganglionic neurons projecting to superior cervical ganglion and stellate ganglion are located more medially or centrally, respectively (Pyner and Coote 1994). The axons of the sympathoadrenal preganglionic neurons arise from the sympathetic trunk as the splanchnic nerves and form the synapses with chromaffin cells of adrenal medulla (Mueller *et al.* 2011). It was proposed that there are two distinct populations of sympathoadrenal preganglionic neurons differing in their sensitivity to various stimuli and also in their conduction velocity. Morrison *et al.* (2000) demonstrated that the first group comprised slowly conducting preganglionic neurons. These neurons were markedly excited during the pseudo-hypoglycemia induced by 2-deoxy-D-glucose, but they exhibited little or no sensitivity to the baroreceptor reflex activation. They probably innervate epinephrine-producing chromaffin cells. By contrast, the second group of sympathoadrenal preganglionic neurons showed rapid conduction velocity similar to barosensitive vasoconstrictor sympathetic preganglionic neurons. These neurons were unaffected by pseudo-hypoglycemia, but they were highly sensitive to baroreceptor reflex activation. They likely regulate norepinephrine release from the adrenal medulla (Morrison *et al.* 2000). This suggests that norepinephrine-producing chromaffin cells of adrenal medulla might be regulated in concert with sympathetic neurons innervating blood vessels. In the spinal cord of SHR, there is a greater basal incidence of Fos-positive sympathoadrenal preganglionic neurons compared to WKY rats (Minson *et al.* 1996), which is in accordance with the increased constitutive activity of the splanchnic nerves in this hypertensive strain (Morrison and Whitehorn 1984, Ricksten *et al.* 1984). Moreover, a greater number of sympathoadrenal preganglionic neurons was activated by nitroprusside administration in SHR (Minson *et al.* 1996) and the enhanced response to hypothalamic stimulation was described in splanchnic

nerves of SHR compared to WKY rats (Takeda and Buñag 1978, Morrison and Whitehorn 1984). Furthermore, SHR exhibited more pronounced activation of sympathoadrenal preganglionic fibers to various stimuli, including ganglionic blockade by trimethaphan, mental stress caused by air-jet and pseudo-hypoglycemia induced by 2-deoxy-D-glucose when compared to normotensive WKY rats (Zhang and Thorén 1998). The enhanced activation of preganglionic neurons observed after various stimuli in SHR is not associated with the augmented activation of RVLM neurons (see above) and the contribution of other brain areas remains to be elucidated. However, excitatory stimulation elicited greater adrenal nerve responses in anesthetized spinally transected SHR compared to WKY rats (Schramm and Chornoboy 1982). Taken together, the hyperexcitability of sympathoadrenal preganglionic neurons in SHR seems to be at least partially independent of the central influence. This abnormality contributes to the greater stress-induced catecholamine release by adrenal medulla in SHR and potentially participates in hypertension development.

The main physiological stimulus for catecholamine secretion from adrenal medulla is acetylcholine (released by preganglionic neurons) which induces depolarization of chromaffin cells and subsequent increase in their intracellular calcium (Burgoyne 1991). Catecholamine release is modulated by substances such as neuropeptide Y, angiotensin II, substance P, cholecystokinin or adrenocorticotrophic hormone (Mravec 2005, Guérineau 2020). It was hypothesized that adrenal medulla might play a role as a sensory organ and thus the non-cholinergic stimulation of chromaffin cells might participate in more complex regulation of catecholamine release (Mravec 2005). Catecholamine release evoked by the stimulation of cholinergic receptors as well as the membrane depolarization was described to be augmented in perfused adrenal glands of SHR in comparison to those of WKY rats (Lim *et al.* 2002, Bomfim *et al.* 2017). This was caused by both enhanced calcium signaling (de Pascual *et al.* 2013) and faster exocytosis of more vesicles as well as by greater quantal catecholamine content in hypertensive rats (Miranda-Ferreira *et al.* 2008). Moreover, the adrenal medulla of SHR contains a greater area of the norepinephrine releasing cell islets. Moreover, the increased number of both norepinephrine granules and vesicles was demonstrated in SHR from the prehypertensive stage to adulthood as compared to

WKY rats (Tabei *et al.* 1988). Thus, the local alterations of catecholamine biosynthesis, storage, release, reuptake and degradation in chromaffin cells might also contribute to the enhanced release of epinephrine and/or norepinephrine from adrenal medulla in hypertensive rats. These aspects will be discussed in detail in the following parts of this review.

Catecholamine biosynthesis

The scheme of catecholamine biosynthesis, storage, release, reuptake and degradation in chromaffin cells is shown in the Figure 1. The catecholamine biosynthesis starts with an import of amino acid L-tyrosine and its hydroxylation by the enzyme tyrosine hydroxylase (TH, encoded by *Th* gene; Nagatsu *et al.* 1964). For catecholamine biosynthesis, TH enzyme requires Fe^{2+} , molecular oxygen and the regulatory cofactor tetrahydrobiopterin (Nagatsu *et al.* 1964) which is synthesized by guanosine triphosphate cyclohydrolase 1 (encoded by *Gchl* gene) and recycled by quinoid dihydropteridine reductase (encoded by *Qdpr* gene; Thöny *et al.* 2000). Tyrosine hydroxylation is a rate-limiting step of catecholamine synthesis and it is a subject of complex regulation, including direct inhibition of the enzyme by catecholamines, post-translational modifications and changes in the transcription of *Th* gene (for review see Tekin *et al.* 2014). The second enzyme involved in catecholamine synthesis is L-DOPA decarboxylase (DDC, encoded by *Ddc* gene) which converts L-DOPA to dopamine (Blaschko 1942). Subsequently, dopamine is converted by the enzyme dopamine β -hydroxylase (DBH, encoded by *Dbh* gene) to form norepinephrine (Friedman and Kaufman 1965). The last enzyme phenylethanolamine N-methyl transferase (PNMT, encoded by *Pnmt* gene), which synthesizes epinephrine from norepinephrine, can be predominantly found in chromaffin cells of adrenal medulla, whereas extra-adrenal PNMT is expressed in a small number of epinephrine-producing neurons in the central nervous system and in some non-neuronal cells of the heart. Morphological studies showing the greatest expression of PNMT and/or epinephrine in the peripheral portion of the medulla, closest to the cortex, suggest that glucocorticoids are critical for efficient epinephrine synthesis in the chromaffin cells (Wong *et al.* 1987, Wong *et al.* 2003). It was shown that the expression and activity of catecholamine biosynthetic enzymes in chromaffin cells is regulated in a stimulus-specific

manner by several mechanisms including acetylcholine (released from sympathetic innervation), glucocorticoids and Ang II (Livett and Marley 1993, Stachowiak *et al.* 1990, Wong 2006). Other experiments using a combination of stress with various interventions (including hypophysectomy, preganglionic denervation of sympathetic nerves or the adrenal medulla, treatment with

hormones or neural agents, e.g. ACTH, glucocorticoid, acetylcholine etc.) further suggest that the induction of *Th* gene may be primarily mediated by the neural activity, whereas the regulation of *Pnmt* gene is dependent mainly on hormonal influences (Axelrod and Reisine 1984, Viskupic *et al.* 1994).

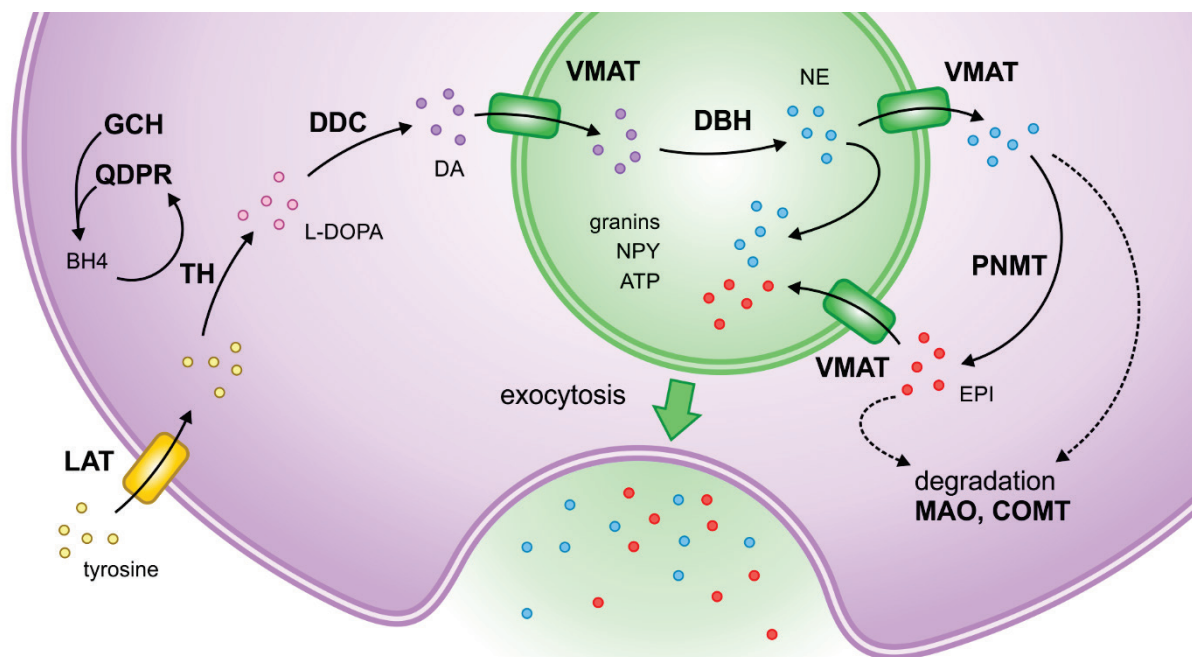


Fig. 1. The scheme of catecholamine biosynthesis, storage, release and degradation in chromaffin cells of the adrenal medulla. The amino acid L-tyrosine is imported into the cytoplasm of chromaffin cells by L-type amino acid transporter (LAT) and converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase (TH). TH requires the regulatory cofactor tetrahydrobiopterin (BH4) which is synthesized by guanosine triphosphate cyclohydrolase (GCH) and recycled by quinoid dihydropteridine reductase (QDPR). L-DOPA is converted by L-DOPA decarboxylase (DDC) to form dopamine (DA). DA is transported to the chromaffin vesicles by the vesicular monoamine transporter (VMAT). The enzyme dopamine β-hydroxylase (DBH) catalyzes DA conversion to norepinephrine (NE). The final step of biosynthesis is performed in the cytoplasm by phenylethanolamine N-methyltransferase (PNMT) which synthesizes epinephrine (EPI) from NE. The content of chromaffin vesicles (NE, EPI, granins, neuropeptide Y (NPY) and ATP) is released from the cells by the process of exocytosis. Monoamine oxidases (MAO) and catechol-O-methyltransferase (COMT) remove catecholamines from the cytoplasm (after vesicular leakage).

Many studies have been published concerning the catecholamine biosynthesis in the adrenal glands of SHR with established hypertension, but their results are quite contradictory (see Table 1). The mRNA and protein expression of *Th* and *Pnmt* genes in the adrenal gland of adult SHR was reported to be higher (Kumai *et al.* 1994, Reja *et al.* 2002a, Reja *et al.* 2002b, Nguyen *et al.* 2009) or lower than in adrenals of WKY rats (Moura *et al.* 2005, Grundt *et al.* 2009, Vavřínová *et al.* 2019a). Moreover, we observed the downregulated adrenal expression of other enzymes involved in catecholamine biosynthesis (*Ddc* and *Dbh*) as well as the enzymes

producing cofactor tetrahydrobiopterin (*Gch1*, *Qdpr*) for TH in adult SHR compared to WKY rats (Vavřínová *et al.* 2019a). The studies concerning adrenal content of dopamine, norepinephrine and epinephrine also provided rather conflicting results (Lee *et al.* 1991a, Korner *et al.* 1993, Moura *et al.* 2005, Vavřínová *et al.* 2019a, Vavřínová *et al.* 2019b). The inconsistent results might be caused by extreme susceptibility of the catecholaminergic system to stressful conditions and the differences in stress response between SHR and WKY rats. It is well known that acute stress elevates plasma levels of catecholamines and induces the

Table 1. The overview of papers evaluating the adrenal mRNA expression, protein expression and the enzyme activity of genes involved in catecholamine biosynthesis in spontaneously hypertensive rats (SHR). Gene symbols referring to mRNA expression are italicized, whereas all letters are in upper-case when describing protein expression or enzyme activity. The information about anesthesia, euthanasia and other important factors can be found in a note section. Symbols ↓, ↑ and = represent lower, higher or similar expression in SHR, respectively, compared to Wistar-Kyoto controls, if not stated otherwise. AG, adrenal gland; AM, adrenal medulla; IP, intraperitoneal; qPCR, quantitative real-time PCR; sqPCR, semiquantitative real-time PCR; w, weeks; WB, western-blot.

Reference	Age	Parameter (method)	Tissue	Gene change in SHR	Note
Friese <i>et al.</i> 2005	4w	mRNA (chip)	AG	<i>Dbh, Pnmt</i> <i>Th, Gchl</i>	↓ = Anesthesia not specified Euthanasia not specified
Grobecker <i>et al.</i> 1982	2w 4w 8w 14w	Enzyme activity	AG	TH, DBH, PNMT TH, DBH, PNMT TH DBH, PNMT TH DBH, PNMT	↓ ↓ = ↓ ↑ = Tail-cuff (timepoint not specified) Decapitation
Grundt <i>et al.</i> 2009	20w	mRNA (qPCR)	AG	<i>Th</i>	↓ Stress-naive Decapitation
Grundt <i>et al.</i> 2009	20w	mRNA (qPCR)	AG	<i>Th</i>	= Immediately after tail-cuff Decapitation
Jirout <i>et al.</i> 2010	6w	mRNA (chip)	AG	<i>Th, Ddc, Dbh</i> <i>Pnmt, Gchl, Qdpr</i>	↓ = Brown-Norway control Cervical dislocation
Kumai <i>et al.</i> 1994	25w	mRNA (Northern blot) Enzyme activity	AM	<i>Th</i> TH	↑ ↑ Tail-cuff (timepoint not specified) Anesthesia not specified Euthanasia not specified
Kumai <i>et al.</i> 2001	15w	Protein (WB) Enzyme activity		TH TH	↑ ↑ Tail-cuff (timepoint not specified) Pentobarbital anesthesia Decapitation
Moura <i>et al.</i> 2005	5w 12w 22w	Protein (WB) Enzyme activity Protein (WB) Enzyme activity Protein (WB) Enzyme activity	AG	TH TH TH TH TH TH	↓ ↓ ↓ ↓ ↓ ↓ Tail-cuff (timepoint not specified) Sodium pentobarbital
Nagatsu <i>et al.</i> 1976	3w	Enzyme activity	AG	DBH	↑ Decapitation
Nagatsu <i>et al.</i> 1977	16w	Enzyme activity	AG	TH, DBH	↑ Tail-cuff (timepoint not specified) Decapitation
Nguyen <i>et al.</i> 2009	16w	mRNA (sqPCR) Protein (WB)	AG	<i>Dbh</i> <i>Th, Pnmt</i> PNMT	= ↑ ↑ Repeated tail-cuff Ketamine-xylazine anesthesia Decapitation
O'Connor <i>et al.</i> (1999)	20-24w	Enzyme activity	AG	DBH PNMT	↓ = Tail-cuff (timepoint not specified) Anesthesia not specified Euthanasia not specified
Reja <i>et al.</i> 2002a	18w	mRNA (qPCR)	AM	<i>Th</i>	↑ Tail-cuff 2 weeks before sampling Euthanasia by sodium pentobarbitone IP
Reja <i>et al.</i> 2002b	20w	mRNA (qPCR)	AM	<i>Pnmt</i>	↑ Tail-cuff 2 weeks before sampling Euthanasia by sodium pentobarbitone IP

Vavřínová <i>et al.</i> 2019a	4w	mRNA (qPCR)	AM	<i>Dbh, Pnmt</i>	↓	Isoflurane Exsanguination
				<i>Th, Ddc, Qdpr</i>	=	
	24w	Protein (WB)	AG		<i>Gchl</i>	↑
					DDC, DBH	↓
				TH, PNMT	=	
		mRNA (qPCR)	AM	<i>Th, Ddc, Dbh, Pnmt,</i>	↓	
				<i>Qdpr, Gchl</i>	↓	
		Protein (WB)	AG	TH, DDC, DBH	↓	
				PNMT	=	
Vavřínová <i>et al.</i> 2019b	22- 24w	mRNA (qPCR)	AM	<i>Th</i>	=	Daily IP injection of saline for 2 weeks Isoflurane Exsanguination
				<i>Ddc, Dbh, Pnmt</i>	↓	

expression of catecholamine biosynthetic enzymes in adrenal medulla (Kvetnansky *et al.* 2004, Kvetnansky *et al.* 2009). By contrast, the chronic stress induced by social isolation decreases basal expression of *Th* gene (Gavrilovic *et al.* 2008), but chronically stressed animals exhibit higher plasma epinephrine levels and the augmented induction of adrenal *Th* expression when exposed to a novel stressor (McCarty *et al.* 1988, Gavrilovic *et al.* 2008). Adult SHR acutely exposed to immobilization show higher plasma levels of norepinephrine and epinephrine compared to WKY rats (Kvetnansky *et al.* 1979). Moreover, the increase in adrenal mRNA expression of *Th*, induced by 25 min of mild stress due to tail cuff measurement of blood pressure, was found to be greater in adult SHR compared to WKY rats (Grundt *et al.* 2009). These findings are in agreement with the above mentioned exaggerated activation of sympathoadrenal preganglionic fibers observed in SHR subjected to physical or psychological stressors (Zhang and Thorén 1998). Actually, several research groups, which reported on the hyperactivation of the adrenomedullary system in SHR, exposed the rats acutely or repeatedly to the stressful procedures such as tail-cuff BP measurement or repeated intraperitoneal administration of drugs (Korner *et al.* 1993, Kumai *et al.* 1994, Nguyen *et al.* 2009). It should be realized that the use of various types of anesthesia (sodium pentobarbital, ketamine-xylazine, isoflurane, etc.) as well as methods of anesthesia (decapitation, cervical dislocation, overdose by anesthetics, etc.) might influence the results, but the details concerning these procedures are often not included in the papers (see notes in Table 1). Besides the differences in the adrenomedullary system, SHR exhibited many other signs of their vulnerability to stress, including higher stress-induced plasma corticosterone

levels (Kvetnansky *et al.* 1979) or ACTH levels (Behuliak and Vavřínová unpublished data). Furthermore, adult stress-naïve SHR showed adrenal hypertrophy, increased locomotion (Vavřínová *et al.* 2019a, Vavřínová *et al.* 2019b), hyperthermia (Hajós and Endberg 1986) and thymic atrophy (Suzuki *et al.* 1999), that might be considered as the hallmarks of adaptation to chronic stress (Ulrich-Lai *et al.* 2006). Brown *et al.* (1988) demonstrated that intracerebroventricular administration of corticotropin-releasing hormone (CRH) produced a greater increase of plasma epinephrine in SHR suggesting that the central stress pathways might trigger the augmented activation of the adrenomedullary system in this rat strain.

The adrenomedullary system might be prone to the greater activation already in the prehypertensive stage. Similar to adult SHR, catecholamine biosynthetic pathway was described to be downregulated, unchanged or upregulated in adrenal gland of 4-week-old prehypertensive SHR compared to WKY rats (Grobeck *et al.* 1976, Nagatsu *et al.* 1976, Friese *et al.* 2005, Moura *et al.* 2005, Vavřínová *et al.* 2019a). Accordingly, the increased cardiac index and heart rate was observed in conscious partially restrained 4-week-old SHR compared to WKY rats (Smith and Hutchins 1979, Behuliak *et al.* 2015, Vavřínová *et al.* 2019a). It seems that the central stress pathways are hyperresponsive in prehypertensive SHR because the exaggerated CRH-induced ACTH response and higher corticosterone levels were described in this strain already at the age of 5-6 weeks (Hattori *et al.* 1986, Hashimoto *et al.* 1989, Sterley *et al.* 2011). Indeed, the acute central administration of tranquilizing agent muscimol (GABA type A receptor agonist) reduced sympathoadrenal activity, lumbar sympathetic nerve activity and BP, the effects being more pronounced in

SHR than in WKY rats (Unger *et al.* 1984, Allen 2002). Moreover, chronic treatment with tranquilizing drug diazepam (positive allosteric modulator of the GABA type A receptors) given from the newborn period markedly reduced BP and vascular resistance in SHR to the levels only slightly above those found in WKY rats (Schieken 1979). Thus, the genetic predisposition of SHR might determine their vulnerability to stress and these rats probably perceive usual care and handling as more stressful. This state of chronic stress can contribute to the above mentioned alterations of the adrenomedullary system in SHR (the decreased basal expression of catecholamine biosynthetic enzymes but the pronounced induction of the expression and the augmented catecholamine release under stress conditions). The alternative explanation is that the downregulation of catecholamine biosynthetic pathway might be a consequence of the high blood pressure, e.g. a compensatory mechanism counteracting the hyperactivity of sympatho-neural system in SHR. Indeed, SHR with transgenically overexpressed *Dbh* gene exhibited higher plasma levels of epinephrine and norepinephrine as well as higher blood pressure compared to non-transgenic SHR controls (Pravenec *et al.* 2016). Guanethidine-induced sympathectomy for 14 days increased the mRNA expression of *Th*, *Dbh* and *Pnmt* genes in adrenal medulla and elevated plasma levels of epinephrine of adult SHR and WKY rats. However, these effects were more pronounced in WKY rats than in SHR, which does not support the idea that the sympathetic hyperactivity is a cause of the downregulation of catecholamine biosynthetic pathway in SHR (Vavřínová *et al.* 2019b).

Taken together, the available evidence indicates that the catecholamine biosynthetic pathway is downregulated at different levels, i.e. mRNA expression, protein expression and the catecholamine content in the adrenal glands of stress-naïve SHR with established hypertension when compared to WKY rats. However, the acute or chronic stressful stimuli cause a more pronounced activation of the adrenomedullary system in SHR, which should be taken into account during the design of experiments and also during the interpretation of results.

Catecholamine vesicles

The speed and the effectiveness of catecholamine release from the chromaffin cells of adrenal medulla depend on the immediate availability of

vesicles filled with high content of catecholamines. The filling of catecholaminergic vesicles is mediated by vesicular monoamine transporters (VMAT) of two types (Blakely and Edwards 2012). Colocalization study showed that VMAT1 (encoded by *Slc18a1* gene) is widely expressed in all rat adrenal chromaffin cells, while VMAT2 (*Slc18a2* gene) is co-localized with TH but not with PNMT enzyme (Tillinger *et al.* 2010). This suggests that VMAT2 is expressed in norepinephrine- but not in epinephrine-synthesizing chromaffin cells. The mRNA expression of both *Vmat1* and *Vmat2* was reported to be decreased in adrenal medulla of adult SHR compared to WKY rats (Vavřínová *et al.* 2019a). The literature concerning expression of *Vmat* genes (and other genes related to catecholamine vesicles, reuptake or degradation which are discussed hereafter) in adrenal gland of SHR is summarized in Table 2. Our research group reported unchanged mRNA expression of *Vmat1* and lower expression of *Vmat2* in adrenal gland of prehypertensive SHR (Vavřínová *et al.* 2019a), whereas Friese *et al.* (2005) demonstrated the attenuated expression of *Vmat1* and the augmented expression of *Vmat2*. The inconsistent results might be caused either by different methods of measurement (quantitative real-time PCR vs. microarray analysis) or by different sampling of tissue (adrenal medulla vs. whole adrenal gland). It would be desirable to verify the expression of *Vmat2* in prehypertensive SHR since the augmented expression of this gene might be a sign of the transition of adrenal chromaffin cells from epinephrine-producing to norepinephrine-producing phenotype.

Catecholamine storage vesicles of the adrenal medulla contain remarkably high concentrations of chromogranins (encoded by *Chga* and *Chgb* genes), secretogranin (*Scg2* gene), neuropeptide Y (*Npy* gene) and adenosine triphosphate (ATP). These molecules might influence the amount of catecholamines ready for exocytosis or when released they can mediate feedback regulation of catecholamine release from chromaffin cells (Burnstock 2014). Granins stabilize the vesicle core osmotically and they are involved in the regulation of exocytosis (Zhang *et al.* 2011). *Chga* knockout mice had the decreased size and number of chromaffin granules as well as the reduced adrenal content of epinephrine and norepinephrine (Mahapatra *et al.* 2005). In the adrenal medulla of prehypertensive SHR, the mRNA expression of *Chga*, *Chgb* and *Scg2* was similar or slightly decreased compared to WKY rats (Friese *et al.* 2005, Vavřínová *et al.* 2019a). We reported the attenuated mRNA

expression of *Chga*, *Chgb* and *Scg2* genes in the adrenal medulla of adult SHR (Vavřínová *et al.* 2019a), whereas the increased mRNA and protein expression of *Chga* was shown in the adrenal medulla of adult SHR by O'Connor *et al.* (1999) and Nguyen *et al.* (2009). Nevertheless, the latter two studies used the repeated tail-cuff measurement

of BP, which might influence the results of these studies. Thus, similarly with the genes involved in the catecholamine biosynthesis, the basal expression of granins seems to be downregulated in adrenal medulla of SHR, but the expression might be activated by stressful conditions such as tail-cuff measurement use.

Table 2. The overview of papers evaluating the adrenal mRNA expression, protein expression and the enzyme activity of genes related to catecholamine vesicles, reuptake or degradation in spontaneously hypertensive rats (SHR). Gene symbols referring to mRNA expression are italicized, whereas all letters are in upper-case when describing protein expression or enzyme activity. The information about anesthesia, euthanasia and other important factors can be found in a note section. Symbols ↓, ↑ and = represent lower, higher or similar expression in SHR, respectively, compared to Wistar-Kyoto controls, if not stated otherwise. AG, adrenal gland; AM, adrenal medulla; IP, intraperitoneal; NB, northern blot; qPCR, quantitative real-time PCR; RIA, radioimmunoassay; sqPCR, semiquantitative real-time PCR; w, weeks; WB, western-blot.

Reference	Age	Parameter (method)	Tissue	Gene change in SHR		Note
Friese <i>et al.</i> 2005	4w	mRNA (chip)	AG	<i>Vmat1</i> , <i>Scg2</i> , <i>Maob</i>	↓	Anesthesia not specified
				<i>Chga</i> , <i>Chgb</i> , <i>Maoa</i> , <i>Net</i>	=	Euthanasia not specified
				<i>Vmat2</i> , <i>Comt</i>	↑	
Guffroy and Strollin Benedetti 1984	14-17w	Enzyme activity	AG	MAOA, MAOB	=	Cervical dislocation
Higuchi <i>et al.</i> 1993	6w	mRNA (NB)	AG	<i>Npy</i>	↓	Decapitation
		Protein (RIA)		NPY	=	
	12w	mRNA (NB)		<i>Npy</i>	↓	
		Protein (RIA)		NPY	↑	
17w	mRNA (NB)	<i>Npy</i>	=			
Jirout <i>et al.</i> 2010	6w	mRNA (chip)	AG	<i>Npy</i> , <i>Comt</i>	↓	Brown-Norway control
				<i>Vmat1</i> , <i>Chga</i> , <i>Chgb</i> , <i>Scg2</i> , <i>Maoa</i> , <i>Maob</i>	=	Cervical dislocation
					=	
Nguyen <i>et al.</i> 2009	16w	mRNA (sqPCR)	AG	<i>Chga</i>	↑	Repeated tail-cuff Ketamine-xylazine anesthesia Decapitation
O'Connor <i>et al.</i> (1999)	4w	Protein (WB)	AG	CHGA	↑	Tail-cuff (timepoint not specified)
	20-24w	mRNA (NB)		<i>Chga</i> , <i>Chgb</i>	↑	Anesthesia not specified
		Protein (WB)		CHGA	↑	Euthanasia not specified
Reja <i>et al.</i> 2002b	18w	mRNA (qPCR)	AM	<i>Net</i>	↑	Tail-cuff 2 weeks before sampling Euthanasia by sodium pentobarbitone IP
Tsunoda and Imai 2004	20-25w	Enzyme activity	AG	COMT	=	Diethyl ether anesthesia
Vavřínová <i>et al.</i> 2019a	4w	mRNA (qPCR)	AM	<i>Vmat2</i> , <i>Npy</i>	↓	Isoflurane
				<i>Vmat1</i> , <i>Chga</i> , <i>Chgb</i> , <i>Scg2</i> , <i>Net</i> , <i>Maoa</i> , <i>Maob</i> , <i>Comt</i>	=	Exsanguination
				<i>Vmat1</i> , <i>Vmat2</i> , <i>Chga</i> , <i>Chgb</i> , <i>Scg2</i> , <i>Npy</i> , <i>Net</i> , <i>Maoa</i>	↓	
24w	mRNA (qPCR)	<i>Comt</i>	↓			
		<i>Maob</i>	=			
			↑			

Catecholamine storage vesicles of the adrenal medulla contain remarkably high concentrations of chromogranins (encoded by *Chga* and *Chgb* genes), secretogranin (*Scg2* gene), neuropeptide Y (*Npy* gene) and adenosine triphosphate (ATP). These molecules might influence the amount of catecholamines ready for exocytosis or when released they can mediate feedback regulation of catecholamine release from chromaffin cells (Burnstock 2014). Granins stabilize the vesicle core osmotically and they are involved in the regulation of exocytosis (Zhang *et al.* 2011). *Chga* knockout mice had the decreased size and number of chromaffin granules as well as the reduced adrenal content of epinephrine and norepinephrine (Mahapatra *et al.* 2005). In the adrenal medulla of prehypertensive SHR, the mRNA expression of *Chga*, *Chgb* and *Scg2* was similar or slightly decreased compared to WKY rats (Friese *et al.* 2005, Vavřínová *et al.* 2019a). We reported the attenuated mRNA expression of *Chga*, *Chgb* and *Scg2* genes in the adrenal medulla of adult SHR (Vavřínová *et al.* 2019a), whereas the increased mRNA and protein expression of *Chga* was shown in the adrenal medulla of adult SHR by O'Connor *et al.* (1999) and Nguyen *et al.* (2009). Nevertheless, the latter two studies used the repeated tail-cuff measurement of BP, which might influence the results of these studies. Thus, similarly with the genes involved in the catecholamine biosynthesis, the basal expression of granins seems to be downregulated in adrenal medulla of SHR, but the expression might be activated by stressful conditions such as tail-cuff measurement use.

Neuropeptide Y (NPY) is a peptide stored together with the catecholamines in the adrenal medulla which acts as a co-transmitter, a neuromodulator and a neurohormone (Lymperopoulos *et al.* 2016). NPY administered intravenously caused prolonged BP increase, which was augmented in SHR compared to WKY rats (Miller and Tessel 1991). Westfall *et al.* (1990) demonstrated that NPY potentiated the vasoconstriction of the mesenteric arterial bed induced by phenylephrine, angiotensin II and arginine vasopressin. This effect was enhanced in SHR compared to WKY rats. On the other hand, NPY decreased norepinephrine release from the mesenteric arterial bed evoked by periarterial nerve stimulation and this NPY action was attenuated in SHR (Westfall *et al.* 1990). Only few studies reported about the effect of NPY on chromaffin cells and their results are contradictory. NPY co-released due to cholinergic stimulation inhibited the parallel catecholamine secretion

from cultured rat chromaffin cells (Shimoda *et al.* 1993). By contrast, Cavadas *et al.* (2006) showed that NPY increased catecholamine release from the cultured mouse chromaffin cells, but the constitutive catecholamine release from adrenal medulla was elevated in NPY Y₁ receptor knockout mice. Furthermore, it was reported that stress-induced increase of catecholamine release is prevented in NPY knockout mice (Wang *et al.* 2013). Apart from the effects on the catecholamine release NPY is also involved in the regulation of catecholamine biosynthesis. Hong *et al.* (1995) showed that acute intravenous NPY administration increased mRNA expression of *Th* gene in rat adrenal medulla. On the other hand, NPY Y₁ receptor knockout mice exhibited higher TH activity in the adrenal glands and the incubation with NPY decreased *Th* promoter activity in Y₁ receptor expressing cells (Cavadas *et al.* 2006). In NPY knockout mice, basal TH immunoreactivity was increased in adrenals compared to wild-type animals suggesting that NPY exerts tonic inhibitory action on *Th* expression (Wang *et al.* 2013). In contrast, NPY knockout mice exhibited smaller stress-induced increase in the adrenal TH immunoreactivity than wild-type mice (Wang *et al.* 2013). Thus, the regulation of adrenal medulla by NPY is very complex and further investigation would be desirable. The decreased mRNA expression of *Npy* gene was found in adrenal gland of young as well as adult SHR (Higuchi *et al.* 1993, Vavřínová *et al.* 2019a). The contribution of lower NPY expression to the observed alterations in the adrenomedullary system in SHR remains to be elucidated.

Catecholamine reuptake and degradation

Catecholamine uptake at the neuroeffector junction is an important mechanism for the regulation of the synaptic norepinephrine concentrations. The catecholamine reuptake by norepinephrine transporter (NET, encoded by *Slc6a2* gene) was enhanced in the blood vessels of SHR where it possibly compensates greater norepinephrine release from the sympathetic nerve endings (Whall *et al.* 1980, Hano and Rho 1989). On the other hand, the adrenal medulla is an endocrine organ and thus there is no reason for reuptake of released epinephrine and norepinephrine. Accordingly, the drugs using NET to enter the target cell (e.g. tyramine, 6-hydroxydopamine or guanethidine) work in the sympathetic

nerve endings but they do not influence the adrenal medulla (Thoenen and Tranzer 1973, Johnson and O'Brien 1976, Wakade and Wakade 1984). However, NET was surprisingly present in epinephrine- but not in norepinephrine-producing cells of rat adrenal medulla (Phillips *et al.* 2001). Moreover, NET was shown to be localized primarily in the cytoplasm rather than in the cell membrane (Kippenberger *et al.* 1999, Phillips *et al.* 2001). Reja *et al.* (2002b) reported higher mRNA expression of *Net* in the adrenal medulla of adult SHR compared to WKY rats. By contrast, we observed lower mRNA expression of *Net* in the adrenal medulla of adult SHR, but similar *Net* expression in 4-week-old SHR and WKY rats (Vavřínová *et al.* 2019a). However, the role of NET in rat adrenal chromaffin cells was not explained yet. Thus, it is not clear whether the altered mRNA expression of *Net* in adrenals of adult SHR might have any physiological effect.

Catecholamines are degraded by the enzymes monoamine oxidases (MAO, encoded by *Maoa* and *Maob* genes) or catechol-O-methyltransferase (COMT, encoded by *Comt* gene). Epinephrine is converted by COMT to more stable metanephrine. Norepinephrine can be converted by both MAO and COMT and thus several metabolites can be produced, e.g. 3,4-dihydroxymandelic acid, normetanephrine, vanillylmandelic acid etc. Sympathetic nerves contain only MAO, while adrenal medulla and other non-neural tissues contain both enzymes – MAO and COMT (Eisenhofer *et al.* 2004a). It was reported that adrenal medulla is a source of about 90 % of circulating metanephrine and 30 % of normetanephrine. However, this is rather a consequence of intracellular catecholamine metabolism following the leakage of norepinephrine and epinephrine from the chromaffin storage granules to the cytoplasm (MAO and COMT protecting the chromaffin cells from catecholamine toxicity) than the clearance of catecholamines from the extracellular space (Eisenhofer *et al.* 1995a, b). This process of catecholamine leakage was proposed to be an important mechanism for “gearing down” the requirement for necessary increases in catecholamine biosynthesis under the stress conditions, which provides a capacity for a more extended range of sustainable rates of catecholamine release (Eisenhofer *et al.* 2004b).

The decreased catecholamine degradation was observed in both neural and in non-neural tissues of SHR (Masuda *et al.* 2006, Tsunoda and Imai 2004). In the adrenal gland of prehypertensive SHR, microarray

analysis revealed more than 30-fold higher mRNA expression of *Comt* but lower expression of *Maob* gene compared to WKY rats (Friese *et al.* 2005). However, these strain differences were not confirmed by our recent study (Vavřínová *et al.* 2019a). In the adrenal medulla of adult SHR, slightly downregulated mRNA expression of *Maoa*, upregulated expression of *Maob* but unchanged expression of *Comt* was described (Vavřínová *et al.* 2019a). The activities of MAOA, MAOB and COMT were reported to be similar in the adrenals of adult SHR and WKY rats (Guffroy and Strollin Benedetti 1984, Tsunoda and Imai 2004). Taken together, the influence of catecholamine reuptake and degradation on the function of the adrenomedullary system is still not well understood. The studies concerning catecholamine reuptake and degradation are scarce and provide inconsistent results.

Adrenergic receptors

Adrenergic receptors are involved in the feedback regulation of catecholamine release. The activation of α_2 -adrenergic receptors inhibits catecholamine release from the sympathetic terminals, neurons in the brainstem as well as from the adrenal medulla (Brede *et al.* 2003, Gilsbach *et al.* 2009, Urban *et al.* 1995), thus exhibiting a hypotensive effect. As the adrenal medulla is an endocrine organ, the physiological role of α_2 -adrenergic receptors-mediated negative feedback in chromaffin cells is still questionable. However, PC12 rat pheochromocytoma cell line, which does not express α_2 -adrenergic receptors, secretes abnormal catecholamine quantities (Taraviras *et al.* 2002). The specific subtypes of α_2 -adrenergic receptors prevailing in the adrenal glands are still unknown and it seems there could be differences between species. In adrenal gland of the rat, mRNA expression of α_{2A} , α_{2B} and α_{2C} was detected (Moura *et al.* 2011, Behuliak and Vavřínová unpublished results). All three types were expressed similarly in the adrenal medulla of young as well as of adult SHR and WKY rats and the α_2 -mediated inhibition of catecholamine release was comparable in normotensive and hypertensive rats (Moura *et al.* 2011). By contrast, Reja *et al.* (2002a) reported lower mRNA expression of α_{2A} -adrenergic receptor subtype in adrenal medulla of adult SHR than in WKY rats. Furthermore, Friese *et al.* (2005) described decreased mRNA expression of α_{2C} - and unchanged expression of α_{2A} - and α_{2B} -adrenergic receptor subtypes in the adrenal gland of

prehypertensive SHR. However, the comparison of mRNA expression results from various laboratories is quite complicated by the use of different conditions of quantitative real-time polymerase chain reaction and different reference genes for data normalization, which might influence the results (Vavřínová *et al.* 2016).

β -adrenergic receptors facilitate norepinephrine release from the sympathetic terminals (Guimarães and Moura 2001). The stimulation of β -adrenergic receptors increases catecholamine release from bovine chromaffin cells (Parramón *et al.* 1995). β_1 -adrenergic receptors mediated the slow potentiation of Ca^{2+} currents into the rat chromaffin cells. By contrast, β_2 -adrenergic receptors caused the fast inhibition of Ca^{2+} currents in the rat adrenal medulla (Cesetti *et al.* 2003). Both mechanisms might be involved in the autocrine regulation of chromaffin cells, but their impact on catecholamine release has not been studied up to now. In rats with heart failure, the chronic treatment with β_1 -adrenergic receptor blocker bisoprolol reduced catecholamine overproduction in the adrenal medulla (Rengo *et al.* 2012). Lower mRNA expression of β_2 - but unchanged expression of β_1 - and β_3 -adrenergic receptor subtypes was reported in adrenal gland of young SHR (Friese *et al.* 2005). However, no physiological study determining the function of β -adrenergic receptors in the adrenal medulla of SHR has been published so far. Taken together, the study on the role of adrenal adrenergic receptors in the regulation of catecholamine release from chromaffin cells of SHR would be desirable since these receptors might alter calcium signaling and contribute to the enhancement of catecholamine release in SHR subjected to physical or psychological stressors.

Adrenal demedullation

There is clear evidence that the adrenomedullary system participates in the development of high blood pressure in SHR, but it is not a crucial cause of hypertension in this model. Adrenal demedullation performed in 4-week-old prehypertensive SHR attenuated but did not prevent the development of high BP in these rats (Borkowski and Quinn 1983, Borkowski 1991), the effect being reversed by epinephrine supplementation (Borkowski 1991). Chronic as well as acute adrenal demedullation decreased BP responses to the electrical stimulation in pithed rats, whereas it did not affect vascular smooth muscle contractility to phenylephrine (Borkowski and Quinn 1983). This suggests that

epinephrine might potentiate norepinephrine release from the sympathetic nerve terminals. Indeed, a combination of sympathectomy with adrenal demedullation (Lee *et al.* 1991a, Lee *et al.* 1991b) or with α_1 -adrenergic blockade (Korner *et al.* 1993) completely prevented the development of high blood pressure in SHR. Moreover, epinephrine produced by the adrenal medulla might be involved in vascular hypertrophy and remodeling in SHR (Lee *et al.* 1991a, Lee *et al.* 1991b, Korner *et al.* 1993). These early structural and functional changes of cardiovascular system induced by pronounced activation of the adrenomedullary system (in cooperation with sympathoneural system) in immature SHR are probably irreversible because the effects of adrenal demedullation became insignificant since the age of 7 weeks (Borkowski 1991). Thus it seems that the adrenomedullary system does not actively contribute to the maintenance of high blood pressure in adult SHR. This is also in line with the age-dependent downregulation of catecholamine biosynthetic pathway described in the adrenal medulla of SHR with established hypertension (Vavřínová *et al.* 2019a). However, the adrenomedullary system might substitute the function of the suppressed sympathoneural system in both young and adult rats. The increased adrenal catecholamine content and the elevated plasma levels of epinephrine were demonstrated in rats treated neonatally or in adulthood with the sympatholytic drug guanethidine (Korner *et al.* 1993, Tipton *et al.* 1984, Vavřínová *et al.* 2019b). The activation of the adrenomedullary system in sympathectomized rats is probably mediated centrally because peripheral sympathectomy by 6-hydroxydopamine increased Fos immunoreactivity in many brain nuclei, including PVN (Callahan *et al.* 1998). Thereby, the activation of the adrenomedullary system might oppose the blood pressure lowering effects of the treatment targeting peripheral sympathetic system in SHR, being one of the reasons for the resistance of adult SHR to the treatment of hypertension.

Conclusions

Spontaneously hypertensive rats (SHR) exhibit numerous abnormalities in the adrenomedullary system from the hyperactivity of brain centers regulating sympathetic outflow, through the exaggerated activation of sympathoadrenal preganglionic neurons, the altered morphology of adrenal medulla, up to the local changes in catecholamine biosynthesis, storage and degradation in

chromaffin cells. Although it is quite difficult to highlight one particular abnormality that would be responsible for the development and maintenance of high blood pressure in SHR, the present evidence suggests that this hypertensive strain is highly prone to various stressful stimuli. This is apparent already from the prehypertensive stage and hence the repeated excessive activation of the adrenomedullary system and the enhanced catecholamine release can promote other pathological changes observed in SHR, including the potentiation of norepinephrine release from the sympathetic nerve terminals or the structural changes of vascular arteries. Indeed, hypertension development is attenuated in animals subjected to adrenal demedullation before the age of 7 weeks and the combination of demedullation with neonatal sympathectomy normalized blood pressure of SHR to the level of normotensive controls. The deleterious effects of the stress are widely accepted and therefore SHR could be a suitable model for studying the connection between the stress susceptibility and the development of cardiovascular diseases. On the other hand, the contribution of the adrenomedullary system to the maintenance of high blood pressure in adult SHR

seems to be minimal. Actually, the expression of genes involved in catecholamine biosynthesis and genes related to catecholamine vesicles is downregulated in adult stress-naïve SHR, but their adrenomedullary system is still more responsive to stressful conditions. Moreover, the activation of the adrenomedullary system is one of the mechanisms opposing the blood pressure lowering effects of treatment targeting peripheral sympathetic system in SHR. This finding points out to the interconnection of particular systems involved in the regulation of blood pressure and their mutual substitution. It also suggests that the treatments targeting central regulation of blood pressure might be effective for the lowering of blood pressure in hypertension in general.

Conflict of Interest

There is no conflict of interest.

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