研究成果の刊行に関する一覧表

書籍

著者名	論文タイトル	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
廣岡 良隆	酸化ストレスと交感神経	横山 光宏、藤田 敏郎	酸化ストレスと心血管病	日本医学出版	東京	2007	113-118
Hirooka Y	Role of nitric oxide and oxidative stress in the brainstem in cardiovascular regulation.	Kubo T	Central Mechanisms of Cardiovascular Regulation.	Research Signport	Kerala, India	2007	139-152

総説

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廣岡　良隆	脳中枢神経系におけるレニン・アンジオテンシン系の役割	ホルモンと臨床	55	49-56	2007
廣岡　良隆	中枢性循環調節機構（NO および Rho-kinase）	自律神経	44	272-276	2007
古閑　靖章 廣岡　良隆	高血圧と食塩感受性：交感神経活動亢進の重要性と中枢性機序	循環制御	28	203-209	2007

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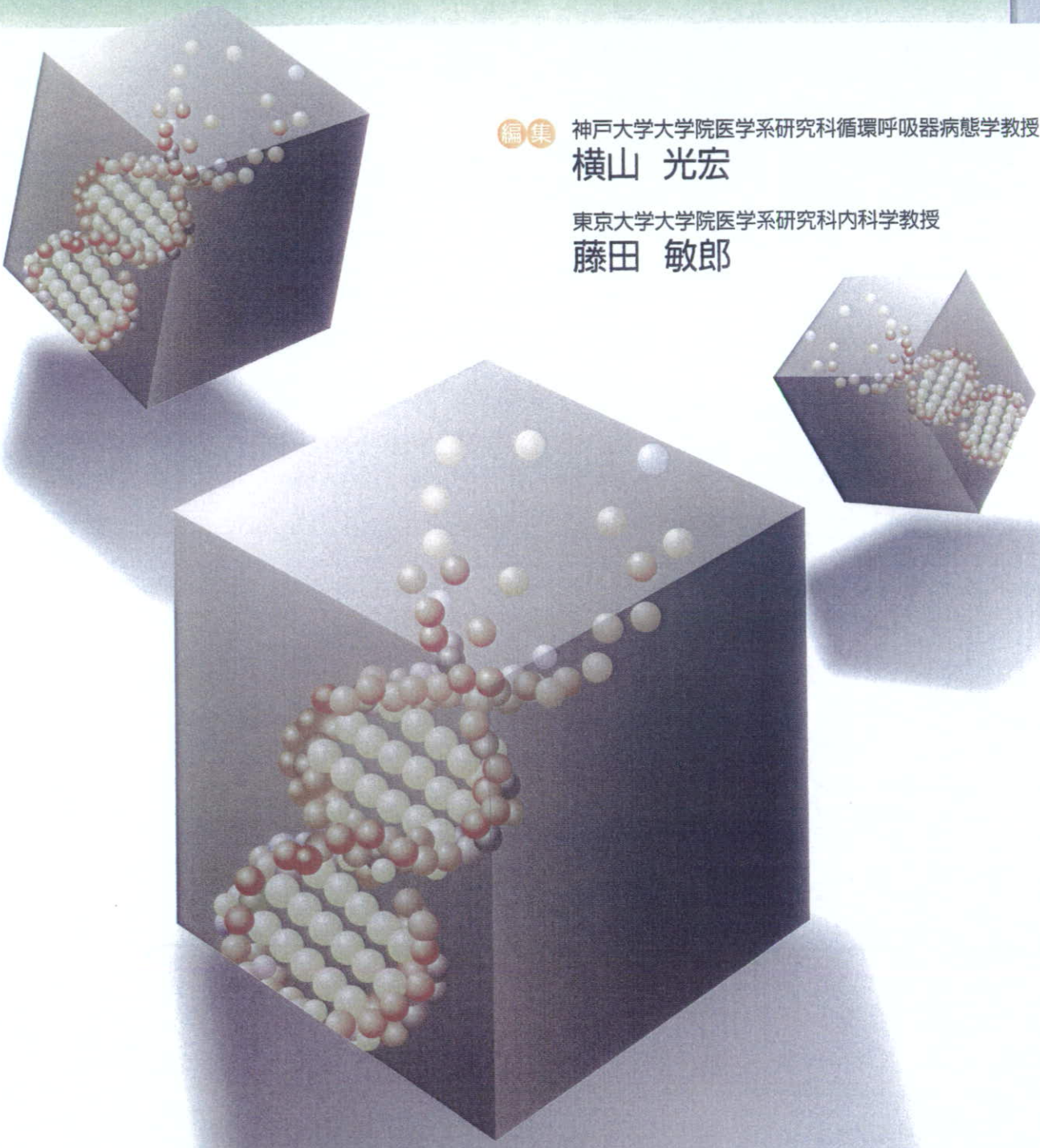
Oxidative Stress and Cardiovascular Disease

酸化ストレスと心血管病



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日本医学出版

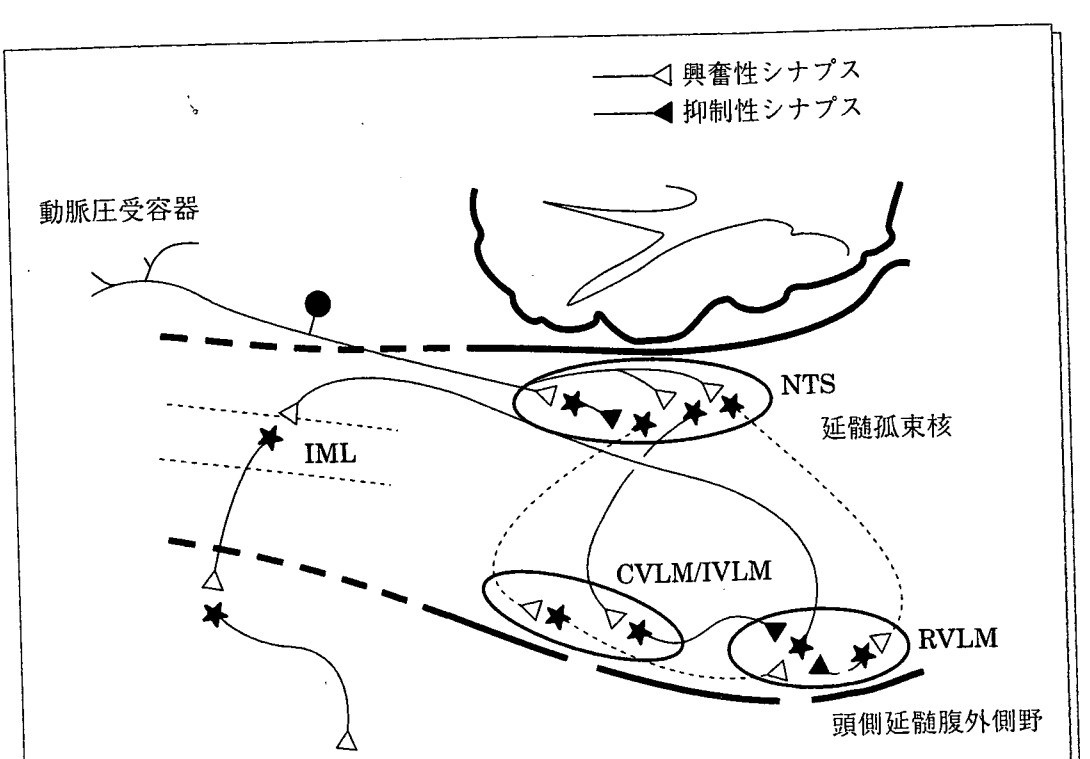
21 酸化ストレスと交感神経

はじめに

近年，心血管病の病態に酸化ストレスが深く関与していることが注目されている。たとえば高血圧・糖尿病・高脂血症やそれらを基盤として生じる動脈硬化性疾患・心不全などである。また，最近話題のメタボリックシンドロームにおいてもその役割が示唆されている。したがって，酸化ストレス増大を抑制する治療が考えられている。その1つとしてレニン-アンジオテンシン系抑制薬の役割が示唆されている。しかし，今1つ忘れてならないのは生体の調節系として根幹をなす交感神経系の役割である。古くから，レニン-アンジオテンシン系と交感神経系の相互作用は知られている。これはアンジオテンシンⅡが血中ホルモンであるという考えが主流であったころから始まっている。すなわち，アンジオテンシンⅡは末梢交感神経からのノルエピネフリンの分泌や副腎からのアドレナリン分泌を促進するというものである。一方，ノルエピネフリンはレニン分泌を促進しレニン-アンジオテンシン系を活性化する。組織レニン-アンジオテンシン系の存在が明らかになり，アンジオテンシン受容体が同定されその拮抗薬が開発されたことによって，レニン-アンジオテンシン系の役割の重要性が再び脚光を浴び，研究の急速な進展がみられた。高血圧・心不全における治療薬としての地位も確立した[1~4]。最近，心血管系を制御するもう1つの重要な柱である交感神経系の研究の進展もみえ始めた。心不全におけるβ遮断薬の有用性やアンジオテンシン受容体拮抗薬（ARB）の交感神経抑制作用が示されてきたからである。また，残念ながらわが国では使用されていないが，新しい副作用の少ない中枢性交感神経抑制薬の登場による。交感神経活動を規定するのは脳幹部に存在する頭側延髄腹外側野（rostral ventrolateral medulla; RVLM）である。そこへは動脈圧受容器からの最初の入力を受ける延髄孤側核（nucleus tractus solitarius; NTS）から尾側延髄腹外側野（caudal ventrolateral medulla; CVLM）を介して刻々の血圧値を認識して一定の血圧を維持するよう，動脈圧受容器反射を介する調節機構が存在する（図1）[1~5]。また，ストレス，運動，その他の刺激に対して上位中枢からの入力を受け，交感神経系を介して血圧・心拍数・臓器灌流を調節している。本稿では，主に酸化ストレスと交感神経系との関係，病態における役割に主眼をおき最近の知見を概説する。

血圧調節・高血圧における酸化ストレスと交感神経活動

高血圧におけるRVLMの活性酸素産生増加が交感神経活動亢進を介した血圧上昇機序に関与していることを示したのは，筆者らの成績が世界で最初である[6]。先行研究により，筆者らは，L-NAMEラットのNTS内へARBであるカンデサルタンを微量注入すると降圧・心拍数低下・交感神経活動抑制が生じること，脳幹部アンジオテンシン変換酵素（ACE）のmRNA発現が増加していることを報告した[7]。すなわち，L-NAMEラッ



実線は確立された主要経路，点線は仮説による追加された経路。

図1　動脈圧受容器反射を示す経路と主要神経伝達物質

〔文献2)より改変引用〕

トでは，末梢血管のみならず脳内レニン-アンジオテンシン系の活性化が中枢性交感神経活動亢進を介する高血圧の機序に関与している。このように脳内NOは一般的には交感神経活動を中枢性に抑制する作用があり，アンジオテンシンⅡとのクロストークが考えられた。脳内には血中ホルモンとしてとは別に独立したレニン-アンジオテンシン系が存在する[8]。それらの成績から，NOの作用を減弱させる活性酸素産生による酸化ストレス増加は脳内において中枢性交感神経活動を増加させる可能性を考えたのである[6]。脳内とくにRVLMにおける活性酸素産生はSHRSPで顕著であり，正常血圧ラット（Wistar-Kyoto rat; WKY）ではほとんど認められなかった[6]（図2）。筆者らの成績を支持する報告はその後もなされている。たとえば，最近，中枢性のアンジオテンシンⅡの昇圧作用が視床下部におけるNAD(P)Hオキシダーゼを介したスーパーオキシド産生によって生じることが示された[9]。また，アンジオテンシンⅡによる中枢性昇圧反応はNAD(P)Hオキシダーゼ由来のスーパーオキシド産生を生じ，p38 mitogen-activated protein kinase (MAPK) を介しているということも示されている[10]。

腎臓病に伴う高血圧は治療抵抗性である場合が多い。これは体液量調節の因子が大きく関わるからである。循環ホルモンの変化も大きい。Capmeseらのグループはフェノールで片側腎傷害により作成した高血圧モデルラットで交感神経系の活性化が生じていること，その機序に脳内活性酸素産生増加が関与していることを一連の研究で呈示してい

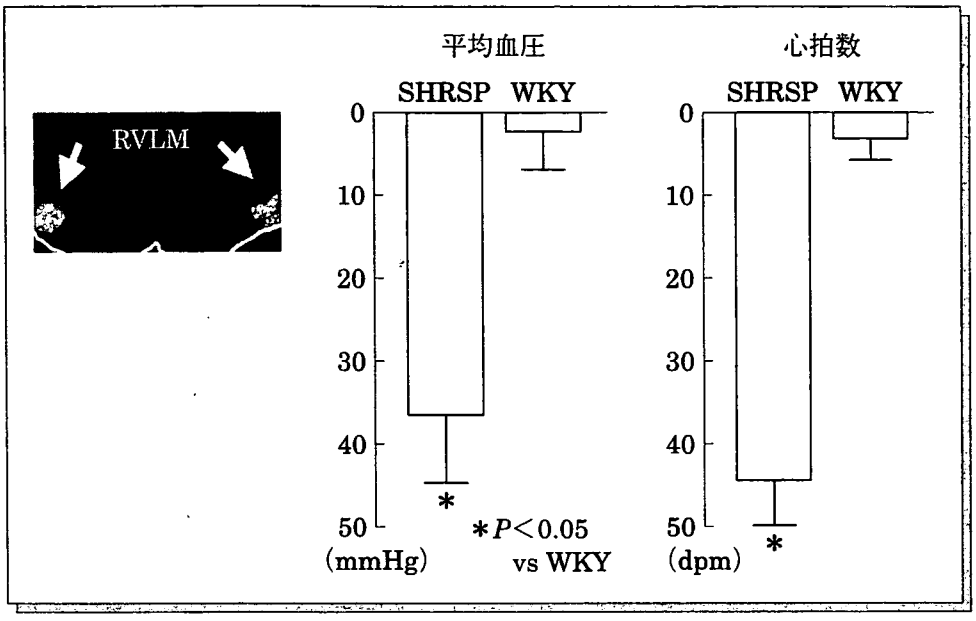


図2　脳内心血管中枢（RVLM; rostral ventrolateral medulla）にMn-SOD遺伝子導入を行った際の遺伝子発現（左：免疫組織化学染色）および血圧・心拍数の変化（右）

〔文献6)より改変引用〕

る[11]。彼らの成績は腎疾患を伴う高血圧における交感神経系活性化と脳内酸化ストレス増大を結びつける点で治療の観点からも重要であると考えられる。事実，腎不全における交感神経の活性化が再び注目されてきている[12]。ここにNOと酸化ストレスの関与が示唆されている。

脳内活性酸素とさまざまな局所因子との相互作用も注目されるところである。たとえば，脳内内因性アドレノメジュリンは脳内酸化ストレス低下を介して交感神経活動を抑制することが報告されている[13]。

心不全における役割

心不全は交感神経系の過剰な亢進がその病態に深く関与していることは周知の事実である[4,5]。また，レニン-アンジオテンシン系の活性化も血液中・組織中ともに重要な役割を果たしている。最近の大規模臨床試験の結果を受けて，アンジオテンシン変換酵素阻害薬は心不全治療の基本薬となり，ARBも有用性が確認されている。また，注目すべきは従来，禁忌と考えられてきたβ遮断薬が積極的に使用されるようになってきたことである。近年の研究により，心不全モデルで，ARBの脳内での交感神経抑制作用が注目されている[14]。その詳細な機序はまだ明らかではないが，心不全では脳内レニン-アンジオテンシン系の活性化が生じており，その抑制によって過剰な交感神経活動の亢進が抑制される。その候補としてNO活性の低下，活性酸素産生増加が考えられている。アンジオテンシンⅡによるNAD(P)Hオキシダーゼを介する活性酸素の増加がRVLMやPVNなどの自律神経系を制御する諸核に作用して交感神経活動亢進を生じていることが報告された[15~17]。脳内AT_1受容体も心不全でアップレギュレーションしている。また，心不全で亢進している心臓交感神経反射もアンジオテンシンⅡが関与しており，AT_1受容体のアンチセンス投与によってその反射は正常化する。最近の最も興味深い報告は，心

不全における脳内AT_1受容体のアップレギュレーションは核内転写因子であるactivator protein 1（AP1）およびJun N-terminal kinaseの活性化を生じるというものである[18]。

交感神経節レベルでの作用

交感神経系の活性化は先の動脈圧受容器反射回路図でも示したように（図1），さまざまなレベルで調節される。その1つとして交感神経節がある。これは脊髄からの交感神経節前線維が交感神経節後線維へシナプスを形成する部位であり，交感神経幹の中にある。最近，deoxycoticosterone acetate（DOCA）食塩高血圧ラットで腹部交感神経節におけるスーパーオキシド産生が増加していることが報告された[19,20]。その機序としては，ET_B受容体のアップレギュレーションが生じており，エンドセリン-1によるET_B受容体刺激によるスーパーオキシド産生増加が示唆されている[19,21]。

末梢交感神経での役割

通常，運動に携わる骨格筋の交感神経による血管収縮は抑制される。これは，局所による代謝性血管拡張作用によるがその生理活性物質の1つとしてNOがある。活性酸素はNOを不活化することによって交感神経系による血管収縮反応を増強する可能性がある。最近，この仮説を支持する成績が慢性アンジオテンシンII注入ラットおよび片腎動脈狭窄ラットで報告された。したがって，少なくとも慢性的にアンジオテンシンIIレベルが上昇している状態では，活性酸素が骨格筋の交感神経による血管収縮反応を増強させることが考えられる[22]。この成績は，とくに血中アンジオテンシンIIレベルが増加しているタイプの高血圧や心不全における運動耐容能などに関与している可能性がある。実際，心不全モデルラットで，骨格筋収縮における交感神経による血管収縮反応を活性酸素がNO活性を抑制することによって増強していることが示唆されている[23]。

最近の研究によると，ARBは末梢交感神経終末ではシナプス前のAT_1受容体に作用して交感神経を抑制するとされている。この作用はどのARBでも認められるが，その種類によって強さは異なる。テルミサルタン，カンデサルタンが比較的強いようである[24,25]。

おわりに

以上述べたように，酸化ストレスは交感神経系と密接に関連していることを支持する報告が集まりつつある。とくに，高血圧・心不全では明らかである。その作用部位として脳・末梢神経両者への作用が考えられる。活性酸素産生源としてはアンジオテンシンII刺激によるNAD(P)Hオキシダーゼの系が最もよく研究されているが，他にもキサンチンオキシダーゼ，ミトコンドリア，uncoupled NO合成酵素などがあり，これから解明されていかなければならない課題であろう。ARBの登場によってレニン-アンジオテンシン系の研究が飛躍的に進み，その理解が深まり臨床における適応が拡大されようとしている。一方，交感神経系は最も重要な調節系であるにもかかわらず“脳”が関係するため循環器研究者に敬遠されがちである。しかし，脳内レニン-アンジオテンシン系の研究が進んできていることもあり，ストレス社会の現代，メタボリックシンドロームも含め，これから交感神経系の研究が進み，至適な調節レベルの決定など臨床への応用が期待される（図3）。とくに，酸化ストレスはその切り口となるであろう。

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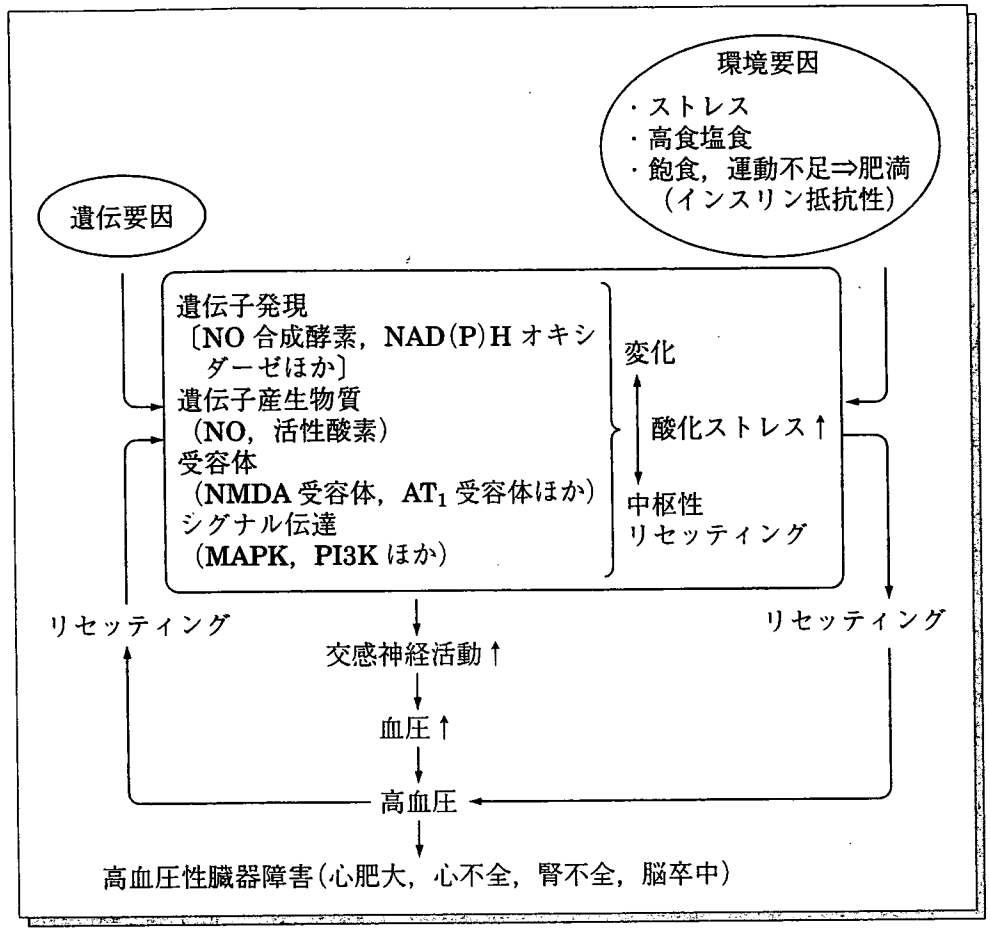

図3　高血圧および高血圧性臓器障害にいかに交感神経系が関わるか，脳が重要か，を示すシェーマ

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(廣岡　良隆)

CENTRAL MECHANISMS OF CARDIOVASCULAR REGULATION

2007

Editors
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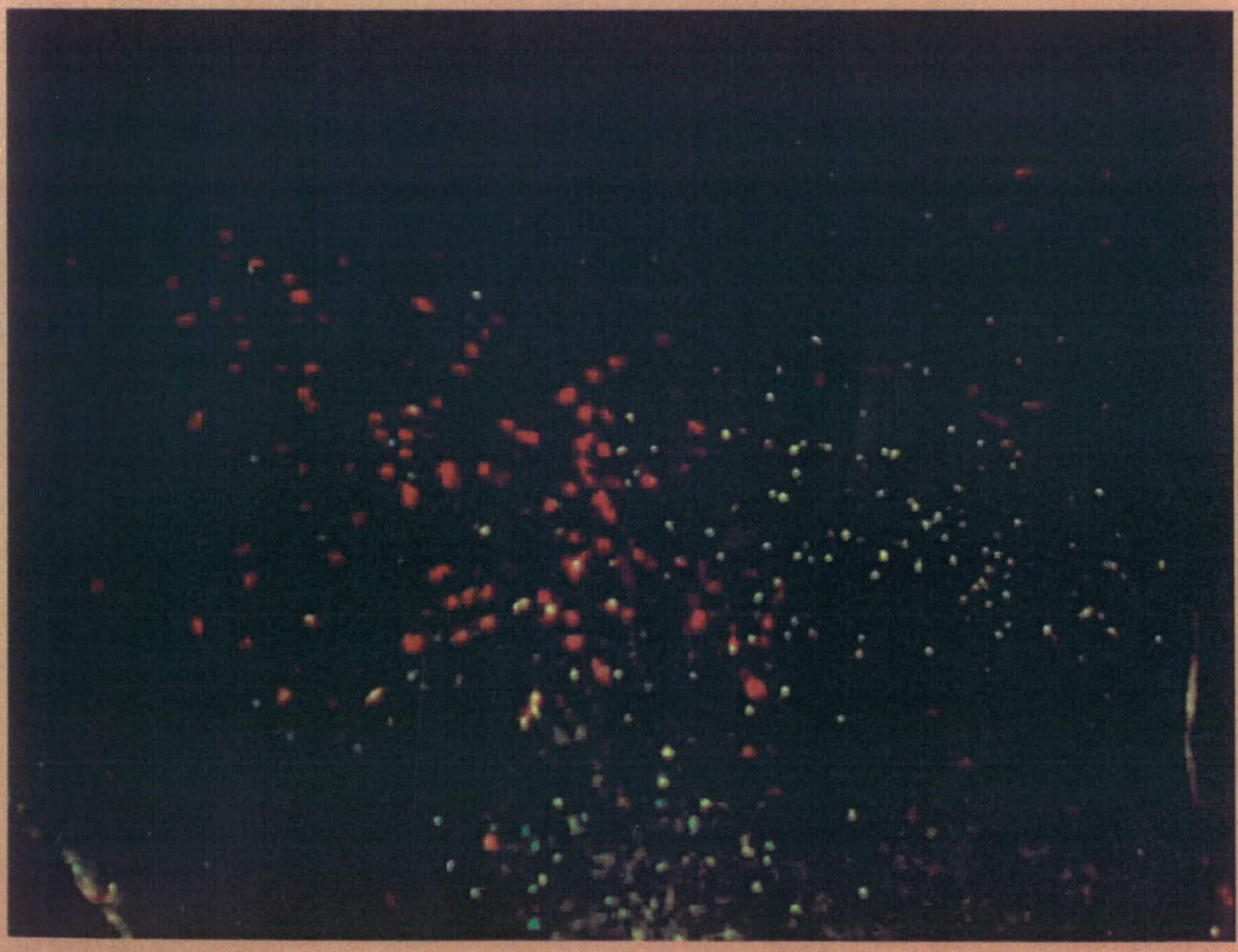
Tomoyuki Kuwaki



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9

Role of nitric oxide and oxidative stress in the brainstem in cardiovascular regulation

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Abstract

Nitric oxide (NO), a gaseous molecule, in the brain stem plays an important role in cardiovascular regulation. In general, it inhibits sympathetic nerve activity thereby reducing blood pressure. Reactive oxygen species (ROS) counteract the action of NO and their production is increased in the pathophysiological states that manifest activation of the sympathetic nervous system, such as hypertension and heart failure. The arterial baroreceptor reflex is the major feedback

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control system that acts to stabilize blood pressure. Abnormalities of this reflex are considered to be an underlying mechanism in the cardiovascular diseases such as hypertension and heart failure. There is accumulating evidence, however, that central nervous system mechanisms are involved in the enhanced sympathetic drive that occurs in these disease states. This article reviews studies performed in our laboratory in which a gene transfer technique, in combination with other methods, was used to determine the functional role of NO and ROS in the brain stem in the central control of cardiovascular regulation. We developed a technique to transfer adenovirus vectors encoding specific genes into the nucleus tractus solitarius (NTS) or the rostral ventrolateral medulla (RVLM) of rats in vivo. We applied this technique to hypertensive rats as well as in mice with heart failure to explore the pathophysiological significance of NO and ROS.

Introduction

Nitric oxide (NO) was originally identified as the endothelium-derived relaxing factor and there is now growing body of evidence that abnormality of NO production contributes to the cardiovascular pathophysiology [1]. NO is also present in the central nervous system (CNS) [1,2]. There are three isoforms of NO synthase (NOS). Two are constitutive enzymes, endothelial NOS (eNOS) and neuronal NOS (nNOS). The other one is inducible NOS (NOS) that is induced by inflammatory stimuli and normally its expression is rare. In neurons, nNOS is abundantly present. Many scientists are interested in its action related to long-term potentiation [3]. We have been studied the role of NO in the brain stem in neural control of circulation and found that it really plays an important role in it [4]. These findings are consistent with studies from other laboratories [1, 2], although there is still controversy [5]. Reactive oxygen species, such as superoxide and hydroxyl radicals, counteract action of NO. Thus, the balance between NO and ROS production determines the final physiological effects. It has been shown that ROS production is increased in the pathophysiological states, such as hypertension and heart failure, which deteriorates the progression of the disease status, particularly from the studies in the field of vascular biology. ROS production also occurs in the CNS and related to degenerative neurological diseases and aging. It is not well understood, however, regarding their role in neural control of circulation. Considering NO's action in the CNS, we hypothesized ROS also plays an important role in central cardiovascular regulation. Therefore, I summarize a series of studies regarding role of NO and ROS within the brain stem in neural control of circulation from our laboratories and describe some interesting studies related to recent progress in this topic from other laboratories.

The arterial baroreceptor reflex is the major mechanism for maintaining stable blood pressure [6, 7]. Based on studies in anesthetized animals, the key

sites (nuclei) along this pathway and the neurotransmitters involved are located in the brainstem [6-9]. Primary afferent fibers from arterial baroreceptors terminate in the nucleus tractus solitarius (NTS). Baroreceptor signals are conveyed from the NTS via an excitatory pathway to amino butyric acid (GABA)ergic neurons in the caudal ventrolateral medulla, which project to spinally projecting neurons in the rostral ventrolateral medulla (RVLM) [6]. The RVLM contains the sympathetic premotor neurons, which then project to the intermediolateral column in the spinal cord where sympathetic preganglionic neurons are located [6]. Finally, postganglionic sympathetic nerves innervate various organs, including the vessels and heart [6]. Therefore, many nuclei are involved in neural regulation of the cardiovascular system. Recent studies in anesthetized animals, however, evidence that a CNS mechanism(s) is involved in the abnormal neural control of circulation that occurs in pathophysiological states, such as hypertension and heart failure, in contrast to previous models [7, 8, 10] (Fig. 1). Although the central nervous system is importantly involved in the neural control of circulation, it is technically difficult to examine the role of the central nervous system in cardiovascular regulation in awake animals. A recent development in molecular biology and bioengineering has enabled us to study the role of specific genes and their products within specific brain nuclei in cardiovascular regulation [11,12]. We developed a technique to transfer gene-encoding adenovirus vectors locally into the NTS or RVLM of rats in vivo [13, 14].

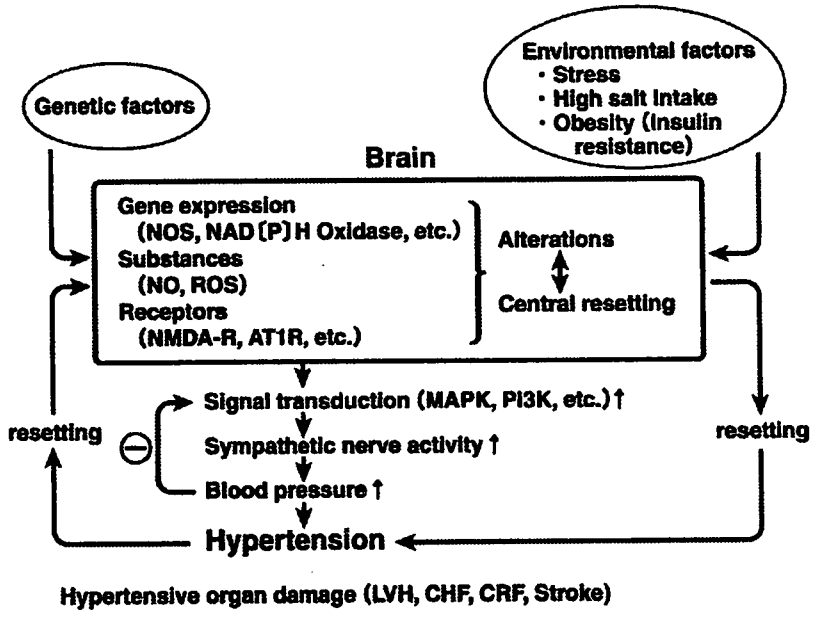

Figure 1. Proposed scheme demonstrating how NO and ROS in the brain lead to hypertension and hypertensive organ damage. NMDA, *N*-methyl-D-aspartate receptors MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3 kinase; LVH, left ventricular hypertrophy; CHF, congestive heart failure; CRF, chronic renal failure.

Blood pressure and heart rate were monitored using a radio-telemetry system. We applied this technique to hypertensive rats and mice with heart failure to explore the pathophysiological role of NO and ROS in these cardiovascular disease states [11].

Role of NO in the brain stem in blood pressure regulation

Administration of N^G-monomethyl-L-arginine (L-NMMA), a non-selective NOS blocker, into the NTS produced an increase in renal sympathetic nerve activity and arterial pressure in anesthetized rabbits [15]. Furthermore, perfusion with L-arginine, a precursor of NO synthesis, or sodium nitroprusside, an NO donor, increased neuronal activity in neurons recorded in the NTS of brain stem slices [16]. The effects of NO in the NTS on the depressor response are caused by the facilitatory release of L-glutamate [17]. Intracisternal administration of N^{ω}-nitro-L-arginine-methyl ester (L-NAME), which inhibits NOS of medullary sites, increased arterial pressure and renal sympathetic nerve activity in anesthetized rabbits [18]. Activation of the renin-angiotensin system in the NTS is involved in the increased sympathetic nerve activity via angiotensin type 1 (AT1) receptors [19].

We extended the series of studies using a gene transfer technique in conscious rats. To increase in NO production locally in the NTS or the RVLM, we transfected an adnovirus vector encoding eNOS [20, 21]. We used adenovirus vectors encoding either the bacterial ß-galactosidase gene or the bovine eNOS gene. These adenoviral vectors were constructed in the Gene Transfer Vector Core Laboratory at the University of Iowa [22, 23]. Adult male Wistar-Kyoto rats (WKY) were used for gene transfer. The rats were anesthetized with sodium pentobarbital and placed in a stereotaxic frame. Microinjections were performed at six sites bilaterally in the NTS. An adenoviral suspension containing $1x10^8$ plaque forming units (pfu)/mL was injected into each injection site over a 5-min period. The RVLM was also identified by monitoring mean arterial pressure after the injection of a small dose of L-glutamate. Identification of the RVLM was confirmed according to the following criteria: (1) an increase in mean arterial pressure occurred immediately after the injection of L-glutamate, (2) the response plateau occurred within 20 seconds of the injection, and (3) the change in mean arterial pressure was greater than 20 mmHg [21]. An adenoviral suspension containing $1x10^8$ pfu/mL was slowly injected into each site over 15 min. The RVLM transfection was confirmed by immunohistochemical staining for phenylethanolamine-*N*-methyltransferase, an enzyme that catalyzes the final step of epinephrine synthesis and is identified specifically in the C1 neurons in the RVLM. After the injection, all rats recovered from anesthesia and were kept unrestrained and free to move in their cages.

On day 7 after the gene transfer, animals were deeply anesthetized with an overdose of pentobarbital. Sections of the medulla (50 μm) were evaluated for either ß-galactosidase expression by X-Gal staining or eNOS expression by immunohistochemistry [20]. Western blot analysis for eNOS protein was performed and the expression level time course was examined [20]. Staining for ß-galactosidase was observed locally in the NTS or the RVLM of the section of the medulla, and ß-galactidase activity was also observed on day 7 after the gene transfer. Immunohistocemistry and Western blot analysis revealed a peak in the local expression of eNOS protein in the NTS (Fig. 2) and the RVLM (Fig. 3) on day 7 after the gene transfer.

The production of NO in the NTS or RVLM was measured as the nitrite and nitrate (nitrite/nitrate) level by in vivo microdialysis before and on day 7 after the gene transfer [20, 24]. The basal level of nitrite/nitrate in the NTS or the RVLM was significantly higher in the AdeNOS-transfected rats than in the Adßgal-transfected rats [20, 24].

The UA-10 telemetry system (Data Sciences International, St. Paul, MN, USA) was used to measure arterial blood pressure and heart rate. Mean arterial pressure and heart rate were recorded continuously for 10 min daily between 10:00 AM and 11:00 AM using a multichannel amplifier and signal converter. We measured the urinary norepinephrine concentration before and on day 7 after the gene transfer by high-performance liquid chromatography, and calculated the urinary 24-h norepinephrine excretion.

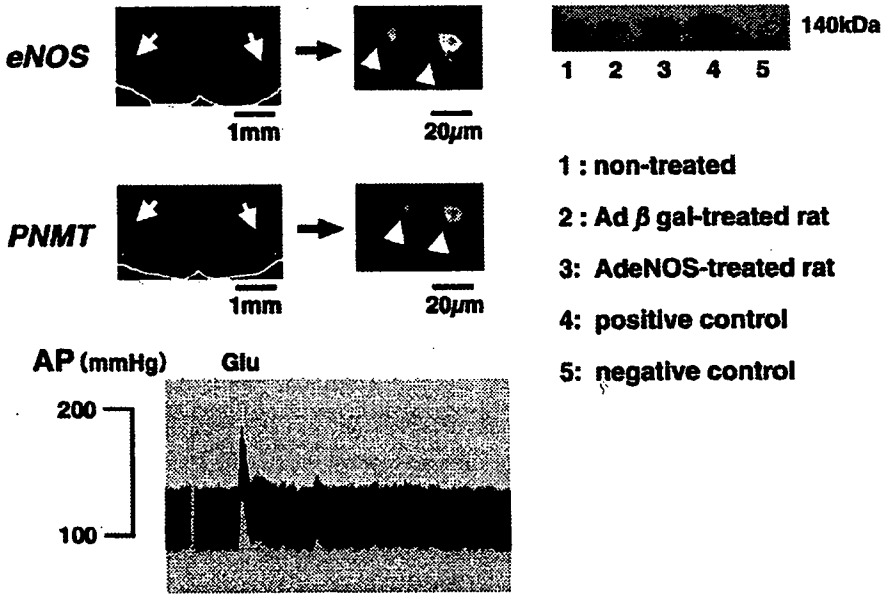

Figure 2. Expression of eNOS protein in the RVLM by immunohistochemistry and Western blot analysis (upper panel). The lower panel shows the pressor site in the RVLM by L-glutamate (Adopted and modified with permission from Kishi, T., et al., 2001).

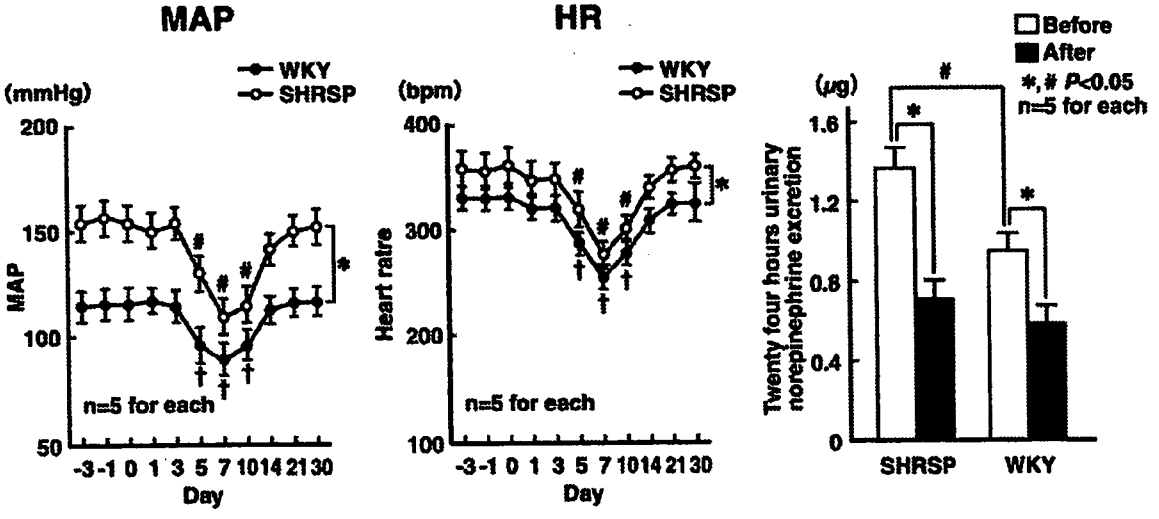

Figure 3. Time courses of mean arterial pressure (MAP) and heart rate (HR) after transfection of AdeNOS into the RVLM in WKY and SHRSP. Urinary norepinephrine excretion before and on day 7 after the gene transfer (Adopted and modified with permission from Kishi, T., et al., 2002).

Blood pressure and heart rate were decreased in rats transfected with AdeNOS into the NTS on days 5 to 10 after the gene transfer. In contrast, these variables did not change in the Adßgal-transfected rats [20]. Urinary norepinephrine excretion on day 7 after eNOS gene transfer was significantly decreased [20], suggesting that the decrease in blood pressure is mediated by the inhibition of sympathetic nerve activity. A similar response was observed in rats transfected with AdeNOS into the RVLM (Fig. 2) [21]. We suggest that these responses induced by the overexpression of eNOS in the NTS or RVLM might be mediated by the enhanced release of glutamate or GABA, respectively [4, 21].

Using an adenovirus vector, a dominant negative eNOS mutant was transfected into the NTS [25, 26]. Expression of a dominant negative eNOS mutant did not affect baseline cardiovascular parameters or baroreflex sensitivity in a rat working heart-brainstem preparation [25]. In conscious rats, however, transfection of a dominant negative eNOS mutant into the NTS decreases heart rate and increases spontaneous baroreflex gain measured using a time-series method [26]. They concluded that endogenous eNOS is constitutively active within the NTS and is involved in setting the baroreflex gain and resting heart rate [26]. The time course of peak changes of these parameters occurred on day 21 after the gene transfer, although complete recovery of these variables after transfection of the gene was not observed. It is not known why the transfected gene expression time courses differed among the studies.

As described above, we examined the effects of AdeNOS in the NTS or RVLM on cardiovascular responses. In a recent study, neuronal NOS (nNOS)

gene transfer into the paraventricular hypothalamus was successfully performed [27], although the peak expression of nNOS was observed on day 3 after the gene transfer. Because endogenous nNOS is normally high in the hypothalamus, however, comparison of the expression levels of nNOS before and after gene transfer might be difficult. Thus, throughout our series of studies, we used eNOS instead of nNOS, which is normally abundant in the brain. In addition, the purpose of our series of studies was to increase NO production locally in the NTS or the RVLM for a much longer period in rats transfected with AdeNOS in an awake state. The role of iNOS in the RVLM in pathophysiological conditions such as hypertension, heart failure, and endotoxic shock remains unknown. Although overexpression of iNOS in the RVLM induces pressor and sympathoexcitatory responses, it might be different in a condition such as endotoxemia, in which iNOS is relatively acutely induced in many organs. In such a condition, NO and superoxide produced from iNOS might elicit caspase-dependent apoptotic cell death, thereby causing fatal cardiovascular depression [28]. Therefore, we examined the effect of iNOS overexpression [29] in the RVLM on blood pressure in vivo and found that it increased blood pressure and sympathetic nerve activity [24]. The results of this study suggest that an increase in oxidative stress induces activation of the sympathetic nervous system. Large amounts of NO might consume L-arginine, a precursor, and tetrahydrobiopterine, a co-factor, thereby inducing superoxide production, instead of NO, by uncoupling NOS. Interestingly, Huang et al. recently suggested that high levels of NO inhibit synaptic transmission in rat RVLM neurons by acting on presynaptic N-type Ca^{2+}-channels [30]. They suggested that this action is mediated by peroxynitrite formation and adenosine release [30]. In contrast, however, they also suggested that NO acts presynaptically to increase synaptic transmission on the RVLM neurons via the activation of presynaptic N-type Ca^{2+}-channels and that this presynaptic action of NO is mediated by the cGMP/PKG-coupled signaling pathway [31].

Role of NO in the brain stem in blood pressure regulation of hypertensive rats

Spontaneously hypertensive rats (SHR) or stroke-prone SHR (SHRSP) exhibit increased sympathetic nerve activity during the development of hypertension and there is a positive correlation between blood pressure and sympathetic nerve activity. Some studies suggest that there is a disorder of the L-arginine-NO pathway in SHR [32]. Therefore, we examined whether the effects of increased NO production in the NTS or RVLM of SHR or SHRSP induced by the overexpression of eNOS on cardiovascular responses are different from those in WKY [33, 34]. We found that the overexpression of

eNOS in the NTS elicited a greater depressor response in SHR than in WKY [33]. The depressor response evoked by microinjection of L-glutamate into the NTS did not differ between SHR and WKY, suggesting that the enhanced depressor response induced by the overexpression of eNOS did not result from a different response to inhibitory stimuli from the sympathetic innervation of peripheral vasculatures. Furthermore, intracisternal injection of L-NMMA elicited a greater pressor response in AdeNOS-transfected SHR than in Adßgal-transfected SHR, indicating that the decrease in blood pressure induced by the overexpression of eNOS is mediated by NO. The release of the major neurotransmitter L-glutamate might be altered by NO activity [17] in the brain of SHR, although we did not address this issue in our study.

In support of our findings, a recent study demonstrated that transfection of AdeNOS into the NTS elicits both hypotension and bradycardia in SHR on days 3 to 14 [34]. Within this period, microinjection of the guanylate cyclase inhibitor 1H-[1,2,4]oxadiazole[4,3-alfa]quinoxalin-1-one reverses the depressor response induced by AdeNOS transfection. They also examined both NO and superoxide production in cultured cells before and after AdeNOS transfection, and reported an increase in NO production and a decrease in superoxide production after AdeNOS transfection, suggesting that AdeNOS transfection generates NO and scavenges superoxide [34].

The decrease in blood pressure, heart rate, and urinary norepinephrine excretion induced by the overexpression of eNOS in the RVLM is greater in SHRSP than in WKY (Fig. 3) [35]. Microinjection of bicuculline bilaterally into the RVLM on day 7 after gene transfer produced a gradual increase in blood pressure. In the non-transfected rats, the increase in blood pressure was significantly smaller in SHRSP than in WKY. In the AdeNOS-transfected rats, however, the pressor response induced by microinjection of bicuculline was significantly greater than that in non-transfected rats and did not differ between SHRSP and WKY [34]. Thus, the decrease in blood pressure induced by AdeNOS transfection was mediated by an increase in GABA release, and the increase in NO improved GABA release in AdeNOS-transfected SHRSP. Therefore, dysfunction of the NO pathway and the resulting disinhibition of the RVLM contribute to activate RVLM premotor neurons and increase sympathetic nerve activity in SHRSP. Furthermore, overexpression of eNOS in the RVLM of SHRSP improved the impaired maximum gain of baroreflex control of heart rate [36]. Interestingly, overexpression of eNOS in the NTS did not affect the baroreflex control of heart rate [36]. Thus, we suggest that GABA has a role in improving baroreflex control of heart rate. From the therapeutic aspects, it is interesting that atorvastatin decreases blood pressure via the reduction of sympathetic nerve activity and this effect may be mediated by an increase in NO production with the upregulation of eNOS expression in the brain [37].

Role of ROS in the brain stem in blood pressure regulation of hypertensive rats

The involvement of reactive oxygen species such as superoxide and hydroxyl radicals is implicated in the pathogenesis of hypertension [38]. The brain contains a high concentration of polyunsaturated fatty acids in its cell membranes. These fatty acids are targets of oxygen-derived free radicals. Thiobarbituric acid-reactive substances (TBARS), end products of lipid peroxidation and an indirect marker of oxidative stress, are increased in the brains of SHRSP compared with those of WKY [39]. In addition, the intensity of electron spin resonance signals taken from the RVLM tissue decreases more rapidly in SHRSP than in WKY [39], suggesting that reactive oxygen species production is greater in the RVLM of SHRSP than in WKY. To confirm the role of reactive oxygen species in the RVLM in SHRSP, we transfected adenovirus vectors encoding the manganese superoxide dismutase (MnSOD) gene (AdMnSOD) bilaterally into the RVLM [39]. Western blot analysis revealed that MnSOD expression was significantly increased in the tissue from the RVLM of the AdMnSOD-transfected SHRSP to the same level as that of WKY on day 10 after the gene transfer. MnSOD activity was also increased in SHRSP on day 10 after the gene transfer. TBARS levels were significantly decreased in the RVLM of AdMnSOD-transfected SHRSP compared with non-treated SHRSP. On day 10 after the gene transfer, mean arterial pressure and heart rate of AdMnSOD-transfected SHRSP were significantly decreased compared with non-treated SHRSP, but not WKY (Fig. 4). Urinary norepinephrine excretion was significantly decreased in AdMnSOD-transfected SHRSP, but not in WKY. Taken together, these results suggest that increases in oxidative stress in the RVLM contribute to the central nervous system mechanisms underlying hypertension in SHRSP.

It is also suggested that interaction between NO production and ROS alters central sympathetic outflow [40, 41]. In support of our findings, a recent study suggests that increased superoxide anions in the RVLM contribute to hypertension in SHR via interactions with NO [42]. They reported a reduction in MnSOD expression and activity in the RVLM [42]. Microinjection of manganese (III)-tetrakis-(4-benzoic acid) porphyrin (MnTBAP), novel superoxide dismutase mimetic, bilaterally into the RVLM induced a greater reduction in blood pressure, heart rate, and sympathetic nerve activity in SHR than in WKY. Microinjecion of MnTBAP on day 10 after transfection of AdeNOS into the RVLM further decreased blood pressure to the level of that in WKY, suggesting that overexpression of eNOS in the RVLM is not sufficient to remove excess superoxide anions. Another important point in their findings is that Cu/ZnSOD expression and activity were similar between SHR and WKY, although MnSOD expression and activity were reduced in SHR

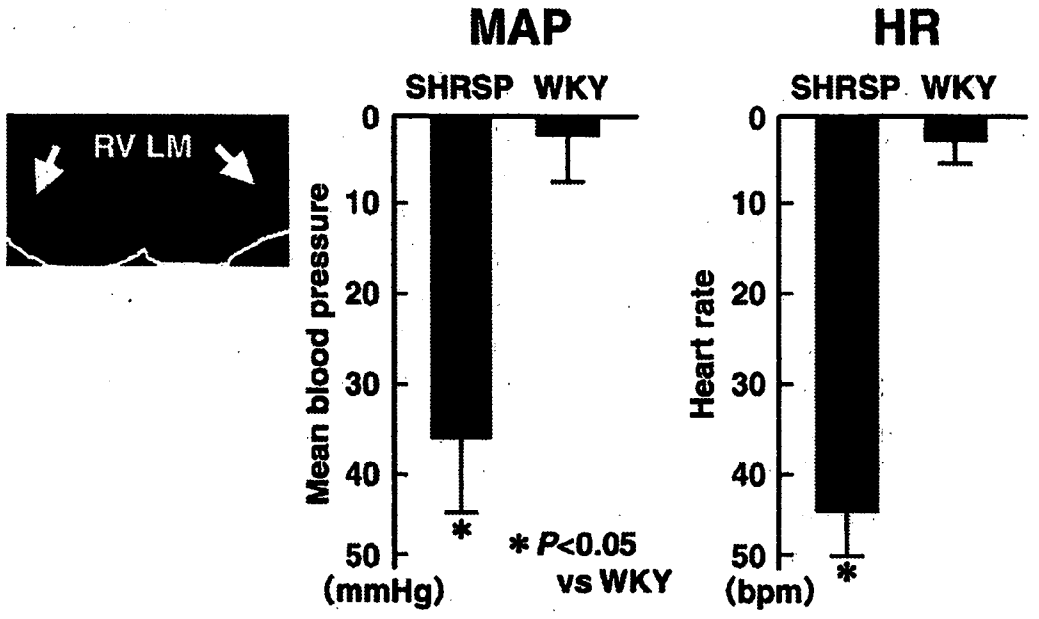

Figure 4. Immunohistochemistry of AdMnSOD expression in the RVLM and changes in mean arterial pressure (MAP) and heart rate (HR) after AdMnSOD gene transfer into the RVLM of SHRSP and WKY (Adopted and modified from Kishi, T., et al., 2004).

compared with WKY, suggesting that the scavenging enzymatic system is different between the two strains.

Changes in blood pressure, heart rate, and drinking behavior elicited by injection of angiotensin II in the brain are abolished by prior treatment with AdMnSOD or AdCu/ZnSOD [43]. Furthermore, a requirement for Rac1-dependent NAD(P)H oxidase in these cardiovascular and dipsogenic actions of angiotensin II in the brain was demonstrated using adenovirus-mediated expression of dominant-negative Rac1 [44]. Thus, the sources of reactive oxygen species in the brain in hypertension should be examined. A recent study suggest that downregulation of gene expression and enzyme activity of the antioxidant Cu/ZnSOD, MnSOD, or catalase may underlie the augmented levels of superoxide and hydrogen peroxide in the RVLM thereby leading to oxidative stress and hypertension in SHR [45]. It is important to examine how oxidative stress increases neuronal activity in the RVLM at the cellular levels. It remains determined, however, some studies suggest that p38 mitogen-activated protein kinase is activated by superoxide [46], inhibition of potassium channels or the increase in intracellular Ca^{2+} concentration are involved [47, 48]. Apparently, further studies needed to clarify this question. It is interesting to examine whether orally administered antihypertensive drugs reduce oxidative stress in the brain. We found that amlodipine reduces ROS production in the brain stem associated with sympatho-inhibitory effect [49].