C. 研究結果

C-1. 刺激電流値と刺激パルス頻度による降圧効果の差異

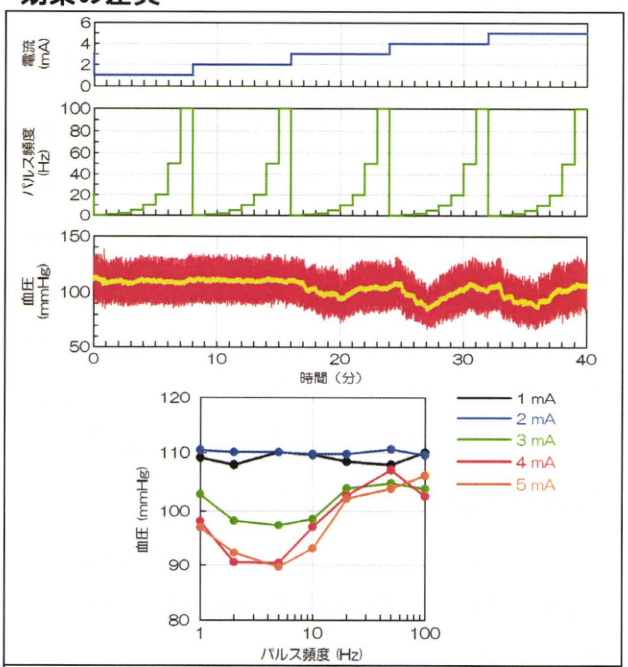


図1　プロトコール1の実験記録（上）と結果（下）

図 1 に示すように、パルス幅 0.5 ms の電気鍼刺激においては、3 mA 以上の電流値で明らかな降圧効果が認められ、刺激パルス頻度 2～5 Hz において最大の降圧効果が得られた。刺激電流が 4 mA または 5 mA の場合は、刺激パルス頻度を 50 Hz または 100 Hz にしたときに、昇圧効果がみられた。

後述の結果でわかるように、刺激パルス幅を 0.5 ms に固定したことが重要であり、他のパルス幅では異なる結果が得られることが予測される。

C-2. 刺激電流値と刺激パルス幅による降圧効果の差異

図 2 に示すように、刺激パルス頻度 2 Hz においては、1 mA ではほとんど血圧に影響が無く、2 ～5 mA において降圧効果が認められ、パルス幅 0.5～2.0 ms の範囲で最大の降圧効果を示した。なお、刺激電流が 4 mA または 5 mA の場合は、パルス幅を 10 ms にしたときに、むしろ昇圧効果がみられた。

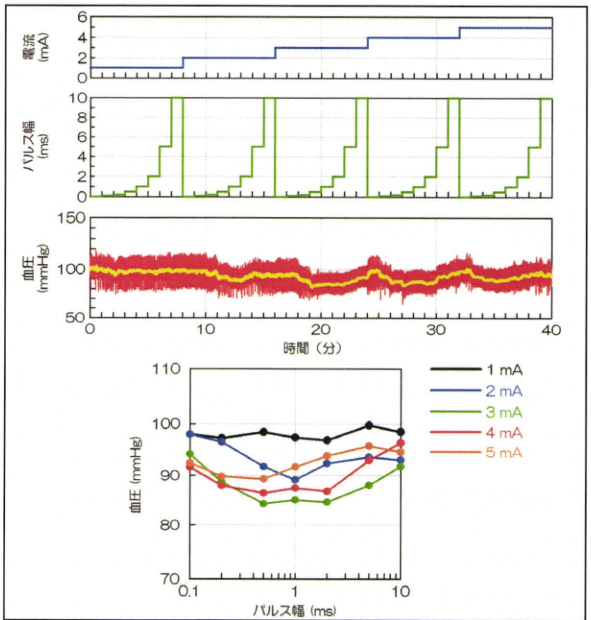


図2　プロトコール2の実験記録（上）と結果（下）

D. 考察

本研究では体表刺激による昇圧を応用した血圧制御システムを開発する。基礎研究において主に低い電気刺激条件において降圧効果がみられる二相性の応答をすることが知られていたので、本課題では降圧効果の条件について体系的に検討した。

理論的には刺激電流値、刺激パルス幅、刺激パルス頻度の 3 つのパラメタの組み合わせが考えられるが、実験の実現可能性の観点から 2 パラメタの組合せによって実験を行った。

本年度は麻酔下のウサギを用いて検討したが、基本的には前年度の麻酔下のネコを用いた検討と同じく、低い刺激パルス頻度で降圧効果、高い刺激パルス頻度で昇圧効果が得られることが確認された。ただし、最大降圧効果が得られる刺激パルス頻度はネコが約 10 Hz であったのに対して、ウサギでは 2～5 Hz であった。

E. 結論

足三里への電気鍼刺激によって降圧効果が見られる条件を可能なかぎり網羅的に検討した。前年度の結果と同じく、刺激パルス幅の変更が降圧または昇圧効果に及ぼす影響は小さく、刺激電流値と刺激パルス頻度の組み合わせで血圧制御を行うことが妥当であると考えられた。

F. 健康危険情報

なし

G. 研究発表

G-1. 論文

1. Hiromi Yamamoto, Toru Kawada, Atsunori Kamiya, Shunichi Miyazaki, Masaru Sugimachi. Involvement of the mechanoreceptors in the sensory mechanisms of manual and electrical acupuncture. *Auton Neurosci.* 2011; 160: 27-31.

2. Kawada T, Meihua Li, Atsunori Kamiya, Shuji Shimizu, Kazunori Uemura, Hiromi Yamamoto, Masaru Sugimachi. Open-loop dynamic and static characteristics of the carotid sinus baroreflex in rats with chronic heart failure after myocardial infarction. *J Physiol Sci.* 2010; 60: 283-298.

G-2. 学会発表

1. Meihua Li, Masashi Inagaki, Can Zheng, Toru Kawada, Kazunori Uemura, Toshiaki Shishido, Masaru Sugimachi. Vagal stimulation markedly suppressed arrhythmic death and prevented cardiac dysfunction in rats after acute myocardial infarction. *CSDS (Cardiovascular System Dynamics Society) Conference, 2010.*

2. Shuji Shimizu, Tsuyoshi Akiyama, Toru Kawada, Masashi Inagaki, Atsunori Kamiya, Toshiaki Shishido, Shunji Sano, Mikiyasu Shirai, Masaru Sugimachi. Medetomidine, an α_2 adrenergic agonist, enhances acetylcholine release from cardiac vagal nerve endings through central action. *ESC (European Society of Cardiology) Congress, 2010.*

G-3. 新聞報道

なし

H. 知的所有権の取得状況

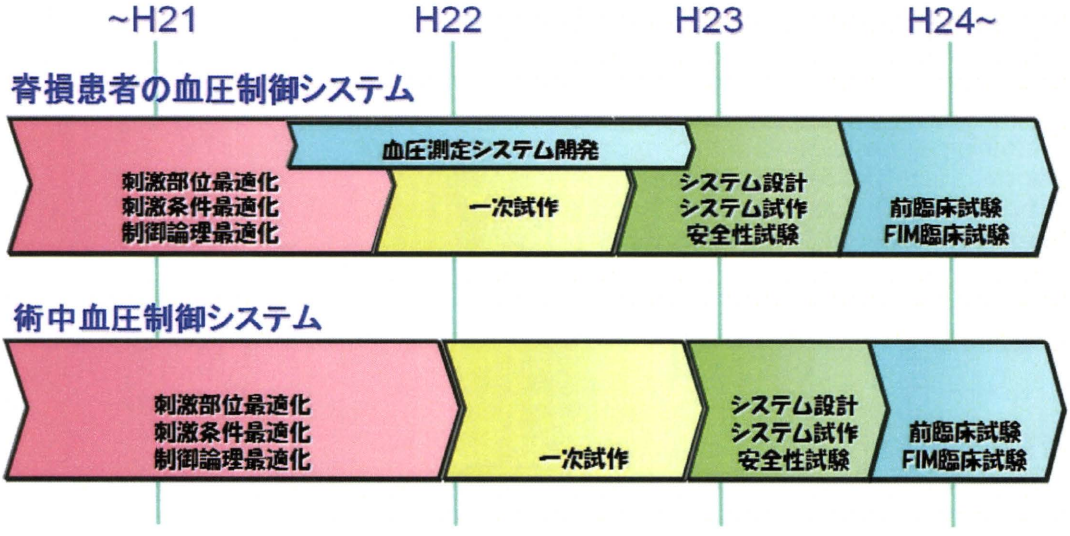
なし

厚生労働科学研究費補助金
（医療技術実用化総合研究事業）
平成２２年度分担研究報告書

バイオニック血圧制御システムの実用化開発
分担研究課題　術中血圧制御システムの開発

分担研究者　佐藤　隆幸（高知大学教育研究部医療学系　教授）

研究要旨：
手術時には、血圧の迅速な制御に大きな役割を果たしている自律神経によるフィードバック制御機構、すなわち動脈圧反射系の機能が麻酔薬等により抑制されるため、少量の出血により、予期せぬ血圧低下を生じ重篤な転機をとることがある。そこで、本研究では、動脈圧反射の機能再建デバイスとして臨床応用可能なバイオニック血圧制御システムを開発する。ヒトの血管運動性交感神経を刺激する方法として，硬膜外カテーテル電極を用いた方法を採用した。
平成２１年度は、圧反射失調の臨床的モデルとなる全身麻酔中の患者を対象に，硬膜外腔からの電気刺激に対する動脈圧応答を伝達関数として同定し、また、硬膜外カテーテル電極の留置をより安全に行うため、カテーテルの経皮的挿入時に電極間インピーダンスを測定しながら電極留置位置を推測することが可能な装置を試作した。
平成２２年度は、研究協力企業（日本光電工業株式会社）とともに、試作器を開発した。研究協力企業の既製電気刺激装置と Windows ベースのノートＰＣを組み合わせることによって、人工的血管運動中枢の一次試作を完了した。また、脊髄硬膜外カテーテル留置位置の電気的確認法の有用性に関する調査を行った。



A. 研究目的

全身麻酔や脊椎麻酔、あるいは体外循環時の術中の血圧管理技術は、手術の成否のみならず、生死を左右する重要な医療技術である。しかし、今なお、術中血圧管理における過誤から植物状態になる症例や虚血による心機能障害のため重篤な後遺症におちいる不幸な症例が後をたたない。その理由の一つとして、これらの手術時には、血圧の迅速な制御に大きな役割を果たしている自律神経によるフィードバック制御機構、すなわち動脈圧反射系の機能が麻酔薬等により抑制されることがあげられる。そのため、少量の出血により、予期せぬ血圧低下を生ずることがある。

血圧低下後に急速輸液・輸血、あるいは昇圧薬を投与しても、血圧回復には一定の時間を要するため、その間に、脳や心臓など重要臓器の潅流障害が生ずる危険がある。また、従来の輸

液・輸血・昇圧薬による血圧管理は、投与速度や量の判断に関するヒューマンファクタによりその成否が左右されることから、より迅速で精度の高い血圧管理技術が求められる。さらに、麻酔科医などのマンパワー不足を補うためにも、フィードバック制御技術を取り入れることにより、ある程度のオートメーション化を図ることが可能になり、臨床のニーズに応えられる。

以上のような臨床上の課題を解決するため、本研究では、術中血圧制御を目的としたバイオニック血圧制御システムを試作し、実用化する。

B. 研究方法

B-1. 開発の原理

動脈圧反射は，さまざまな外乱による脳の潅流圧変化を抑制する機構としてはたらく極めて重要なフィードバック制御システムである。時々刻々と変化する動脈圧は，頸動脈洞や大動脈弓の圧受容器で検知され，圧受容器神経活動として血管運動中枢にフィードバックされる。血管運動中枢はこの圧受容器神経活動に応じて，交感神経活動を変化させる。その結果，血管の収縮・弛緩が生じ，外乱の影響が抑制されることになる。

本研究で開発するバイオニック血圧制御システムの動作原理は，図 B-1 のように，「血圧を常時監視しながら，実時間演算で交感神経の電気刺激頻度を決定する」というものである。すなわち，本装置は，圧センサー→人工的血管運動中枢→電気刺激装置→交感神経→血管床からなるフィードバック血圧制御装置である。

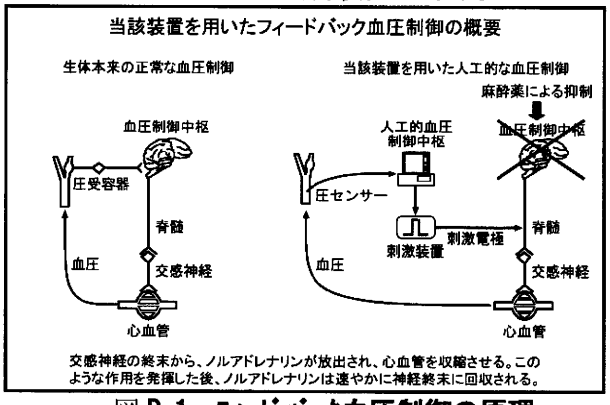
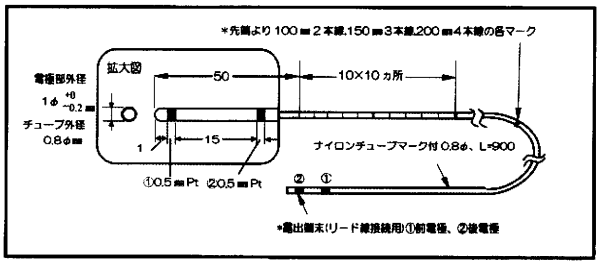


図 B-1　ﾌｨｰﾄﾞﾊﾞｯｸ血圧制御の原理

B-2. 硬膜外カテーテル電極

脊髄・脊椎手術中の脊髄機能モニターの検査に用いられることがある硬膜外カテーテル電極（図 B-2）を採用し、バイオニック血圧制御システムにおける脊髄刺激電極とした。



B-3. 交感神経刺激論理の開発

高知大学医学部倫理委員会に承認された手続きにしたがって，インフォームドコンセントが得られた人工膝関節置換術を行う患者を対象とした。吸入ガス（セボフルレン）による全身麻酔の導入後，経皮的に硬膜外カテーテル電極を挿入し，カテーテル電極のリード線を誘発電位検査装置に接続した。カテーテル電極部を確認するために，1Hz の微弱な電気刺激を行い，傍脊柱筋の局所的な収縮部位を観察しながらカテーテル先端を頭側にすすめ，第９ないし第１２胸椎レベルに電極を留置した。電極位置をエックス線検査により確認した

ついで，誘発電位検査装置からの刺激パルスのパラメータをパルス幅 0.1 ミリ秒，刺激頻度 20Hz に設定した。刺激強度は，この刺激パルスにより平均動脈圧がおおむね 10mmHg だけ上昇する電流値に調整した。誘発電位検査装置からの刺激パルスが外部トリガー入力で駆動されるように設定した。また，観血的に動脈圧を記録するために，橈骨動脈にテフロン留置針を挿入し固定した。コンピュータから誘発電位検査装置に，白色雑音様の不規則なトリガー信号を入力しながら，動脈圧の変動を 15 分間記録した。刺激パルスの頻度は，0 か 20Hz かのいずれかになるように８秒間隔毎に不規則に切り替えた。下部胸髄の不規則刺激に対する動脈圧応答の記録を 20 例の患者から得た。

B-4. 電極間インピーダンスモニター機能付電気刺激装置の試作

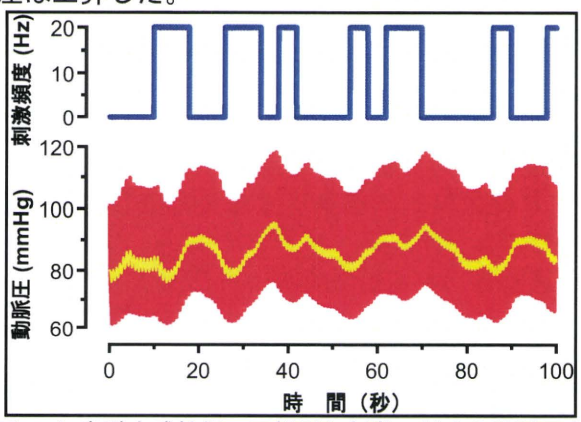
経皮的に硬膜外腔にカテーテル電極を挿入し、効果的に昇圧反応を引き起こすためには、胸髄下部の脊髄交感神経を刺激する必要がある。本血圧制御システムの実用化のためには、カテーテル電極を挿入する際にその位置を実時間でモニターできることが望ましい。

そこで、微弱交流を用いて電極間インピーダンスをモニターしながら、同時に、脊髄運動神経刺激に伴う局所筋収縮を目視で確認しながらカテーテル電極を挿入する手技を考案した。

C. 研究結果

C-1. 交感神経刺激論理の開発

図C-1に示すように、刺激に反応して、動脈圧は上昇した。



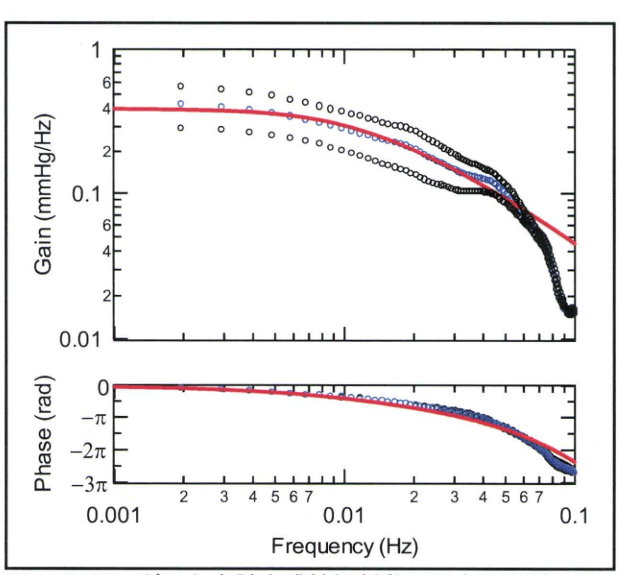
図C-1. 脊髄交感神経の不規則な刺激に対する動脈圧応答

上記のようなデータから刺激頻度の変化を入力，動脈圧の変動を出力とした伝達関数を求めたところ，図C-2のような結果が得られた。

平均的な伝達関数 $H_2(f)$ を下記の二次の低域通過フィルターへの曲線近似法を用いて解析した。

$$H_2(f)=\frac{a}{1+2\zeta\left(\frac{f}{f_N}j\right)+\left(\frac{f}{f_N}j\right)^2}exp\left(-2\pi fjL\right)$$

なお、a は定常ゲイン、・は減衰係数、f_N は固有周波数、L はラグ時間である。その結果、それぞれ、0.4、2.6、0.06 Hz、9 秒という結果が得られた。



図C-2. ランダムな脊髄交感神経刺激に対する動脈圧の応答特性（○印でプロットしたデータは平均±標準偏差である。赤実線は曲線近似の結果を示している。）

C-2. 電極間インピーダンスモニター機能付電気刺激装置の試作

これまでの術中脊髄機能モニター検査に関するデータにもとづき、次のような仕様とした。

①電源　直流 1.5［ｖ］（乾電池×2）

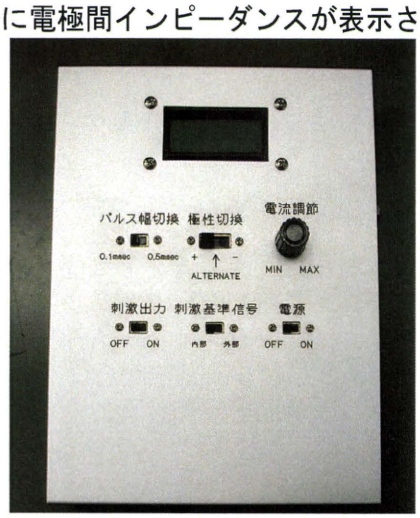
②局所脊髄運動神経刺激

電流パルス最大振幅	20 [mA]
刺激電流パルス幅	0.1, 0.5[ms]
刺激電流周波数	1 [Hz]
極性	交互・双極

③電極間インピーダンス測定用

励起電流実行値	100［μA］
励起電流周波数	1 [kHz]
最大測定インピーダンス	10 [kΩ]

図 C-3 に試作装置の外観を示す。上部液晶表示部分に電極間インピーダンスが表示される。



D. 考察

D-1. 交感神経刺激論理の開発

動脈圧のコントロールを目的として交感神経を刺激する場合には、交感神経刺激に対する動脈圧の反応にみられる過渡応答がわかっていなければならない。今回求められた伝達関数を用いて、シミュレーション等により、ヒト血管運動中枢の動作原理をパラメトリックモデルで模倣設計する予定である。

D-2. 電極間インピーダンスモニター機能付電気刺激装置の試作

経皮的なカテーテル電極の挿入時に誤って、硬膜を突き破り、脊髄を損傷する場合がある。硬膜を突き破った場合には、髄液が電極周囲を浸すため、電極間インピーダンスが急激に低下することが予想される。今後、今回試作した装置の有効性の検証を行う予定である。

E. 結論

ヒトの脊髄交感神経刺激によって、迅速な血圧制御が可能であることを示す臨床結果を得ることができた。また、安全な電極留置術を支援するための電極間インピーダンスモニター機能付電気刺激装置の試作に成功した。

F. 健康危険情報

なし

G. 研究発表

G-1. 論文

1. Sato K, Urbano R, Yu C, Yamasaki F, Sato T, Jordan J, Robertson D, Diedrich A. The effect of donepezil treatment on cardiovascular mortality. Clin Pharmacol Ther. 2010; 88: 335-338.

2. 佐藤隆幸. アルツハイマー病に用いられるドネペジルの抗心不全作用. 循環器内科. 2010; 68: 496-498.

3. Furuno T, Yamasaki F, Yokoyama T, Sato K, Sato T, Doi Y, Sugiura T. Effects of various doses of aspirin on platelet activity and endothelial function. Heart Vessels. 2011; in press.

4. Yamauchi K, Nagafuji H, Nakamura T, Sato T, Kohno N. Feasibility of ICG fluorescence-guided sentinel node biopsy in animal models using the HyperEye Medical System. Ann Sur Oncol. 2011; in press.

G-2. 学会発表

1. 鄭 燦, 李 梅花, 杉町 勝, 佐藤隆幸. A new intelligent technique of fluid restriction in small animals. 第 31 回日本循環制御医学会総会 大阪府 (2010.05.28-29)

2. 柿沼由彦, 秋山 剛, 有川幹彦, 半田武巳, 佐藤隆幸. Effects of a non-central and non-neuronal acetylcholine synthesis system equipped for cardiomyocytes, as a molecular brake, on overshooting cardiac energy metabolism. 第 31 回日本循環制御医学会総会 大阪府 (2010.05.28-29)

3. 久保 亨, 西永正典, 柿沼由彦, 佐藤隆幸, 土居義典. 塩酸ドネペジル内服による心血管系への影響に関する前向き登録調査研究. 第 11 回 Neurocardiology Workshop. 東京都 (2010.07.31)

4. Kakinuma Y, Sato T. The new era of therapeutic modalities against heart failure, focusing on pharmacological intervention of the cardiovascular cholinergic system. 19th International Conference of the Cardiovascular System Dynamics Society. Fukuoka, Japan (2010.09.23-26)

5. Yamasaki F, Nakajima N, Ikeuchi M, Kamimoto Y, Sugiura T, Sato T. Bionic baroreflex system for stabilizing arterial pressure by spinal cord stimulation and abdominal cuff. 19th International Conference of the Cardiovascular System Dynamics Society. Fukuoka, Japan (2010.09.23-26)

6. Arikawa M, Kakinuma Y, Zheng C, Yamasaki F, Sato T. Donepezil, an acetylcholinesterase inhibitor, reduces the risk of left ventricular free wall rupture during an acute phase of myocardial infarction by attenuating macrophage matrix metalloproteinase-9. 19th International Conference of the Cardiovascular System Dynamics Society. Fukuoka, Japan (2010.09.23-26)

7. Kakinuma Y, Akiyama T, Arikawa M, Sato T. Effects of a non-neuronal cholinergic system equipped for cardiomyocytes, as a molecular brake, on overshooting cardiac energy metabolism. 19th International Conference of the Cardiovascular System Dynamics Society. Fukuoka, Japan (2010.09.23-26)

8. 佐藤隆幸. 日本医工ものつくりコモンズへの期待. 第 19 回日本コンピュータ外科学会,シンポジウム「日本のもの作りを医療へ生かす取り組み」福岡県 (2010.11.03)

9. 山本正樹, 西森秀明, 割石精一郎, 福冨敬, 佐藤隆幸, 笹栗志朗. 冠動脈バイパス術におけるＨＥＭＳ－ＩＣＧ造影法を用いた新血流評価法. 第 41 回日本心臓血管外科学会学術総会. 千葉県 (2011.02.23-25)

G-3. 新聞報道等

(新聞)

1. 「手術時、目的部位が発光　高知大など、赤外線カメラ開発」日本経済新聞 2010.06.05.

2. 「リンパ節や血管、手術中くっきり　高知大、蛍光ナビ開発」朝日新聞（大阪）2010.06.05.

3. 「高知大医学部　近赤外線でリンパ管“透

視"世界初 手術用ナビ製品化」高知新聞 2010.06.05.

4. 「高知大が蛍光カメラ開発ー外科切除部を可視化」日刊工業新聞 2010.06.05.
5. 「高知大、手術システムを製品に 患部を近赤外透過で可視化」FujiSankei Business 2010.06.11.
6. 「手術用カメラ：血流くっきり 世界初、高知大医学部・佐藤教授チームが製品化」毎日新聞（高知）2010.06.15.
7. 「瑞穂医科など、近赤外光利用新システム、術中に微細切除組織を可視化」化学工業日報 2010.06.16.
8. 「外科手術の切除部分をリアルタイムで可視化」科学新聞 2010.06.18.
9. 「リンパ節や血管の状態、手術時、カラーで観察、高知大など、近赤外蛍光を撮影」日経産業新聞 2010.06.23.
10. 「認知症薬 心臓病予防に効果 高知大など」日本経済新聞（朝刊）2010.12.05.
11. 「認知症薬 心臓病に効果 高知大と米合同チーム 心筋細胞再生促す」高知新聞（朝刊）2010.12.05.

（テレビ）

1. 「手術患部 カラー映像で確認」ＮＨＫ総合放送（高知）「こうち情報いちばん」2010.06.07.
2. 「光る患部で手術を変える」ＮＨＫ総合放送（高知）「おはようこうち」2010.07.06.
3. 「光る患部が医療を変える」ＮＨＫ総合放送「ニュースウオッチ９」2010.08.06.
4. 「体内の手術のポイントを可視化．最新手術ナビゲーションシステム完成」 JST Science News 2010.09.
5. 「地域発 がん治療革命～医療格差を解消せよ～」ＮＨＫ総合放送（四国）「四国羅針盤」2010.10.29.

（雑誌）

1. 「近赤外蛍光を利用した血管・血流、リンパ管・リンパ流・リンパ節のカラーイメージング装置の開発」 MEDICAL PHOTONICS 2010.07.
2. 「カラー動画による手術ナビゲーションシステムの誕生」 ＪＳＴ News , 2010.07.
3. 医工連携を歩く.「近赤外蛍光と可視光の同時撮影が可能なカラーイメージング装置の開発」映像情報メディカル･インダストリアル 2010.0 9.
4. 「生体深部の血液・リンパ管を近赤外蛍光カラーで可視化.」医療タイムス 2010.11.

（講演）

1. 「世界初！身体的･精神的に負担の少ない外科手術の実現へ ～近赤外蛍光を捕捉する術中ナビゲーションカラーイメージングシステムの開発 ～」"知"と"地"の協奏 地域貢献を目指す高知発の科学技術 ～JST イノベーションサテライト高知 研究成果報告会～ 2010.09.09.
2. 近赤外蛍光カラーイメージング装置による手術支援ー生体深部の可視化ー. HOSPEX JAPAN 2010 特別講演.2010.11.17.
3. 血圧をコンピュータで自在にあやつる. ヒューマンストレス産業技術研究会. 2011.03.08.

H. 知的所有権の取得状況

1. 特許第 4544917 号「生体圧迫装置及び血圧測定装置」発明者：小椋敏彦、佐藤隆幸、山崎文靖. 登録日：2010.07.09.

厚生労働省科学研究補助金
（医療技術実用化総合研究事業）
平成２２年度分担研究報告書

バイオニック血圧制御システムの実用化研究
瞬時血圧測定法の開発（分担課題名）

分担研究者　山越　憲一（金沢大学 理工研究域　教授）

研究要旨：
脊髄損傷患者の自律神経障害に起因する起立性低血圧症を予防するためのバイオニック血圧制御システムの実用化研究として，前年度までの成果をさらに発展させて足背動脈を対象とした非侵襲瞬時血圧計測システムの設計開発を行った。特に，今年度は実用化のための重点開発項目として，「各被測定者の足型に合わせたカフ・光電センサ部を含む血圧計測インターフェース部の改良」を行った。また，上記の主課題に付随する副課題として「血圧計測インターフェース部の速やかな開発・改良のためのラピッドプロトタイピングと CAD システム導入による試作システムの開発」を行った。以上の結果，血圧計測インターフェースの小型化が達成され，健常者を対象とした性能試験において，非侵襲瞬時血圧計測システムの有効性も確認された。

A. 研究目的

現在，世界における脊髄損傷患者(以下脊損者)は毎年約 13 万人以上増加しており，累計 120 万人以上にのぼり，このうち日本においては 10 万人以上の脊損者がいるとされている。脊損者の多くは自律神経障害などの合併症を発症し，特に脊損者の 70%以上が起立性低血圧症による QOL の低下に悩まされている。

本分担研究では，脊損者の QOL 向上のためのバイオニック血圧制御システムの実用化開発を目指し，非侵襲瞬時血圧計測・制御システムの開発研究を進めている。今年度はこれまでの成果をさらに発展させて，足背動脈を対象とした瞬時血圧計測システムの設計・試作開発を行うことを目的とした。

B. 研究方法

B-1. 非侵襲瞬時血圧計測システムの開発に関する研究

図 B-1 にバイオニック血圧制御システムの全体概要ブロック図を示す。本システムでは，脊損者の瞬時血圧を計測し，目標血圧との差分から血圧制御装置により脊損者の血圧を制御することで，上体起こしや起立などの姿勢変化の支援を行うものである。今年度(平成 22 年度)は，昨年度に引き続き，分担研究者が提案した容積補償法に基づき，脊損者の負担にならない足背動脈を対象とした非侵襲瞬時血圧計測プロトタイプシステムの試作開発を行った。特に本年度では主たる課題として，血圧計測用のカフ・光電センサ部を含む血圧計測インターフェース部に焦点をあて，血圧計測インターフェース部の改良を行った。また，主たる課題に付随する副課題として，血圧計測インターフェース部の速やかな開発・改良のためのラピッドプロトタイピングと CAD システム導入による試作システムの開発を行った。

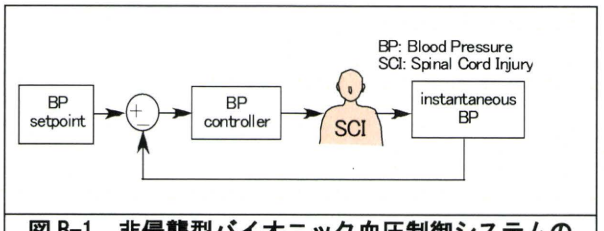


図 B-1　非侵襲型バイオニック血圧制御システムの基本ブロック図

B-1.1. ラピッドプロトタイピングと CAD システム導入による新規試作システムの開発

上記 B-1 の速やかな実現のために，前年度に導入した３次元造型機によるラピッドプロトタイピングと３次元 CAD を組み合わせた新規試作システムを開発した。

C. 研究結果

C-1. 非侵襲瞬時血圧計測システムの開発に関する研究

図 C-1 に新たに改良した血圧計測インターフェースを含む血圧計測部を示す。今回改良した

システムは，前年度までに開発したものと比較して装着性に優れており，実用化に向けて大きく前進したものと考えられた。また，今回開発したシステムにより，瞬時血圧も正しく測定可能であった。

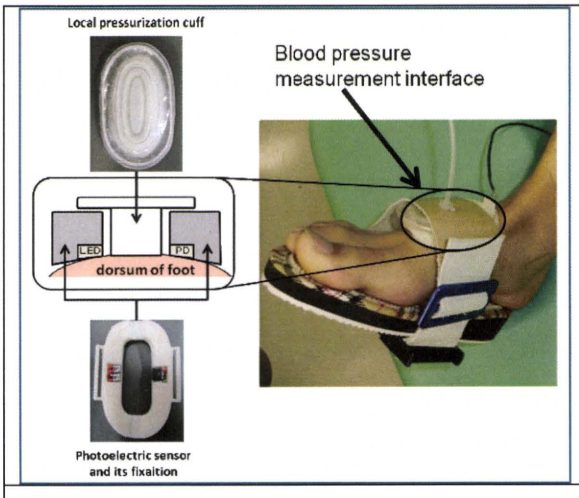


図 C-1　足背血圧計測部

C-1.1. ラピッドプロトタイピングと CAD システム導入による試作システム

ラピッドプロトタイピングと３次元 CAD を組み合わせた新規試作システムにより，血圧計測インターフェースの速やかな開発・改良が可能となり，研究の進展に大きく貢献することができた。図 C-2 に，試作システムによって作製され，順次改良された血圧計測インターフェースを示す。各世代（図 C-2 では第１～第４世代として示す）間の改良を３次元 CAD で行い，試作を３次元造型機によるラピッドプロトタイピングで行うことによって，速やかな改良を行うことが可能であった。

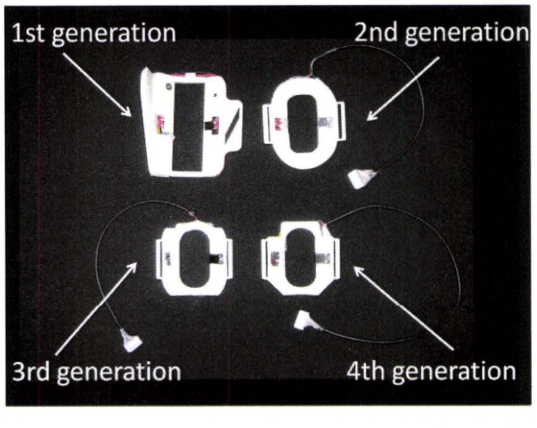


図 C-2　順次改良された血圧計測インターフェース

D. 考察

バイオニック血圧制御システムの実用化のための非侵襲瞬時血圧計測システムの試作開発は，順調に進んでいるものと考えられた。また，さらにその開発をスピードアップし，さらには各被測定者に合わせた血圧計測インターフェースを作製するための試作システムの開発も行い，その有用性も実証された。今後，実際の脊損者において，瞬時血圧計測システムを組み込んだバイオニック血圧制御システムの開発・評価を進める予定である。

E. 結論

バイオニック血圧制御システムの実用化研究として，足背動脈における非侵襲瞬時血圧計測システムの試作開発および評価を行った。本年度は，血圧計測インターフェースに焦点をあて，その改良を行った。また，血圧計測インターフェース改良のための試作システムも開発した。

F. 健康危険情報

特になし

G. 研究発表

G-1. 論文

1. Yamakosi K. Bioinstrumentation, IEEE Rev. Biomed. Eng., 3: 3-6, 2010

2. Ogawa M, Nogawa M, Yamakoshi T, and Yamakoshi K. Evaluation of cardiovascular stress reaction using HPCD method on a beat-by-beat basis, Advances in Natural Science, 3(2): 128-132, 2010

3. Yamakoshi K. Current status of non-invasive bioinstrumentation for healthcare, Sensors and Materials, 20:1-20, 2010

G-2. 学会発表

1. Ogawa M, Nogawa M, Yamakoshi T, and Yamakoshi K. Evaluation of cardiovascular stress reaction using HPCD method on a beat-by-beat Basis, The 3rd International Conference of Bionic Engineering (September 14-16, 2010, Zhuhai, China)

2. Nogawa M, Ogawa M, Yamakoshi T, Sunagawa K and Yamakoshi K. Development of non-invasive instantaneous arterial pressure measurement and its control system for spinal injury patients, The 6th

International Symposium on Precision Engineering Measurements and Instrumentation (August 8-11, 2010, Hangzhou, China)

3. 小川充洋, 野川雅道, 大兼政天平, 田中志信, 山越憲一. Preliminary study on beat-by-beat HPCD method for non-invasive hemodynamics evaluation, 第 48 回日本生体医工学大会, (2010 年 5 月 25-27 日, 東京)

4. 長瀬樹生, 小野崇貴, 野川雅道, 小川充洋, 山越健弘, 坂本隆史, 戸伏倫之, 村山佳範, 田中志信, 山越憲一, 砂川賢二. Noninvasive beat-by-beat blood pressure measurement for preventing severe orthostatic hypotension with spinal cord injury, 第 48 回日本生体医工学大会, (2010 年 5 月 25-27 日, 東京)

G-3. 新聞報道

なし

H. 知的所有権の取得状況

なし

研究成果の刊行に関する一覧表

書籍

なし

雑誌

1. Hashimoto T, Ichiki T, Ikeda J, Narabayashi E, Matsuura H, Miyazaki R, Inanaga K, Takeda K, Sunagawa K. Inhibition of MDM2 Attenuates Neointimal Hyperplasia via Suppression of Vascular Proliferation and Inflammation. Cardiovasc Res. In press, 2011.

2. Kamiya A, Kawada T, Shimizu S, Sugimachi M. Closed-loop spontaneous baroreflex transfer function is inappropriate for system identification of neural arc but partially appropriate for peripheral arc: a predictability analysis. J Physiol. In press, 2011.

3. Uemura K, Kawada T, Sunagawa K, Sugimachi M. Peak Systolic Mitral Annulus Velocity Reflects the Status of Ventricular-Arterial Coupling-Theoretical and Experimental Analyses. J Am Soc Echocardiogr. In press, 2011

4. Furuno T, Yamasaki F, Yokoyama T, Sato K, Sato T, Doi Y, Sugiura T. Effects of various doses of aspirin on platelet activity and endothelial function. Heart Vessels. In press, 2011

5. Yamauchi K, Nagafuji H, Nakamura T, Sato T, Kohno N. Feasibility of ICG fluorescence-guided sentinel node biopsy in animal models using the Hyper Eye Medical System. Ann Sur Oncol. In press, 2011.

6. Hirooka Y, Kishi T, Sakai K, Takeshita A, Sunagawa K. Imbalance of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. Am J Physiol Regul Integr Comp Physiol. 300: R818-26, 2011.

7. Chen L, Nakano K, Kimura S, Matoba T, Iwata E, Miyagawa M, Tsujimoto H, Nagaoka K, Kishimoto J, Sunagawa K, Egashira K. Nanoparticle-mediated delivery of pitavastatin into lungs ameliorates the development and induces regression of monocrotaline-induced pulmonary artery hypertension. Hypertension. 57: 343-50, 2011.

8. Fujino T, Nishizaka M, Yufu T, Sunagawa K. A case of multiple focal nodular hyperplasia in the liver which developed after heart transplantation. Intern Med. 50: 43-46, 2011.

9. Kawada T, Shimizu S, Kamiya A, Sata Y, Uemura K, Sugimachi M. Dynamic characteristics of baroreflex neural and peripheral arcs are preserved in spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol. 300: R155-165, 2011

10. Mizuno M, Kawada T, Kamiya A, Miyamoto T, Shimizu S, Shishido T, Smith SA, Sugimachi M. Exercise training augments the dynamic heart rate response to vagal but not sympathetic stimulation in rats. Am J Physiol Regul Integr Comp Physiol. 300: R969-977, 2011

11. Yamamoto H, Kawada T, Kamiya A, Miyazaki S, Sugimachi M. Involvement of the mechanoreceptors in the sensory mechanisms of manual and electrical acupuncture. Auton Neurosci 160: 27-31, 2011.

12. Kishi T, Sunagawa K. Baroreflex sensitivity might predict responders to milrinone in patients with heart failure. Int Heart J. 51: 411-415, 2010.

13. Sunagawa K, Sugimachi M. Development of artificial bionic baroreflex system. Conf Proc IEEE Eng Med Biol Soc. 2010: 3446-3448, 2010.

14. Sunagawa K. The pressure-volume relationship of the heart: past, present and future. Conf Proc IEEE Eng Med Biol Soc. 2010: 3554-3555, 2010.

15. Sugimachi M, Sunagawa K, Uemura K, Shishido T. Physiological significance of pressure-volume relationship: a load-independent index and a determinant of pump function. Conf Proc IEEE Eng Med Biol Soc. 2010: 3553, 2010.

16. Uemura K, Sugimachi M, Kawada T, Sunagawa K. Automated drug delivery system for the management of hemodynamics and cardiac energetic in acute heart failure. Conf Proc IEEE Eng Med Biol Soc. 2010: 5222-5225, 2010.

17. Sugimachi M, Sunagawa K, Uemura K, Kamiya A, Shimizu S, Inagaki M, Shishido T. Estimated venous return surface and cardiac output curve precisely predicts new hemodynamics after volume change. Conf Proc IEEE Eng Med Biol Soc. 2010: 5205-5208, 2010.

18. Sakamoto T, Murayama Y, Tobushi T, Sakamoto K, Tanaka A, Tsutsumi T, Sunagawa K. How to quantitatively synthesize dynamic changes in arterial pressure from baroreflexly modulated ventricular and arterial properties. Conf Proc IEEE Eng Med Biol Soc. 2010: 2869-2871, 2010.

19. Inanaga K, Ichiki T, Miyazaki R, Takeda K, Hashimoto T, Matsuura H, Sunagawa K. Acetylcholinesterase inhibitors attenuate atherogenesis in apolipoprotein E-knockout mice. Atherosclerosis. 213: 52-58, 2010.

20. Ito K, Hirooka Y, Sunagawa K. Blockade of mineralocorticoid receptors improves salt-induced left-ventricular systolic dysfunction through attenuation of enhanced sympathetic drive in mice with pressure overload. J Hypertens. 28: 1449-1458, 2010.

21. Oda S, Nagahama R, Nakano K, Matoba T, Kubo M, Sunagawa K, Tominaga R, Egashira K. Nanoparticle-mediated endothelial cell-selective delivery of pitavastatin induces functional collateral arteries (therapeutic arteriogenesis) in a rabbit model of chronic hind limb ischemia. J Vasc Surg. 52: 412-420, 2010.

22. Hirooka Y, Sagara Y, Kishi T, Sunagawa K. Oxidative stress and central cardiovascular regulation. - Pathogenesis of hypertension and therapeutic aspects -. Circ J. 74: 827-835, 2010.

23. Kishi T, Hirooka Y, Konno S, Ogawa K, Sunagawa K. Angiotensin II type 1 receptor-activated caspase-3 through ras/mitogen-activated protein kinase/extracellular signal-regulated kinase in the rostral ventrolateral medulla is involved in sympathoexcitation in stroke-prone spontaneously hypertensive rats. Hypertension. 55: 291-297, 2010.

24. Miyashita H, Aizawa A, Hashimoto J, Hirooka Y, Imai Y, Kawano Y, Kohara K, Sunagawa K, Suzuki H, Tabara Y, Takazawa K, Takenaka T, Yasuda H, Shimada K. Cross-sectional characterization of all classes of antihypertensives in terms of central blood pressure in Japanese hypertensive patients. Am J Hypertens. 23: 260-268, 2010.

25. Kishi T, Hirooka Y, Konno S, Sunagawa K. Sympathoinhibition induced by centrally administered atorvastatin is associated with alteration of NAD(P)H and Mn superoxide dismutase activity in rostral ventrolateral medulla of stroke-prone spontaneously hypertensive rats. J Cardiovasc Pharmacol. 55: 184-190, 2010.

26. Takemoto M, Nakashima A, Muneuchi J, Yamamura K, Shiokawa Y, Sunagawa K, Tominaga R. Para-Hisian pacing for a pediatric patient with a congenitally corrected transposition of the great arteries (SLL). Pacing Clin Electrophysiol. 33: e4-e7, 2010.

27. Kamiya A, Kawada T, Mizuno M, Shimizu S, Sugimachi M. Parallel resetting of arterial baroreflex control of renal and cardiac sympathetic nerve activities during upright tilt in rabbits. Am J Physiol Heart Circ Physiol. 298: H1966-H1975, 2010

28. Kawada T, Li M, Kamiya A, Shimizu S, Uemura K, Yamamoto H, Sugimachi M. Open-loop dynamic and static characteristics of the carotid sinus baroreflex in rats with chronic heart failure after myocardial infarction. J Physiol Sci. 60: 283-298, 2010

29. Kawada T, Akiyama T, Shimizu S, Kamiya A, Uemura K, Sata Y, Shirai M, Sugimachi M. Large conductance Ca2+-activated K+ channels inhibit vagal acetylcholine release at the rabbit sinoatrial node. Auton Neurosci. 156: 149-151, 2010

30. Mizuno M, Kawada T, Kamiya A, Miyamoto T, Shimizu S, Shishido T, Smith SA, Sugimachi M. Dynamic characteristics of heart rate control by the autonomic nervous system in rats. Exp Physiol. 95: 919-925, 2010

31. Shimizu S, Shishido T, Une D, Kamiya A, Kawada T, Sano S, Sugimachi M. Right ventricular stiffness constant as a predictor of postoperative hemodynamics in patients with hypoplastic right ventricle: a theoretical analysis. J Physiol Sci. 60: 205-212, 2010

32. Takahama H, Asanuma H, Sanada S, Fujita M, Sasaki H, Wakeno M, Kim J, Asakura M, Takashima S, Minamino T, Komamura K, Sugimachi M, Kitakaze M. A histamine H receptor blocker ameliorates development of heart failure in dogs independently of beta-adrenergic receptor blockade. Basic Res Cardiol. 105: 787-794, 2010

33. Uemura K, Zheng C, Li M, Kawada T, Sugimachi M. Early short-term vagal nerve stimulation attenuates cardiac remodeling after reperfused myocardial infarction. J Card Fail. 16: 689-699, 2010

34. Une D, Shimizu S, Kamiya A, Kawada T, Shishido T, Sugimachi M. Both skeletonized and pedicled internal thoracic arteries supply adequate graft flow after coronary artery bypass grafting even during intense sympathoexcitation. J Physiol Sci. 60: 407-413, 2010

35. Yokokawa M, Chugh A, Ulfarsson M, Takaki H, Han L, Yoshida K, Sugimachi M, Morady F, Oral H. Effect of linear ablation on spectral components of atrial fibrillation. Heart Rhythm. 7: 1732-1737, 2010

36. Sato K, Urbano R, Yu C, Yamasaki F, Sato T, Jordan J, Robertson D, Diedrich A. The effect of donepezil treatment on cardiovascular mortality. Clin Pharmacol Ther. 88: 335-338, 2010

37. Yamakoshi K Bioinstrumentation. IEEE Rev. Biomed. Eng 3: 3-6, 2010

38. Ogawa M, Nogawa M, Yamakoshi T, and Yamakoshi K. Evaluation of cardiovascular stress reaction using HPCD method on a beat-by-beat basis. Advances in Natural Science. 3: 128-132, 2010

39. Yamakoshi K. Current status of non-invasive bioinstrumentation for healthcare. Sensors and Materials. 20: 1-20, 2010

40. 佐藤隆幸 アルツハイマー病に用いられるドネペジルの抗心不全作用循環器内科 68: 496-498, 2010

Am J Physiol Regul Integr Comp Physiol 300: R818–R826, 2011.
First published February 2, 2011; doi:10.1152/ajpregu.00426.2010.

Imbalance of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension

Yoshitaka Hirooka, Takuya Kishi, Koji Sakai, Akira Takeshita,† and Kenji Sunagawa

Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan

Submitted 29 June 2010; accepted in final form 29 January 2011

Hirooka Y, Kishi T, Sakai K, Takeshita A, Sunagawa K. Imbalance of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. *Am J Physiol Regul Integr Comp Physiol* 300: R818–R826, 2011. First published February 2, 2011; doi:10.1152/ajpregu.00426.2010.— Nitric oxide (NO) and reactive oxygen species (ROS) play important roles in blood pressure regulation via the modulation of the autonomic nervous system, particularly in the central nervous system (CNS). In general, accumulating evidence suggests that NO inhibits, but ROS activates, the sympathetic nervous system. NO and ROS, however, interact with each other. Our consecutive studies and those of others strongly indicate that an imbalance between NO bioavailability and ROS generation in the CNS, including the brain stem, activates the sympathetic nervous system, and this mechanism is involved in the pathogenesis of neurogenic aspects of hypertension. In this review, we focus on the role of NO and ROS in the regulation of the sympathetic nervous system within the brain stem and subsequent cardiovascular control. Multiple mechanisms are proposed, including modulation of neurotransmitter release, inhibition of receptors, and alterations of intracellular signaling pathways. Together, the evidence indicates that an imbalance of NO and ROS in the CNS plays a pivotal role in the pathogenesis of hypertension.

blood pressure; sympathetic nervous system; central nervous system; nitric oxide; oxidative stress

ACTIVATION OF THE SYMPATHETIC nervous system is critically involved in the pathogenesis of hypertension, from initial occurrence to the development of target organ damage, such as heart failure, stroke, and renal failure (35, 36). The importance of the effects of the renin-angiotensin system on the sympathetic nervous system in the pathogenesis of hypertension is recently highlighted (30, 31). This is not surprising because both the autonomic nervous system and hormonal factors are the major regulators of blood pressure; therefore, abnormalities of either system are likely to be involved in the pathogenesis of essential hypertension (30, 31, 37). Esler (30) reported that the sympathetic nervous system is activated in ~50% of patients with hypertension, particularly in patients with essential hypertension. Central sympathetic outflow is determined by several important nuclei and their circuits in the central nervous system (CNS) (9, 81). These pathways involve many neurotransmitters and neuromodulators (16, 25, 38, 99). In particular, the brain stem circuitry is now considered crucial for the pathogenesis of hypertension, including both excitatory and inhibitory inputs from the supramedullary nuclei and the baroreceptors (16, 25, 38, 100, 115). In this review, we focus on the role of nitric oxide (NO) and reactive oxygen species (ROS) in the brain stem as factors constituting the neural mechanisms of hypertension. Because of the close relationship between NO and ROS, we discuss the individual roles of NO and ROS in the brain stem in central mechanisms of hypertension, and then the relationship between the two. Finally, we will discuss the possibility of targeting some cardiovascular drugs to improve the imbalance of NO and ROS.

NO in the Brain

NO is an important mediator of intracellular signaling in various tissues, including the CNS (32, 118, 119). NO acts via the second messenger cyclic GMP (32). Thus, soluble guanylate cyclase is its receptor. NO is synthesized from its precursor, L-arginine, by endogenous NO synthase (NOS). There are three NOS isoforms: constitutive enzymes, such as neuronal NOS (nNOS) and endothelial NOS (eNOS), and inducible enzymes such as inducible NOS (iNOS). A number of studies have demonstrated the localization of the nNOS, eNOS, and iNOS within the CNS using in situ hybridization and histochemical staining with NADPH-diaphorase or immunohistochemistry (8). nNOS is abundant in neurons. Considerable evidence indicates that NOS acts on central and peripheral sites throughout the autonomic nervous system, which controls the cardiovascular system, including the receptors and effectors of the baroreflex pathway (70, 95, 129).

Role of NO in the Brain Stem in Controlling Blood Pressure

Chronic administration of the NO synthesis inhibitor N^{w}-nitro-L-arginine methyl ester (L-NAME) in drinking water induces a large increase in blood pressure in rats (29). Gangli-

† Deceased March 15, 2009.

Address for reprint requests and other correspondence: Y. Hirooka, Dept. of Cardiovascular Medicine, Kyushu Univ., Graduate School of Medical Sciences, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan (e-mail: hyoshi@cardiol.med.kyushu-u.ac.jp).

onic blockade elicits a greater fall in blood pressure in L-NAME-treated rats compared with controls, suggesting that the level of central sympathetic outflow in L-NAME-treated rats is greater than that in control rats. Microinjection of an ANG II type 1 (AT_1) receptor blocker (candesartan), but not that of an AT_2 receptor blocker (PD123319), into the nucleus tractus solitarius (NTS) elicits a greater decrease in blood pressure, heart rate, and renal sympathetic nerve activity (RSNA) in L-NAME-treated rats than in control rats. These results suggest that increased RSNA contributes to hypertension induced by chronic NOS inhibition and that activation of the renin-angiotensin system in the NTS is involved, at least in part, in the increased RSNA via AT_1 receptors (29). The rostral ventrolateral medulla (RVLM), the vasomotor center, is also activated in this model of hypertension, suggesting enhanced central sympathetic outflow (9). Pharmacological inhibition of NOS evoked by N^G-monomethyl-L-arginine (L-NMMA) or L-NAME also induces large increases in blood pressure that are partially sympathetically mediated in humans (109).

Immunohistochemical studies have revealed a rich distribution of nNOS in the NTS (8). Microinjection of L-NMMA into the NTS elicits an increase in blood pressure and RSNA, regardless of whether the baroreceptors are intact in anesthetized rabbits (39). The neurons in the NTS are activated by NO projecting to the caudal ventrolateral medulla, thereby activating the inhibitory neurons in the caudal ventrolateral medulla, which project to the RVLM, and may ultimately result in decreased sympathetic nerve activity (SNA). Single-unit extracellular recordings of NTS neurons in rat brain stem slices revealed that L-arginine increases neuronal activity dose-dependently, but D-arginine does not (80, 116). L-NMMA blocks the L-arginine-induced increases in the neuronal activity. Sodium nitroprusside, an NO donor, also increases neuronal activity. Consistent with the findings from the in vivo studies (39), these results suggest that NO increases the neuronal activity in the NTS through an increase in cyclic GMP. It has been proposed that NO acts in an ultrashort feedback loop, in which the release of L-glutamate activates nNOS and subsequently the production of NO (32). The NO, in turn, diffuses to presynaptic terminals, where it modulates the release of L-glutamate in response to neuronal activation. Studies using in vivo microdialysis demonstrated that activation of NMDA receptors in the NTS induces the release of NO, and NMDA-induced NO production stimulates L-glutamate release (74, 75, 82). In addition, this mechanism is involved in the depressor and bradycardic responses evoked by NMDA receptor activation in anesthetized rats (82). To determine the effects of increased NO production in the NTS for much longer periods on blood pressure, heart rate, and urinary norepinephrine excretion, we developed an in vivo technique for eNOS gene transfer into the NTS of rats (43, 44, 46, 107). In this study, the successful transfer of the eNOS gene into the NTS was confirmed by several methods, including immunohistochemistry, Western blot analysis, and nitrite/nitrate concentration measurements (107). Changes in blood pressure and heart rate were observed using a radio-telemetry system. It is important to note that we used eNOS instead of nNOS, which is normally abundant in the CNS, because the purpose of the study was to increase NO production from constitutively expressed NOS. The results indicated that NO in the NTS exerts an inhibitory effect on SNA in vivo.

Compared to studies of the NTS, studies of the RVLM in both acute and anesthetized models have produced more conflicting results (42, 53, 66, 81, 112, 120, 131). Therefore, we applied the technique described above to studies of the RVLM (57, 58). In those studies, blood pressure, heart rate, and urinary norepinephrine excretion were decreased after eNOS gene transfer. Microinjection of either L-NMMA or bicuculine, a GABA receptor antagonist, into the RVLM after eNOS gene transfer increased blood pressure to greater levels in the eNOS gene transfer group compared with the mock gene transfer control group. GABA levels in the RVLM after the eNOS gene transfer measured by in vivo microdialysis were also increased in the eNOS gene transfer group. These results indicate that the increased NO production evoked by the overexpression of eNOS in the bilateral RVLM decreases blood pressure, heart rate, and SNA in awake rats. Furthermore, these responses are mediated by an increased release of GABA in the RVLM. These studies provided convincing evidence that chronic changes in neurotransmitters/neuromodulators in the RVLM have a sustained impact on blood pressure in awake animals.

There is no clear explanation for the different modulatory effects of NO on neurons between the NTS and RVLM. NO increases both excitatory and inhibitory amino acids in the RVLM (43, 57). NO has also been shown to increase both L-glutamate and GABA in the paraventricular nucleus of hypothalamus (49). Microinjection of kynurenic acid into the RVLM, however, did not alter blood pressure after eNOS gene transfer, although microinjection of bicuculline into the RVLM augmented the increase in blood pressure (57). Therefore, we consider that GABAergic inhibition of the RVLM neurons might be more powerful than the glutamatergic activation in the resting condition (43, 57). In contrast, the glutamatergic input into the NTS neurons might be more powerful than the GABAergic input. In the NTS, there are close anatomic connections between nNOS and glutamatergic receptors (75). Furthermore, increases in NO induce L-glutamate release and microinfusion of NMDA and AMPA increase NO levels, suggesting that there are facilitatory interactions between L-glutamate and NO (27, 74, 82), although there are no studies measuring GABA levels induced by NO in the NTS. Furthermore, higher concentrations of NO are required to directly engage GABAergic inhibition, while lower concentrations of NO might be important for glutamatergic transmission in the NTS (125). Thus, it is still difficult and complicated to explain the physiological response induced by NO in the NTS (119). With regard to the action of NO on neuronal activity, NO induces both excitatory and inhibitory postsynaptic currents that likely depend on the neuron examined (6, 7, 126, 127).

Effects of NO in the Brain System in Experimental Models of Hypertension

Neurogenic mechanisms are dominant in the pathogenesis of essential hypertension in ~50% of patients (30). Spontaneously hypertensive rats (SHR) or stroke-prone SHR (SHRSP) exhibit increased RSNA during the development of hypertension, and blood pressure and RSNA are positively correlated (52, 79). The L-arginine-NO pathway is disrupted in SHR and SHRSP. The depressor response to an intracerebroventricular injection of an NO donor is greater in SHRSP than in normo-

tensive control rats, whereas the pressor response to intracerebroventricular injection of L-NAME is smaller (13). Semiquantitative RT-PCRs and in situ hybridization in SHR and Wistar-Kyoto (WKY) rats at 4 (prehypertensive) and 14 (established hypertension) wk of age (101) indicate that eNOS mRNA expression changes with the development of hypertension. Although there are no differences between the groups at 4 wk of age, nNOS gene expression increases in the hypothalamus, dorsal medulla, and caudal ventrolateral medulla of SHR compared with WKY rats at 14 wk of age. In the RVLM, there are no differences between the groups. In the SHRSP, there are also no differences in nNOS expression levels in the RVLM compared with WKY rats (101). A recent study demonstrated that NOS activity, measured by the ability of tissue homogenate to convert [^{3}H]L-arginine to [^{3}H]L-citrulline in a calcium- and NADPH-dependent manner, is impaired in the cerebral cortex and brain stem of prehypertensive SHR (104). In contrast, NOS activity is increased in the hypothalamus and brain stem in SHR rats with established hypertension compared with WKY rats (104). Thus, attenuated NOS activity in the cortex and brain stem of prehypertensive SHR might play a role in the pathogenesis of hypertension, and the up-regulated NOS activity in the hypothalamus and brain stem of SHR with established hypertension might serve to compensate for the hypertension. The expression of iNOS mRNA and protein is under the limits of detection in the hypothalamus of both WKY rats and SHR (40). Decreased NOS activity measured by the nitrite and nitrate contents was also demonstrated in the hypothalamus of SHR (1). In hypertensive SHRSP, nNOS protein expression levels in the hypothalamus and brain stem were enhanced compared with those in WKY (59). In a renovascular hypertensive rat model, mRNA expression levels of nNOS and soluble guanylate cyclase genes are reduced in the hypothalamus but not in the dorsal medulla (69). Together, these results suggest that the L-arginine-NO pathway is impaired in hypertensive rats, including SHR, possibly because of a posttranscriptional abnormality (70). Overexpression of eNOS in the NTS results in a greater depressor response in SHR than in WKY rats in the awake state (44). In that study, eNOS was used instead of nNOS to increase NO production locally in the NTS. Findings from another study suggest that the depressed NO modulation is consistent with the lower NOS activity in the dorsal brain stem (103). Therefore, the abnormality in the L-arginine-NO pathway in the NTS might be involved in the maintenance of hypertension of SHR. A recent study by Waki et al. (121) demonstrated that endogenous eNOS activity in the NTS plays a major role in determining the blood pressure set point in SHR and contributes to maintaining high arterial blood pressure in this model, suggesting the possible involvement of neurovascular coupling (96). In the RVLM of SHRSP, overexpression of eNOS elicits greater depressor and sympathoinhibitory responses than in WKY (58). Furthermore, the increase in NO production evoked by the overexpression of eNOS in the RVLM enhances the inhibitory action of GABA on the RVLM neurons (58). The results indicate that NO dysfunction and the resulting disinhibition of the RVLM contribute to increase RSNA in SHRSP.

Effects of NO in the Brain Stem on Baroreflex Function

As described earlier, NO activity in the NTS and RVLM influences cardiovascular regulation. We examined the role of endogenous NO in the brain stem in the rapid central adaptation of baroreflex control of RSNA in anesthetized rabbits (41). Bilateral carotid sinuses were isolated, and a stepwise increase in pressure was applied to the carotid sinuses, while arterial pressure and RSNA were recorded. The procedure was performed after intracisternal injection of L-NAME, D-NAME, L-arginine, or the vehicle solution. L-NAME enhances the rapid adaptation of the arterial baroreflex control of renal sympathetic nerve activity in rabbits (41). Transmission of arterial baroreflex signals depends on NO (27, 118). It was reported that the baroreceptor reflex gain in awake animals was increased by NO in the bradycardic component, although in these studies NOS inhibitors were administered systemically to examine the role of NO on baroreflex function (78, 87). Furthermore, overexpression of eNOS in the RVLM improves impaired baroreflex control of heart rate in SHRSP (60).

In summary, NO in the brain stem, particularly in the NTS and RVLM, has a sympathoinhibitory function, thereby reducing blood pressure. NO in the brain stem also facilitates the baroreflex function. The sympathoinhibitory effects of NO are impaired in animal models of hypertension, and supplementation of NO in the brain stem in hypertensive rats attenuates the abnormality, thereby decreasing blood pressure. The facilitory release of neurotransmitters induced by NO might be involved in the synaptic transmission mechanism.

ROS in the Brain

Substantial evidence also indicates that increased oxidative stress is involved in the pathogenesis of hypertension (12, 47, 48, 94, 99). ROS, such as superoxide anions and hydroxyl radicals, increase oxidative stress. There are several sources of ROS generation, such as NADPH oxidase, xanthine oxidase, mitochondria, and NOS uncoupling (12, 47, 48, 94, 99). On the other hand, reduction of antioxidant enzymes, such as superoxide dismutases (SOD), also induces an increase in oxidative stress (47, 48, 99). Although the role of ROS in the regulation of blood pressure in the normotensive state is not clear, increased ROS generation in the brain stem contributes to neural mechanisms of hypertension (47, 48). For example, although there is evidence of an increase in oxidative stress in the vasculature in hypertension, we showed, for the first time, that increased ROS in the RVLM contributes to SNA, leading to the neural mechanisms of hypertension in SHRSP (61). Zimmerman et al. (133) demonstrated that hypertension caused by low doses of circulating ANG II depends on the production of superoxide in the circumventricular organs (133). It was demonstrated that physiological responses to brain ANG II involve ROS production (15, 132, 133). Considering the importance of the brain ANG II system (2, 10, 26, 28, 83, 85, 86, 108), ROS play an important role in the neural regulation of blood pressure because ROS production largely depends on AT_1 receptor stimulation (47, 48, 99).

Role of ROS in Neural Mechanisms of Hypertension

As described earlier, on the basis of results demonstrating that microinjection of Tempol or overexpression of manga-

nese-superoxide dismutase in the RVLM markedly decreases blood pressure in SHRSP, but not in WKY, increased oxidative stress in the RVLM contributes to the neural mechanisms of hypertension in SHRSP (61). Oxidative stress levels in the RVLM were determined by measuring thiobarbituric acid-reactive substances (TBARS) levels and electron spin resonance (ESR) spectroscopy with a spin trapping technique (47, 48, 61). In SHR, oxidative stress in the RVLM plays an important role in hypertension via activation of the sympathetic nervous system (19, 66, 106, 117). An increase in oxidative stress in the RVLM also contributes to hypertension via activation of the sympathetic nervous system in rats with renovascular hypertension (two-kidney one-clip hypertensive model) (92). This model is an ANG II-dependent model of hypertension. Therefore, it is conceivable that ANG II increases oxidative stress by acting both centrally and peripherally, thereby activating the sympathetic nervous system and leading to hypertension as one of the hypertensive mechanisms in this model. AT_1 receptor expression levels in the RVLM and the paraventricular nucleus of the hypothalamus are enhanced in this rat renovascular model of hypertension (93). Interestingly, NADPH oxidase activity is increased, but Cu/Zn-SOD expression in the RVLM is unchanged. In a subsequent study, the authors showed that oxidative stress increased in both the RVLM and paraventricular nucleus, as well as systemically in this hypertensive model (93). These results suggest that systemic activation of the renin-angiotensin system activates AT_1 receptors in the brain, including the RVLM and paraventricular nucleus, thereby increasing SNA, leading to hypertension, as one of the mechanisms.

Sources of ROS Generation in the Brain Stem

NADPH oxidase is a major source of ROS in hypertension (71, 72) and has a critical role in generating ROS in the brain (5, 14, 51, 90, 122, 134). ANG II is upstream of NADPH oxidase activation, which requires Rac1 (48, 90, 122, 134). NADPH oxidase-derived ROS are involved in the effects of ANG II on Ca^{2+} influx in the NTS neurons receiving vagal afferents (122). Importantly, the essential subunit of NADPH oxidase, gp91phox, is present in somatodendritic and axonal profiles containing AT_1 receptors (122). The potentiation of Ca^{2+} currents indicates that ANG II increases neuronal excitability and spontaneous activity in some neurons (135). ANG II failed to increase ROS production or to potentiate L-type Ca^{2+} currents in the dorsomedial portion of the NTS neurons of mice lacking Nox2 (123). Thus, the excitatory actions of ANG II in the NTS neurons are caused, at least, in part, by the activation of L-type Ca^{2+} channels. It should be noted that ANG II-induced inhibition of neuronal delayed rectifying potassium current (I_{KV}) is mediated by ROS in primary neurons isolated from the hypothalamus and brain stem, because both NAD(P)H oxidase inhibition and Tempol prevented the ANG II inhibition of I_{KV} (113).

Mitochondria are another source of ROS generation in the brain. Chan et al. (21) examined the role of the mitochondrial electron transport chain in the RVLM of SHR and found that mitochondrial electron transport chain dysfunction in the RVLM of SHR depressed complex I or III activity and reduced the electron transport capacity (ETC) between complexes I and III or II and III (21). Interestingly, microinjection of coenzyme Q_{10} into the RVLM of SHR reversed the depressed ETC activity and enhanced superoxide generation. In addition, microinjection of antisense oligonucleotide against the p22phox subunit of NADPH oxidase into the RVLM reduced the enhanced ROS production in SHR (21). It is also important to note that microinjection of coenzyme Q_{10} into the RVLM of SHR decreases blood pressure (21). These results suggest that impairment of mitochondrial ETC complexes contributes to chronic oxidative stress in the RVLM of SHR, leading to enhanced central sympathetic drive and hypertension (21, 136). Consistent with their observation, we also found that ANG II induced the mitochondria-derived ROS production via activation of NADPH oxidase, although we did not find differences in the mitochondrial respiratory complexes between SHRSP and WKY (91), thus suggesting a feedforward system for ROS generation (21, 91, 136) (Fig. 1). Mitochondrial produced superoxide mediates the ANG II inhibition of I_{KV} (128). Recently, Chan et al. (22) suggested that transcriptional upregulation of mitochondrial uncoupling protein 2 (UCP2) in response to an increase in superoxide plays an active role in the feedback regulation of ROS production in the RVLM (22). Furthermore, oral treatment with rosiglitazone enhances a central antihypertensive effect via an upregulation of peroxisome proliferator-activated receptor-γ (PPAR-γ) and reduced oxidative stress in the RVLM of SHR (23). Stimulation of PPAR-γ results in the upregulation of UCP2, thereby reducing oxidative stress. The dose of rosiglitazone used in that study,

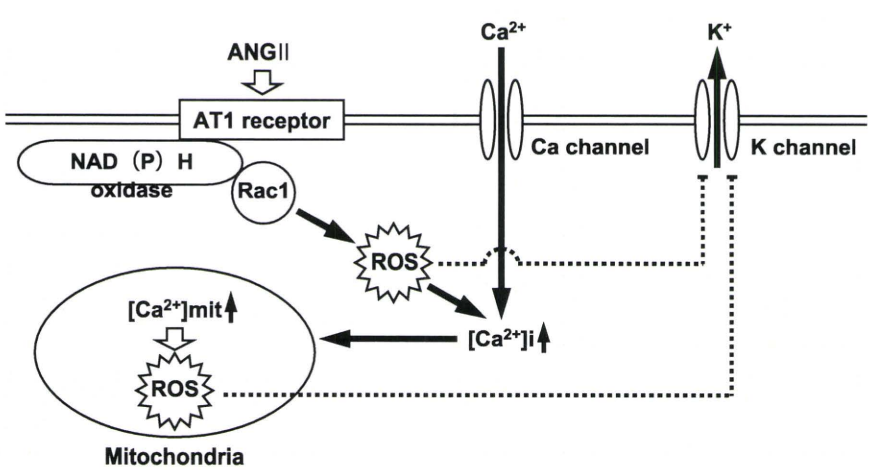


Fig. 1. A suggested scheme demonstrating that ANG II stimulation increases reactive oxygen species (ROS) generation via NAD(P)H oxidase and related mechanisms. [Modified from Nozoe et al. (91).]

however, was fairly high, and this does not necessarily relate to the clinical setting.

Downstream Signaling Pathway of the AT_1 Receptor Stimulation in the RVLM Involving ROS Production

As described above, activation of the AT_1 receptor produces superoxide anions as an initial step of ROS generation through NADPH oxidase. Thus, the signaling pathway should be pivotal for neuronal activation leading to hypertension via central sympathetic outflow. NAD(P)H oxidase-derived ROS production mediates the ANG II-induced pressor response via activation of p38 MAPK and ERK in the RVLM (18, 20). Chan et al. (20) demonstrated that intracerebroventricular infusion of ANG II elicits the long-term pressor response, and this pressor response is mediated by protein kinase C/ERK/cyclic adenosine monophosphate response element binding protein and c-*fos* induction (20). It should be noted that the ANG II-induced pressor response might not necessarily be related to ROS production in the RVLM. The ANG II-induced pressor response, however, is significantly inhibited by ROS scavenging, and endogenous blockade of AT_1 receptors in the brain stem of SHRSP reduces ROS and blood pressure (48, 91). Activation of caspase-3 acting through the Ras/p38 MAPK/ERK pathway in the RVLM might be involved in sympathoexcitation of SHRSP (65). In addition, the apoptotic proteins Bax and Bad are activated, and the antiapoptotic protein Bcl-2 is inhibited in the RVLM of SHRSP (65). The Ras inhibitor substantially attenuated these changes, thereby attenuating caspase-3 associated with the decrease in blood pressure. In contrast, however, c-Jun N-terminal kinase activity was not altered in the RVLM of SHRSP compared with that of WKY (65). It should be noted that the possibility of caspase-3-independent neuronal apoptosis in the RVLM or of a direct link between ROS and caspase-3 activation was not examined in that study (65). However, this finding is consistent with the results demonstrating that microinjection of ANG II induces AT_1 receptor-dependent ROS production and phosphorylation of p38 MAPK and ERK, but not stress-activated protein kinase/Jun N-terminal kinase in the RVLM of Sprague-Dawley rats (18). Interestingly, this is not the case in the RVLM of heart failure rabbits (77). Stress-activated protein kinase/Jun N-terminal kinase activity was increased in the RVLM of these heart failure rabbits (77). The increased phosphorylation of Jun N-terminal kinase may lead to activation of the transcription factor AP-1, which is a dimer of Jun and c-Fos family members. It is not clear why these differences between hypertension and heart failure occur. It is possible that signal transduction changes in the progression from hypertension to heart failure, thereby leading to further enhanced central sympathetic outflow. Further studies are needed to establish a more direct link between these signaling pathways, redox sensitivity, and the development and/or progression of hypertension.

Imbalance of Brain NO and ROS

Superoxide derived from NADPH oxidase reacts with and inactivates NO and thereby modulates its bioavailability (32, 97, 114) (Fig. 2). The converse is also true; that is, NO reduces superoxide, which may be beneficial (32, 99) (Fig. 2). An increase in NO in the RVLM decreases blood pressure and sympathetic nervous system activity to a greater extent in

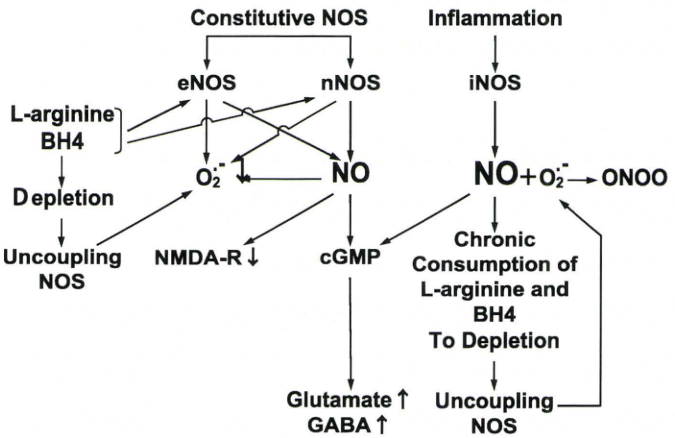


Fig. 2. A scheme demonstrating the interaction between nitric oxide (NO) and ROS generation. NMDA-R, *N*-methyl-D-aspartate receptors; GABA, γ-aminobutyric acid; BH4, tetrahydrobiopterin; NOS, nitric oxide synthase; eNOS, endothelial NOS; iNOS, inducible NOS; nNOS, neuronal NOS. [Modified from Hirooka (47).]

SHRSP than in WKY rats (58). This might be due to a reduction in superoxide via NO in the RVLM of SHRSP, which is increased in the RVLM of SHRSP (61). All three NOS isoforms generate superoxide depending on substrate (L-arginine) and cofactor (tetrahydrobiopterin) availability (32, 97, 114). The induction of both iNOS and ROS during inflammation is well established (88, 97). A recent study suggested that ROS and reactive nitrogen species, such as peroxynitrite dose-dependently regulate iNOS function (114). Overexpression of iNOS in the RVLM causes sympathoexcitation via an increase in oxidative stress (54). As expected, the release of more nitrite/nitrate (NO_x) in RVLM dialysate is induced by iNOS overexpression than by eNOS overexpression (54). Relative to the constitutive isoforms, iNOS has approximately five-fold higher NO production (97). NO_x release, however, is increased by approximately twofold higher by iNOS overexpression than by eNOS overexpression (54). We considered that the precursor of NO production, L-arginine, and its cofactor, tetrahydrobiopterin, might be consumed and insufficient when iNOS is chronically expressed, thereby iNOS would produce superoxide instead of NO (Fig. 2). Otherwise, chronic overexpression of iNOS increases levels of NO chronically, which, in turn, reacts with superoxide in a diffusion-limited reaction to produce peroxinitrite (Fig. 2). In fact, we found an increase in the TBARS levels in the RVLM and the pressor response after overexpression of iNOS. The increased pressor response was, however, abolished by iNOS inhibitors or Tempol. Once ROS production is increased, ROS enhance superoxide production from iNOS, indicating that ROS promote iNOS uncoupling. Further, peroxynitrite, produced from the reaction between NO and superoxide, reduces both NO and superoxide generation, indicating that peroxynitrite causes iNOS dysfunction enzymatically. In our study, we detected some iNOS-positive cells with the antinitrotyrosine antibody (54). Furthermore, iNOS expression levels were increased in the RVLM of SHRSP compared with WKY (56). Kung et al. (68) suggested that mitochondrial respiratory enzyme complexes in the RVLM were cellular targets of NO and ROS interaction after eNOS gene transfer. This concept is problematic, however, in that they suggest that superoxide and per-

oxynitrite are produced after eNOS gene transfer into the RVLM (68). Another recent study suggested that NMDA receptor activation increases ROS production through NO and Nox2 (33). Further studies are needed to explore whether this mechanism functions via ubiquitous glutamatergic synaptic transmission in vivo.

Sympathoinhibitory Effects of Antihypertensive Drugs and Statins

NADPH oxidase, which is activated by AT_1 receptor stimulation, is a major source of ROS (11, 17, 113, 135). The specific brain nuclei that regulate SNA, such as the anteroventral third ventricle, paraventricular nucleus of the hypothalamus, NTS, and the RVLM, are rich in AT_1 receptors (2, 10, 26, 28, 83). AT_1 receptor expression levels are upregulated in the RVLM of hypertensive animal models compared with normotensive controls (105). Thus, it is possible that AT_1 receptor blockers reduce oxidative stress in the brain, as well as in the peripheral vasculature. It is also possible that AT_1 receptor blockers inhibit ROS production by blocking AT1 receptor-mediated intracellular signaling (11, 48, 50) and that this antioxidant action accounts for the absence of reflex-induced sympathoexcitation after treatment with AT_1 receptor blockers. We evaluated the effects of AT_1 receptor blockers, olmesartan and telmisartan, on brain oxidative stress in SHRSP (4, 48). Both AT_1 receptor blockers have antioxidant properties in the brain without stimulating reflex-mediated SNA in SHRSP. We used in vivo ESR spectroscopy to examine the effect of oral olmesartan on oxidative stress in the brain (4), because the in vivo ESR method is a powerful technique for evaluating oxidative stress (3, 110, 111). The effects of peripherally administered olmesartan or telmisartan on central sympathetic outflow have been demonstrated in other studies (34, 76). Are these antioxidant effects of olmesartan or telmisartan specific for each drug or the AT_1 receptor blocker class? Other angiotensin receptor blockers, such as losartan or candesartan, have similar sympatho-inhibitory effects in the CNS, although there are some differences among angiotensin receptor blockers (24, 89, 102, 124). The differences of the central effects of each angiotensin receptor blocker might depend on its lipophilicity, phamacokinetics, and the transporter system (24, 34, 48, 124). Furthermore, systemically administered candesartan reduces brain ANG II via downregulation of the brain renin-angiotensin system (98). This finding provides new mechanistic insight into the treatment of hypertension by the AT_1 receptor blockers (84). Unfortunately, however, these effects of AT_1 receptor blockers, that is, reduction of brain oxidative stress and sympatho-inhibitory effects, even when administered systemically, are usually ignored by researchers or clinicians, but should be considered as potential therapeutic candidates.

Considering the inhibitory effects of AT_1 receptor blockers on brain oxidative stress and sympathetic nervous system activity, it would be interesting to know whether other cardiovascular drugs have similar effects. We found that atorvastatin causes depressor and sympathoinhibitory effects with upregulation of NOS in SHRSP (59), which is consistent with the effects of statins on eNOS upregulation in the vasculature (55). Atorvastatin also reduces oxidative stress in the RVLM of SHRSP (62, 63, 64). With regard to the central sympathoinhibitory effects of calcium channel blockers, lipophilic dihydropyridine calcium channel blockers, such as nifedipine, nisoldipine, and amlodipine, readily cross the blood-brain barrier, thereby presumably blocking brain L-type Ca^{2+} channels leading to central sympathoinhibition (73). It is generally considered that an arterial baroreflex-mediated increase in sympathetic activity is responsible for the unfavorable effects of short- and strong-acting dihydropyridine calcium channel blockers; therefore, the intrinsic sympathoinhibitory effects of calcium channel blockers have been ignored. These findings together suggest that increased NOS activity and antioxidant effects in the brain stem might be involved in the central sympathoinhibitory effects of some calcium channel blockers (45, 55, 67). The precise mechanisms involved, however, remain unknown, and further studies are required.

Summary and Conclusions

In summary, accumulating evidence indicates that an imbalance of NO and ROS in the CNS, particularly in the brain stem, is crucially involved in hypertension via the activation of central sympathetic outflow. Upstream and downstream consequences of the precise mechanisms are discussed. Several questions remain, however, because the interactions between NO and ROS are complex. Further studies are required to gain a better understanding of the role of brain NO and ROS in autonomic cardiovascular regulation and potential therapeutic targets.

GRANTS

This series of studies was supported by the Grants-in Aid for Scientific Research from the Japan Society for Promotion of Science and, in part, by a Grant from Kimura Memorial Foundation Research.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. **Alaghband-Zadeh J, Das I, Hanson MR, MacGregor CAL, de Wardener HE, Laycock JF.** Hypothalamic and plasma total nitrate/nitrite concentrations in spontaneously hypertensive rats. *Exp Physiol* 81: 881–883, 1996.
2. **Allen AM, O'Callaghan EL, Chen D, Bassi JK.** Central neural regulation of cardiovascular function by angiotensin: a focus on the rostral ventrolateral medulla. *Neuroendocrinology* 89: 361–369, 2009.
3. **Anzai K, Saito K, Takeshita K, Takahashi S, Miyazaki H, Shoji H, Lee MC, Masumizu T, Ozawa T.** Assessment of ESR-CT imaging by comparison with autoradiography for the distribution of a blood-brain-barrier permeable spin probe, MC-PROXYL, to rodent brain. *Magn Reson Imaging* 21: 765–772, 2003.
4. **Araki S, Hirooka Y, Kishi T, Yasukawa K, Utsumi H, Sunagawa K.** Olmesartan reduces oxidative stress in the brain of stroke-prone spontaneously hypertensive rats assessed by an in vivo ESR method. *Hypertens Res* 32: 1091–1096, 2009.
5. **Bai Y, Jabbari B, Ye S, Campese VM.** Regional expression of NAD(P)H oxidase and superoxide dismutase in the brain of rats with neurogenic hypertension. *Am J Nephrol* 29: 483–492, 2009.
6. **Bains JS, Ferguson AV.** Nitric oxide depolarizes type II paraventricular nucleus neurons in vitro. *Neuroscience* 79: 149–159, 1997.
7. **Bains JS, Ferguson AV.** Nitric oxide regulates NMDA-drived GABAergic inputs to type I neurons of the rat paraventricular nucleus. *J Physiol* 499: 733–746, 1997.
8. **Batten TFC, Atkinson L, Deuchars J.** Nitric oxide systems in the medulla oblongata and their involvement in autonomic control. In: *Functional Neuroanatomy of the Nitric Oxide System. Handbook of Chemical Neuroanatomy*, edited by Steinbusch HWM, De Vente J, Vincent SR, Amsterdam, The Netherlands: Elsevier, 177–213, 2000.

9. **Bergamaschi CT, Campos RR, Lopes OU.** Rostral ventrolateral medulla: a source of sympathetic activation in rats subjected to long-term treatment with L-NAME. *Hypertension* 34: 744–747, 1999.
10. **Bourassa EA, Sved AF, Speth RC.** Angiotensin modulation of rostral ventrolateral medulla (RVLM) in cardiovascular regulation. *Mol Cell Endocrinol* 302: 167–175, 2009.
11. **Brandes RP.** Vascular functions of NADPH oxidases. *Hypertension* 56: 17–21, 2010.
12. **Briones AM, Touyz RM.** Oxidative stress and hypertension: current concepts. *Curr Hypertens Rep* 12: 135–142, 2010.
13. **Cabrena CL, Bealer SL, Bohr DF.** Central depressor action of nitric oxide is deficient in genetic hypertension. *Am J Hypertens* 9: 237–241, 1996.
14. **Campese VM, Sindhu RK, Ye S, Bai Y, Vaziri ND, Jabbari B.** Regional expression of NO synthase, NAD(P)H oxidase and superoxide dismutase in the rat brain. *Brain Res* 1134: 27–32, 2007.
15. **Campese VM, Shaohua Y, Huiquin Z.** Oxidative stress mediates angiotensin II-dependent stimulation of sympathetic nerve activity. *Hypertension* 46: 533–539, 2005.
16. **Campos RR, Bergamaschi CT.** Neurotransmission alterations in central cardiovascular control in experimental hypertension. *Curr Hypertens Rev* 2: 193–198, 2006.
17. **Campos RR.** Oxidative stress in the brain and arterial hypertension. *Hypertens Res* 32: 1047–1048, 2009.
18. **Chan SHH, Hsu KS, Huang CC, Wang LL, Ou CC, Chan JYH.** NADPH oxidase-derived superoxide anion mediates angiotensin II-induced pressor effect via activation of p38 mitogen-activated protein kinase in the rostral ventrolateral medulla. *Circ Res* 97: 772–780, 2005.
19. **Chan SHH, Tai MH, Li CY, Chan JYH.** Reduction in molecular synthesis or enzyme activity of superoxide dismutase and catalase contributes to oxidative stress and neurogenic hypertension in spontaneously hypertensive rats. *Free Radic Biol Med* 40: 2028–2039, 2006.
20. **Chan SHH, Wang LL, Tseng HL, Chan JYH.** Upregulation of AT_1 receptor gene on activation of protein kinase Cβ/nicotinamide adenine dinucleotide diphosphate oxidase/ERK1/2/c-*fos* signaling cascade mediates long-term pressor effect of angiotensin II in rostral ventrolateral medulla. *J Hypertens* 25: 1845–1861, 2007.
21. **Chan SHH, Wu KLH, Chang AYW, Tai MH, Chan JYH.** Oxidative impairment of mitochondrial electron transport chain complexes in rostral ventrolateral medulla contributes to neurogenic hypertension. *Hypertension* 53: 217–227, 2009.
22. **Chan SHH, Wu CA, Wu KLH, Ho YH, Chang AYW, Chang JYH.** Transcriptional upregulation of mitochondrial uncoupling protein 2 protects against oxidative stress-associated neurogenic hypertension. *Circ Res* 105: 886–896, 2009.
23. **Chan SHH, Wu KLH, Kung PSS, Chan JYH.** Oral intake of rosiglitazone promotes a central antihypertensive effect via upregulation of peroxisome proliferator-activated receptor- and alleviation of oxidative stress in rostral ventrolateral medulla of spontaneously hypertensive rats. *Hypertension* 55: 1444–1453, 2010.
24. **Culman J, Blume A, Gohlke P, Unger T.** The renin-angiotensin system in the brain: possible therapeutic implications for AT_1-receptor blockers. *J Hum Hypertens* 16: S64–S70, 2002.
25. **Dampney RAL.** Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* 74: 323–364, 1994.
26. **Dampney RAL, Fontes MAP, Hirooka Y, Potts PD, Tagawa T.** Role of angiotensin II receptors in the regulation of vasomotor neurons in the rostral ventrolateral medulla. *Clin Exp Pharmacol Physiol* 29: 467–472, 2002.
27. **Dias AC, Vitela M, Colombari E, Mifflin SW.** Nitric oxide modulation of glutamatergic, baroreflex, and cardiopulmonary transmission in the nucleus of the solitary tract. *Am J Physiol Heart Circ Physiol* 288: H256–H262, 2005.
28. **Dupont AG, Brouwers S.** Brain angiotensin peptides regulate sympathetic tone and blood pressure. *J Hypertens* 28: 1599–1610, 2010.
29. **Eshima K, Hirooka Y, Shigematsu H, Matsuo I, Koike G, Sakai K, Takeshita A.** Angiotensin in the nucleus tractus solitarii contributes to neurogenic hypertension caused by chronic nitric oxide synthase inhibition. *Hypertension* 36: 259–263, 2000.
30. **Esler M.** Sympathetic nervous activation in essential hypertension: commonly neglected as a therapeutic target, usually ignored as a drug side effect. *Hypertension* 55: 1090–1091, 2010.
31. **Esler M.** The 2009 Carl Ludwig Lecture. Pathophysiology of the human sympathetic nervous system in cardiovascular diseases: the translation from mechanisms to medical management. *J Appl Physiol* 108: 227–237, 2010.
32. **Garthwaite J.** Concepts of neural nitric oxide-mediated transmission. *Eur J Neurosci* 27: 2783–2802, 2008.
33. **Girouard H, Wang G, Gallo EF, Anthrather J, Zhou P, Pickel VM, Iadecola C.** NMDA receptor activation increases free radical production through nitric oxide and Nox2. *J Neurosci* 29: 2545–2552, 2009.
34. **Gohlke P, Weiss S, Jansen A, Wienen W, Stangier J, Rascher W, Culman J, Unger T.** AT1 receptor antagonist telmisartan administered peripherally inhibits central responses to angiotensin II in conscious rats. *J Pharmacol Exp Ther* 298: 62–70, 2001.
35. **Grassi G.** Assessment of sympathetic cardiovascular drive in human hypertension: achievements and perspectives. *Hypertension* 54: 690–697, 2009.
36. **Grassi G.** Sympathetic neural activity in hypertension and related diseases. *Am J Hypertens* 23: 1052–1060, 2010.
37. **Grassi G, Seravalle G, Quarti-Trevano F.** The 'neurogenic hypothesis' in hypertension: current evidence. *Exp Physiol* 95: 581–586, 2010.
38. **Guyenet PG.** The sympathetic control of blood pressure. *Nat Rev Neurosci* 7: 335–346, 2006.
39. **Harada S, Tokunaga S, Momohara M, Masaki H, Tagawa T, Imaizumi T, Takeshita A.** Inhibition of nitric oxide formation in the nucleus tractus solitarius increases renal sympathetic nerve activity in rabbits. *Circ Res* 72: 511–516, 1993.
40. **Haüser W, Sassmann A, Qadri F, Jöhren O, Dominiak P.** Expression of nitric oxide synthase isoforms in hypothalamo-pitutary-adrenal axis during the development of spontaneously hypertension in rats. *Mol Brain Res* 138: 198–204, 2005.
41. **Hironaga K, Hirooka Y, Matsuo I, Shihara M, Tagawa T, Harasawa Y, Takeshita A.** Role of endogenous nitric oxide in the brain stem on the rapid adaptation of baroreflex. *Hypertension* 31: 27–31, 1998.
42. **Hirooka Y, Polson JW, Dampney RAL.** Pressor and sympathoexcitatory effects of nitric oxide in the rostral ventrolateral medulla. *J Hypertens* 14: 1317–1324, 1996.
43. **Hirooka Y, Kishi T, Sakai K, Shimokawa H, Takeshita A.** Effect of overproduction of nitric oxide in the brain stem on the cardiovascular response in conscious rats. *J Cardiovasc Pharmacol* 41 *Suppl* 1: S119–S126, 2003.
44. **Hirooka Y, Sakai K, Kishi T, Ito K, Shimokawa H, Takeshita A.** Enhanced depressor response to endothelial nitric oxide synthase gene transfer into the nucleus tractus solitarii of spontaneously hypertensive rats. *Hypertens Res* 26: 325–331, 2003.
45. **Hirooka Y, Kimura Y, Nozoe M, Sagara Y, Ito K, Sunagawa K.** Amlodipine-induced reduction of oxidative stress in the brain is associated with sympatho-inhibitory effects in stroke-prone spontaneously hypertensive rats. *Hypertens Res* 29: 49–56, 2006.
46. **Hirooka Y.** Localized gene transfer and its application for the study of central cardiovascular control. *Auton Neurosci* 126–127: 120–129, 2006.
47. **Hirooka Y.** Role of reactive oxygen species in brainstem in neural mechanisms of hypertension. *Auton Neurosci* 142: 20–24, 2008.
48. **Hirooka Y, Sagara Y, Kishi T, Sunagawa K.** Oxidative stress and central cardiovascular regulation: pathogenesis of hypertension and therapeutic aspects. *Circ J* 74: 827–835, 2010.
49. **Horn T, Smith PM, McLaughlin BE, Bauce L, Marks GS, Pittman QJ, Ferguson AV.** Nitric oxide actions in paraventricular nucleus: cardiovascular and neurochemical implications. *Am J Physiol Regul Integr Comp Physiol* 266: R306–R313, 1994.
50. **Inaba S, Iwai M, Furuno M, Tomono Y, Senba I, Okayama H, Mogi M, Higaki J, Horiuchi M.** Continuous activation of renin-angiotensin system impairs cognitive function in renin/angiotensinogen transgenic mice. *Hypertension* 53: 356–362, 2009.
51. **Infanger DW, Shrama RV, Davisson RL.** NADPH oxidases of the brain: distribution, regulation, and function. *Antioxid Redox Signal* 8: 1583–1596, 2006.
52. **Judy WV, Watanabe AM, Henry DP, Besch HR, Murphy WR, Hockel GM.** Sympathetic nerve activity: role in regulation of blood pressure in the spontaneously hypertensive rat. *Circ Res* 38: I-121–I-129, 1976.
53. **Kagiyama S, Tsuchihashi T, Abe I, Fujishima M.** Cardiovascular effects of nitric oxide in the rostral ventrolateral medulla of rats. *Brain Res* 757: 155–158, 1997.
54. **Kimura Y, Hirooka Y, Sagara Y, Ito K, Kishi T, Shimokawa H, Takeshita A, Sunagawa K.** Overexpression of inducible nitric oxide synthase in rostral ventrolateral medulla causes hypertension and sym-