▶【本薬剤の特徴・臨床上の使用ポイント】

基本的にはベラパミルと同様である.

▶【薬剤相互作用】

CYP3A4 で代謝されるためベラパミルと同様の注意が必要となる.

ジギタリス, テオフィリンの血中濃度を上昇させる.

▶【重大な副作用】

うっ血性心不全, 完全房室ブロック, 高度徐脈が現れることがある.

Stevens-Johnson 症候群が現れることがある.

▶【禁忌・慎重投与】

- 重篤なうっ血性心不全, 第 II 度以上の房室ブロック, 洞不全症候群のある患者には禁忌.
- 妊娠又は妊娠している可能性のある婦人, 本剤に対し過敏症の既往歴のある患者には禁忌.
- 高度の徐脈, うっ血性心不全, 低血圧のある患者には慎重投与である.

MEMO

- ベラパミルより使用経験が少ない. 海外のガイドラインでは効果不十分として推奨されていない.

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〈戸高浩司　砂川賢二〉

特集

急性冠症候群治療の最前線を知る

識る 9

急性冠症候群に対する抗血栓薬－わが国における新しい抗血栓薬の必要性は？：開発の現状

Antithrombotic therapy for ACS－Do we need new antithrombotic drugs in Japan? Current status of development

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急性冠症候群(acute coronary syndrome；ACS)症例に対してアスピリンやヘパリンのような抗血栓薬(血小板凝集阻害薬・血液凝固阻害薬)の早期投与が推奨されることは周知の事実であり，国内外のガイドラインに明記されている[1-4]。米国では医療機関における急性心筋梗塞診療の質の評価(performance measurement)を行うガイドライン[5]が別途存在し，これらの投与が適切にされたかどうかを指標としていることからもいかに強固な推奨であるかがわかる。国内外のガイドラインとも個々の薬剤のもつエビデンスにより推奨される病態や程度は異なっているが，残念ながら根拠データのほとんどは海外のものである。この領域では一般に「民族差がある」と仮定されている節があり，海外のデータが受け入れられずにわが国では上市されていない薬剤，導入が遅れている薬剤(drug lag)が少なくない。そこで本稿ではわが国未導入の抗血栓薬が海外でいかに使われているか，および有望視される新薬の開発状況を概説し，わが国における今後のACS治療の方向性を探る。

なお抗血栓薬の一種である血栓溶解薬についてはACSに対する経皮的冠動脈インターベンション(percutaneous coronary intervention；PCI)治療全盛の今日にあってわが国での役割は小さく，新しい動きもほとんどないため割愛する。

 1342-6591/09/¥400/論文/JCOPY

わが国の血栓薬使用の現状

わが国において正規にACSに使用できる抗血栓薬(血栓溶解薬を除く)とその適応は以下のとおりである。

①アスピリン：狭心症(慢性安定狭心症，不安定狭心症)心筋梗塞，PCI施行後における血栓・塞栓形成の抑制

②チクロピジン：血管手術および血液体外循環に伴う血栓・塞栓の治療ならびに血流障害の改善(平成19年の厚生労働省医療課長通知で冠動脈ステント留置後の血栓予防に適応ありと解釈)

③クロピドグレル：PCIが適用されるACS(不安定狭心症，非ST上昇型心筋梗塞)

④ヘパリン：血栓塞栓症(静脈血栓症，心筋梗塞症，肺塞栓症，脳塞栓症，四肢動脈血栓塞栓症，手術中・術後の血栓塞栓症など)の治療および予防

ほかにシロスタゾールやサルポグレラートなど一部の薬剤でわが国独自にステントの血栓予防などを目的として適応外使用されてきたものもあるが，一般に海外と比較すると使用できる薬剤は少ない。

クロピドグレルについては用法・用量が「通常，成人には，投与開始日にクロピドグレルとして300mgを1日1回経口投与し，その後，維持量として1日1回75mgを経口投与する」とあり，ACSという急性の病態に対する適応でいつまで維持投与が許されるのか不明確である。一方，薬剤溶出性ステント(drug-eluting stent；DES)の添付文書には「術後少なくとも3ヵ月(製品によっては6ヵ月)間のクロピドグレル硫酸塩製剤またはチクロピジン塩酸塩製剤の投与を推奨する。ただし，留置後1年を超えての遅発性ステント血栓症が報告されていることから，出血などの副作用のリスクに留意しながら，患者の状態に応じて当該製剤の投与期間延長の必要性を検討すること」とあり，DESが入った症例にはACSでなくともクロピドグレルを3～6ヵ月以上の長期にわたって投与するよう推奨されている。実際には支払基金ごとに適応の判断が行われているものと考えられる。平成21年3月30日薬事・食品衛生審議会薬事分科会で議論されているので公開された議事録[6]を参照されたい。

また，下記の薬剤については適応があるとされているが現実的にはほとんど使われない。

①ジピリダモール：狭心症，心筋梗塞，その他の虚血性心疾患，うっ血性心不全

②トラピジル：狭心症

③ワルファリン：血栓塞栓症(静脈血栓症，心筋梗塞症，肺塞栓症，脳塞栓症，緩徐に進行する脳血栓症など)の治療および予防

ジピリダモールはわが国で心筋梗塞症に対する適応をもっているが，欧米ではメタ解析にて効果が否定されており，ACSには一般的に使用されない。ST上昇型心筋梗塞(ST elevation myocardial infarction；STEMI)に対しては国内ガイドラインでも単独投与に関してクラスⅢである[3]。注射薬についてはACSのような虚血の急性期に使用すべきでないのは周知のとおりである。トラピジルについては世界的にみて一部の国でしか承認されておらずエビデンスは少ない。わが国のガイドラインではアスピリンが投与できない場合に二次予防として推奨されているのみである。ワルファリンは循環器学会ガイドライン[2]にも記載されているが効果発現の遅さから実際にACSに使われることはまれと思われる。

わが国未承認で欧米のガイドラインで推奨されている薬剤

(1)血小板凝集阻害薬(抗血小板薬)

●グリコプロテインⅡb/Ⅲa受容体阻害薬

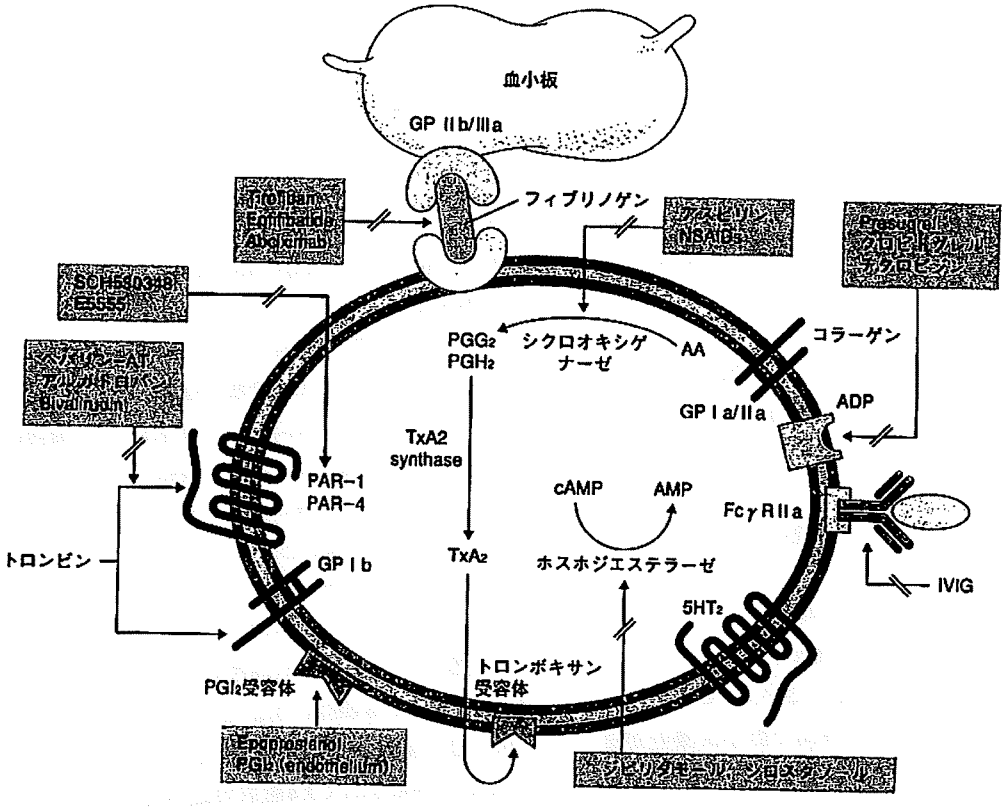
①Abciximab

「-ximab」という接尾辞からわかるようにヒト・マウスのキメラモノクローナル抗体Fab部分であり，血小板細胞膜上グリコプロテイン(GP)Ⅱb/Ⅲa受容体に結合して血小板とフィブリノーゲンとの結合を阻害することにより，血小板凝集を抑制する(図1)。

欧米では1995年発売。早期侵襲的治療戦略(early invasive strategy)でPCIを予定する場合クロピドグレルとともに投与することが推奨されている。これらはEPIC[7]やCAPTUREといったPCIを予定されたACS患者を対象とした試験により30日後のイベント(死亡・心筋梗塞・緊急血行再建)を減少させた成績を基にしているが1990年代前半

実施のやや古い試験である。保存的治療戦略(conservative strategy)であっても虚血発作を繰り返したり，トロポニンが陽性であるなどの高リスク群には投与してもよいとされている。後者は複数試験のメタ解析の結果によっている。

わが国でもEPIC試験にならって1990年代後半に二重盲検による治験(JEPPORT)[8]が9百数十例で行われたがプラセボに対してイベント抑制に有意差がつかなかった。異なる試験の数字だけの比較は危険であるがEPICにおける複合エンドポイント発生がプラセボ群12.8%，実薬群8.3%であったのに対してJEPPORTではプラセボ群3.6%，実薬群2.8%とイベント発生がもともと低かったことが大きく影響しているようにみえる。軽症例が組み入れられたというsampling bias，血栓形成における民族差の可能性，試験時期の違いによるPCI全般のリスク低減



抗血小板薬	作用部位
アスピリン	シクロオキシゲナーゼ(COX)
Prasugrel，クロピドグレル，チクロピジン	ADP受容体
ジピリダモール，シロスタゾール	PDE3
Epoprostenol	PGI_2受容体
ヘパリン-AT，アルガトロバン，Bivalirudin	トロンビン
トロンビン受容体拮抗薬，SCH530348，E5555	PAR-1
Tirofiban，Eptifibatide，Abciximab	GP IIb/IIIa受容体

図1 抗血小板薬の作用部位
(文献15より一部改変引用)
右上から時計回りに各種抗血小板薬と主な作用部位を示す。

などが原因としては考えられるがいずれも推測の域を出ない。2004年に開発者である日本セントコア社と藤沢薬品(当時)の共同開発契約が解消され実質的に国内上市はほぼ断念されているものと思われる。

ステント留置に伴ってクロピドグレル負荷投与がルーチンとなっている欧米では以前のようなAbciximabの有用性が薄れているのではないかとの疑問が呈されており，ISAR-REACT2[9]ではトロポニン陽性でのみ有用，STEMI対象のBRAVE-3[10]では梗塞サイズを縮小しないとの結果となった。本薬のような高価な抗体製剤使用を再考するきっかけになっている。

②Eptifibatide

蛇毒から誘導された環状のオリゴペプチド製剤でGPⅡb/Ⅲa受容体をブロックする。

③Tirofiban

合成非ペプチド性GPⅡb/Ⅲa受容体阻害薬である。

Abciximabは24時間以上GPⅡb/Ⅲa受容体と結合し血小板機能回復に中止後24～48時間かかるが，これら2剤は投与中止後4～8時間で血小板機能が戻るといわれている。薬剤同士をhead-to-headで比べた試験は少なくAHAガイドライン[1]では静注GPⅡb/Ⅲa受容体阻害薬としてひとまとめに扱われている。根拠とした試験成績から心カテ・PCIまでに遅れがない(実施される)場合にAbciximabを推奨し，それ以外はこれらの2剤という使い分けが提示されている。

静注GPⅡb/Ⅲa受容体阻害薬は糖尿病症例で特に有用であることが示されている。いずれもわが国における開発はされていないようである。

経口GPⅡb/Ⅲa受容体阻害薬(xemilofiban，orbofiban，sibrafiban)についてはいずれも90年代後半，欧米の試験で有用性が示されず開発中止となっている。

(2)血液凝固阻害薬(抗凝固薬)

●低分子ヘパリン(low molecular weight heparin；LMWH)

①エノキサパリン，ダルテパリン，nadroparin，tinzaparin(後二者は米国でも適応外)

ガイドライン[1]では抗血小板薬投与とともになんらかの抗凝固療法が推奨されている。具体的薬剤については未分画ヘパリン(unfractionated heparin；UFH)，LMWH，フォンダパリヌクス，bivalirudinのいずれかとされている。

ヘパリンはアンチトロンビンⅢと結合することによりⅡa(トロンビン)を最も阻害し，次いでXa(活性化第Xa因子)を阻害する(図2，3)。UFHに含まれる分子量の小さい分画(LMWH)はⅡa阻害作用が弱くXa阻害の選択性が高くなる(図3)。これにより出血が少なくなると期待されると同時にUFHがもつ欠点の一部，血栓に結合したトロンビンの不活化ができない，血漿蛋白との結合率が高く効果にばらつきがあるなどが回避される。結果としてACSにおけるイベントをUFHと同等かそれ以上に抑制した成績が特にエノキサパリンで多く出されている。

一方プロタミンによる中和が不完全，活性化部分トロンボプラスチン時間(activated partial thromboplastin time；APTT)などによる薬力学的モニターができない(必要がないので利点と考える向きもある)といった欠点も生じる。

わが国においてはエノキサパリン，ダルテパリン，パルナパリン，レビパリンなど多くが上市されているが，いずれも播種性血管内凝固症候群(disseminated intravascular coagulation syndrome；DIC)や血液透析時の凝固防止といった適応しか取得していない。ACSでの想定使用量に比して薬価がさして高くないためか適応拡大の動きはないようである。

●活性化第X因子阻害薬

①フォンダパリヌクス

本薬はアンチトロンビンに結合するヘパリンの5単糖構造に類似した合成型のヘパリン類似化合物である。アンチトロンビンⅢに依存して間接的にXaを選択阻害する(図2，3)。

エノキサパリンとの直接比較で同等のイベント抑制と大出血の少なさを併せもっており保存的治療戦略に特に推奨されている(ただし米国では適応外である)。ヘパリンと異なり1日1回の皮下投与でよく簡便である。PCIを予定される場合などは主にカテーテル血栓症に対するエビデンスの不足により単独の抗凝固薬としては推奨されていない。

わが国では2007年に術後の静脈血栓予防で承認されたがACSの適応は

もっていない。

●直接トロンビン抑制因子

①Bivalirudin

蛭の唾液から発見されたHirudinの合成類似化合物でアンチトロンビンを介さずにトロンビンの触媒部位と結合し活性を可逆的に阻害する(図1～3)。Hirudin，BivalirudinのいずれもUFHよりイベントを低減したが，前者で大出血が多かったのに対し後者では少なかった。PCIを早期に実施した症例に有効性は限られており，ガイドライン[1]では侵襲的治療戦略の場合本薬をUFH，LMWH，フォンダパリヌクスよりも推奨している。またクロピドグレルを十分早期(6時間以上前)に併用投与できた場合はGPⅡb/Ⅲa受容体阻害薬を省略できるとしている。

わが国での開発はされていないようである。

欧米やわが国で開発中(または未確立)の薬剤

(1)血小板凝集阻害薬

●Prasugrel

クロピドグレルと同じチエノピリジン誘導体であり体内で代謝されるとP2Y12タイプのADP受容体を阻害することにより血小板凝集を抑制する(図1)。クロピドグレルで問題となった抵抗性・低反応性がなく効果発現が早いとされており，TRITON-TIMI 38[11]においてイベント抑制はクロピドグレルに勝っていたが大出血は多かった。欧州では2009年2月に承認・上市，米国では同7月にPCIを受けるACS患者に対し血栓性心血管イベントの抑制が適応として承認されたばかりである。米国の添付文書には出血リスクに対する枠組み警告(boxed warning)で脳卒中既往や75歳以上などには使うべきでないとされた。

わが国では製薬協の情報サイト「開発中の新薬」[12]によればACS対象に第Ⅱ相の段階である。

ほかに同じくP2Y12阻害薬であるCangrelorやAZD6140(Ticagrelor)は海外で開発されている(第Ⅲ相)が，わが国ではこのサイトに記載がなく情勢不明である。

●トロンビン受容体拮抗薬

①SCH530348

②E5555

血小板表面のトロンビン受容体PAR-1(protease-activated receptor 1)に対して拮抗作用を有し血小板凝集を抑制する(図1)。これら2剤は経口薬である。

ACS適応に対して前者は国内外とも第Ⅲ相にあり，FDAからFast track(優先審査)の指定を受けている。最近発表された第Ⅱ相の結果[13]では選択的PCIを受ける患者でアスピリン＋クロピ

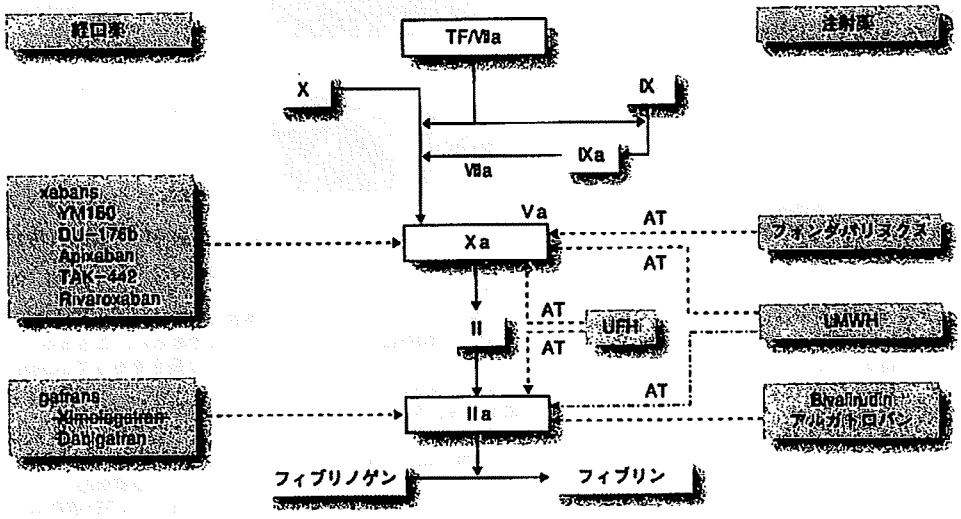


図2 凝固系カスケードと抗凝固薬の作用点
(文献17より一部改変引用)
左側に経口薬，右側に注射薬を示す。
Xa(活性化第X因子)の阻害薬として経口薬が多く開発されている。Xabansと総称されることがある。
IIa(トロンビン)の阻害薬も経口薬の開発が盛んである。Gatransと総称されることがある。
注射薬としてはXa阻害薬としてantithrombin(AT)を介するフォンダパリヌクス，LMWH(低分子ヘパリン)，UFH(未分画ヘパリン)がある。
IIa阻害薬としてはATを介するUFHと，直接阻害薬であるBivalirudin，アルガトロバンがある。

ドグレルに追加しても出血を増加させず主要心血管イベント(major adverse cardiovascular event；MACE)を減少させる傾向が示唆されている。後者については国内外とも第Ⅱ相段階である。

(2)血液凝固阻害薬

●直接トロンビン抑制因子

①アルガトロバン

トロンビンの活性部位と可逆性に結合することにより直接阻害する(図1～3)。米国ではヘパリン起因性血小板減少症(heparin-induced thrombocytopenia；HIT)のときの血栓症予防・PCI随伴薬物療法として用いられる。BivalirudinとことなりACSにおけるイベント抑制がみられなかったためガイドラインでは推奨されていない。わが国ではHITに限らず脳血栓症，末梢動脈疾患などに適応をもつ。ACSの適応はない。

②Ximelagatran

ACSに対する適応も含め開発していたが残念ながら肝毒性により中止，2006年にほかの適応で上市済みの欧州からも撤退してしまったのは記憶に新しい。

③Dabigatran

心房細動の血栓塞栓症予防の開発が行われている。2008年に欧州の承認を受けた。

後二者などの経口薬は集合的にgatransとよばれることがある。

●活性化第Ⅹ因子阻害薬

①Rivaroxaban

②Apixaban

③TAK-442

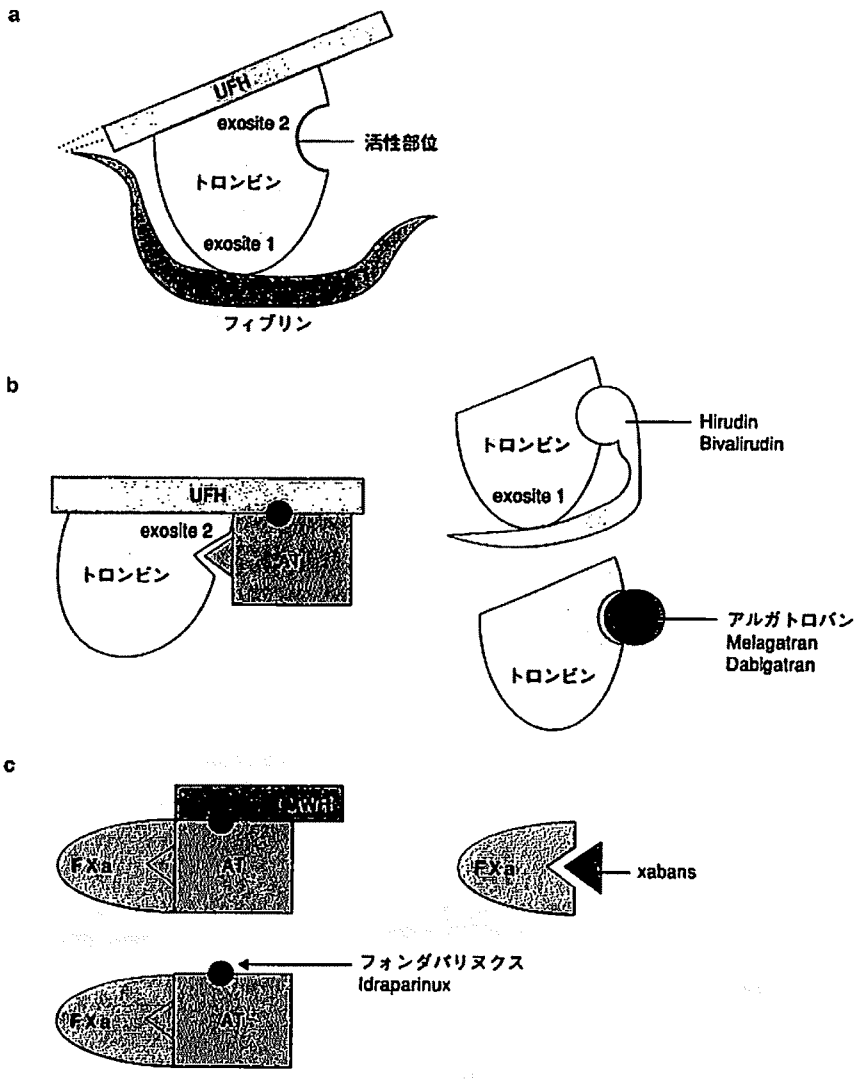


図3 トロンビン阻害薬，活性化第Ⅹ因子阻害薬の作用機序概念図(文献19より一部改変引用)

a：UFH(未分画ヘパリン)はフィブリンに結合したトロンビンと複合体をつくり，AT(アンチトロンビン)と共役してトロンビン活性部位の阻害をすることができない。

b：UFHは5単糖構造部位(●)でATと結合して(フィブリンと結合していない)トロンビンの活性部位を阻害する。LMWH(低分子ヘパリン)も同じであるが，こちらはトロンビンがフィブリンに結合しているかどうかにこだわらない。UFHのみ十分な長さをもってexosite 2に結合しATを通じての阻害作用が強くなる。長さの短いLMWHはトロンビンとの結合が弱い。Hirudin，BivalirudinはトロンビンのⅠ活性部位を直接阻害するとともにexosite 1にも結合しフィブリンとトロンビンとの結合を阻害する。アルガトロバンなどはトロンビンの活性部位を直接阻害する。

c：FXa(活性化第Ⅹ因子)に対してLMWHはUFHと同様にATと共役して阻害し，結果的にFXaに対する選択性がUFHに比較して相対的に高くなる。5単糖構造のみのフォンダパリヌクスはATと共役してFXaのみを阻害する。Xabansとよばれる第Ⅹa因子阻害薬は活性部位に直接結合して阻害するためATを必要としない。

④YM-150
⑤Edoxaban DU-176b

いずれもアンチトロンビンに依存せず活性化第X因子を直接阻害する経口薬である(図2, 3)。集合的にxabansとよばれることがある。ワルファリンの代替薬とすべく静脈血栓の予防を中心に国内外で盛んに開発され，一部ACSに対する開発も行われている。Rivaroxabanは米国で股関節，膝関節置換術後の血栓症予防で既承認である。

おわりに

以上，ACSに対して特にPCIに随伴して用いられる抗血栓薬，開発中の薬剤について概説した。欧米では使用可能な薬剤が多数あり，どの病態・タイミングでどのような組合せが予後を最も改善するのか，治療戦略(strategy)による違いも含めて複雑な問題が盛んに議論されている。ガイドラインでの記載はいろいろな組合せの臨床試験エビデンスに依拠する必要があり，結果として複雑化しわかりにくい。わが国では適応をもった薬剤が少ないため，あまり問題にされてこなかったが今後重要な点になる。

ステント血栓症がわが国では少ない可能性が指摘され[14]，ACSやPCIに対して新しい抗血栓薬の必要性を疑問視する向きもあるが，糖尿病症例，右冠動脈plaque-burdenの大きな症例など有用性が推測される例も少なからず存在する。わが国では欧米と比較して同じコレステロールレベルで冠動脈疾患の発生率が低いため治療必要例数(number needed to treat；NNT)の違いはあるものの，スタチン投与により欧米と同様にリスクを低減し，大きな予後改善効果があった。同様に，世界的に標準とされているGPⅡb/Ⅲa受容体阻害薬などの抗血栓薬はわが国においても重要と考えられ，わが国のACS，PCI治療の成績をさらによくすることが期待される。

この分野だけでもわが国で使えない薬剤は前述したようにかなりの数にのぼる。Abciximabの例に象徴されるように絶対リスクが比較的低いとされているわが国の冠動脈疾患患者群においてアウトカムを主要評価項目にした臨床試験・治験で有意差を出すのは容易ではない。global studyに積極的に参加するなどの企業，臨床現場が一体となった努力や承認行政のなんらかの新機軸がないと，今後もこの領域のdrug lagは払拭されずわが国が最新の治療手段から取り残される懸念がある。

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REVIEW

Oxidative Stress and Central Cardiovascular Regulation

– Pathogenesis of Hypertension and Therapeutic Aspects –

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Oxidative stress is a key factor in the pathogenesis of hypertension and target organ damage, beginning in the earliest stages. Extensive evidence indicates that the pivotal role of oxidative stress in the pathogenesis of hypertension is due to its effects on the vasculature in relation to the development of atherosclerotic processes. It remains unclear, however, whether oxidative stress in the brain, particularly the autonomic nuclei (including the vasomotor center), has an important role in the occurrence and maintenance of hypertension via activation of the sympathetic nervous system. The aim of the present review is to describe the contribution of oxidative stress in the brain to the neural mechanisms that underlie hypertension, and discuss evidence that brain oxidative stress is a potential therapeutic target. (*Circ J* 2010; **74:** 827–835)

Key Words: Blood pressure; Brain; Heart rate; Hypertension; Sympathetic nervous system

Accumulating evidence indicates that the sympathetic nervous system plays an important role in the pathogenesis of hypertension.[1–3] Activation of the sympathetic nervous system is involved in the stages, clinical forms, 24-h blood pressure patterns, end-organ damage, and metabolic abnormalities of hypertension.[1–3] Although peripheral factors are also involved, the central nervous system (CNS) mechanisms are considered crucial.[3–7] The results of recent studies strongly suggest that central sympathetic outflow is increased in hypertension.[3–7] Increased oxidative stress is also involved in the pathogenesis of hypertension.[8] Although there have been many studies regarding target organ damage in hypertension, relatively few studies have addressed the role of oxidative stress in sympathetic nervous system activation.[9–11] Based on the role of angiotensin II (Ang II) in the generation of reactive oxygen species (ROS), the relationship between brain angiotensin and central sympathetic outflow has been examined.[12,13] Our group was the first to report that increased ROS generation in the brainstem contributes to the neural mechanisms of hypertension in hypertensive rats,[14] and we and other investigators have reported additional evidence to support this concept and the potential therapeutic aspects.[9–11] This review focuses on the role of oxidative stress within the brain in the neural pathogenesis of hypertension.

Increased Oxidative Stress in the Brain in Hypertension

Among the target organs of hypertensive vascular diseases, the brain is most affected by aging and oxidative stress.[15,16] Cell membranes in the brain contain a high concentration of polyunsaturated fatty acids. These fatty acids are targeted by ROS, which elicit chain reactions of lipid peroxidation. Oxidative stress is determined by measuring levels of thiobarbituric acid-reactive substances (TBARS), end products of lipid peroxidation. The levels of TBARS reflect those of malondialedehyde, although the assay is not specific for malondialedehyde.[15,17] There are some important points, however, for assessing the levels of TBARS.[17] The medium used for tissue preparation needs to contain a chelating agent and an antioxidant, and conditions for the assay must be kept constant. Therefore, we used another method for assessing the ROS production, which is electron spin resonance (ESR) spectroscopy. The amount of ROS was quantified by monitoring the time-dependent decay of the amplitude of the ESR spectra produced by the nitroxide radical 4-hydroxy-2,2,6,6-tetramethyl-piperidine-*N*-oxyl (hydroxyl-TEMPO) as a spin probe.[9,14] The signal decay of ESR spectroscopy reflects oxidative stress more directly. Also, it has an advantage for in vivo study.[18] We evaluated oxidative stress in the brains of stroke-prone spontaneously hypertensive rats (SHRSP) compared with normotensive Wistar–Kyoto (WKY) rats.[9,14] The rostral ventrolateral medulla (RVLM) is the major vasomotor center that determines basal sympathetic nervous system activity and it is essential for the maintenance of basal vasomotor tone.[3–7] Spontaneously hypertensive rats (SHR) or SHRSP exhibit increased sympathetic nervous system activity during the development of hypertension and are commonly used in experimental studies as models of human essential hypertension.[3–7] We previously investigated whether ROS are increased in the RVLM of SHRSP.[14] First, we found that ROS levels measured by TBARS and ESR spectroscopy were increased in the RVLM of SHRSP compared with WKY

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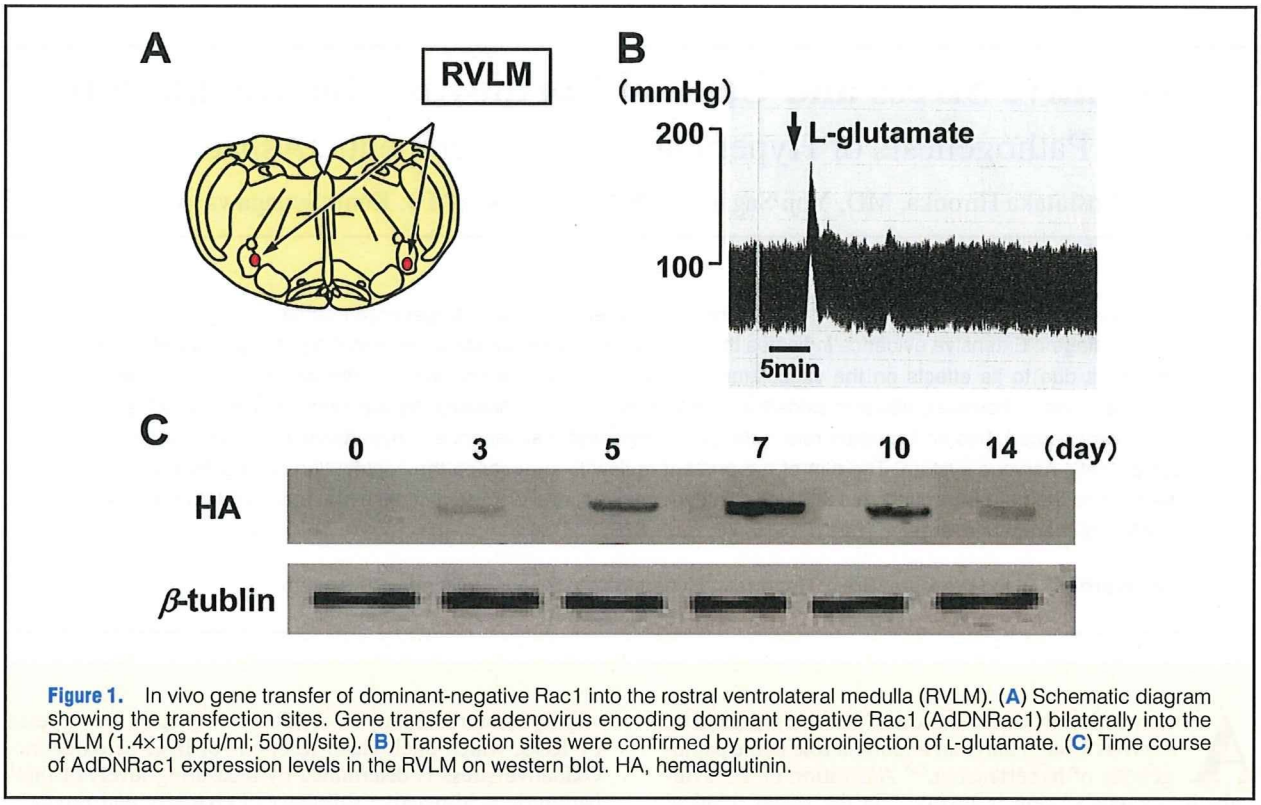

Figure 1. In vivo gene transfer of dominant-negative Rac1 into the rostral ventrolateral medulla (RVLM). (**A**) Schematic diagram showing the transfection sites. Gene transfer of adenovirus encoding dominant negative Rac1 (AdDNRac1) bilaterally into the RVLM (1.4×10^9 pfu/ml; 500 nl/site). (**B**) Transfection sites were confirmed by prior microinjection of L-glutamate. (**C**) Time course of AdDNRac1 expression levels in the RVLM on western blot. HA, hemagglutinin.

rats. In addition, superoxide dismutase (SOD) expression and activity, which are ROS scavenging factors, were decreased in the RVLM of SHRSP compared with WKY rats. Functionally, microinjection of the membrane-permeable radical scavenger tempol into the RVLM decreased blood pressure, heart rate, and sympathetic nervous system activity in SHRSP but not in WKY rats. More importantly, overexpression of Mn-SOD, an antioxidant enzyme, in the RVLM of SHRSP decreased blood pressure and sympathetic nervous system activity. These findings strongly indicate that oxidative stress in the RVLM is increased in SHRSP and contributes to the neural mechanisms of hypertension. As described here, brain ROS is one of the results of generalized target organ damage, appearing earlier in the brain due to its susceptibility. The brain ROS would increase blood pressure via activation of the sympathetic nervous system and this would ultimately result in a vicious cycle. It would be possible, however, that brain ROS is involved in the early stage of hypertension in SHR or SHRSP, because we found that oxidative stress in the brain assessed on in vivo ESR was enhanced in young (6-week-old) SHR or SHRSP compared with age-matched WKY rats (unpublished data). The levels of TBARS were not different, probably because the levels of TBARS reflect lipid peroxidation caused by ROS. Other investigators also found that an increase in superoxide anions in the RVLM is associated with hypertension in SHR,[19] and reduced expression and activity in Cu/Zn-SOD and Mn-SOD within the RVLM contribute to oxidative stress and neurogenic hypertension in SHR.[20] An increase in oxidative stress within the RVLM also plays an important role in maintaining high arterial blood pressure and sympathetic activation in 2-kidney 1-clip (2K-1C) hypertensive rats, which is a renovascular hypertension model.[21] In that study, Oliveira-Sales et al demonstrated that the mRNA expression of NAD(P)H oxidase subunits ($p47^{phox}$ and $gp91^{phox}$) in the RVLM was greater in 2K-1C than in the control group. Interestingly, there were no differences in Cu/Zn-SOD expression between the two groups. TBARS levels in the RVLM were significantly greater in the 2K-1C than in the control group, suggesting enhanced oxidative stress. Functionally, microinjection of vitamin C into the RVLM decreased blood pressure and renal sympathetic nerve activity in 2K-1C but not in controls. Importantly, in a subsequent study, these authors suggested that the paraventricular nucleus of the hypothalamus is also involved.[22] Notably, although 2K-1C is a model of renovascular hypertension, suggesting that circulating Ang II is increased, angiotensin type I (AT1) receptor gene expression levels within the RVLM and paraventricular nucleus were upregulated in this model, indicating that ROS was produced via the activation of nicotinamide-adenine dinucleotide phosphate [NAD(P)H] oxidase.

Sources of ROS Production in the Brain

As a source of ROS production in the CNS, NAD(P)H oxidase is a major player. NAD(P)H oxidase is composed of two membrane-bound subunits, $gp91^{phox}$ and $p22^{phox}$; several cytoplasmic subunits, $p47^{phox}$, $p40^{phox}$, and $p67^{phox}$; and the small G-protein Rac1.[23–26] Stimulation of AT1 receptors activates NAD(P)H oxidase by which the cytoplasmic subunits of Rac1NAD(P)H oxidase such as Rac1 bind to the membrane subunits, thereby activating the enzyme leading to superoxide generation. Rac1 requires lipid modification to migrate from the cytosol to the plasma membrane, which is a necessary step for activating ROS-generating NAD(P)H oxidase. NAD(P)H oxidase activity is greater in the brainstem of SHRSP than in that of WKY.[27,28] We transfected adenovirus

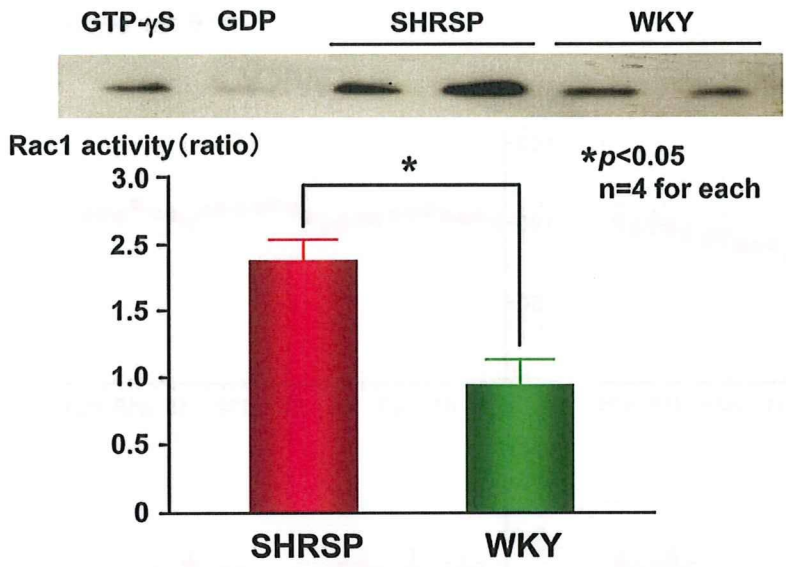

Figure 2. Rac1 activity is elevated in the rostral ventrolateral medulla of stroke-prone spontaneously hypertensive rats (SHRSP) compared to Wistar–Kyoto (WKY) rats. GDP, guanosine 5'-diphosphate; GTP-γS, guanosine 5'-*O*-(3-thiotriphospahte).

encoding dominant-negative Rac1 into the RVLM of SHRSP and WKY rats (Figure 1).[27] Rac1 activity in the RVLM tissue was increased in SHRSP compared to WKY rats (Figure 2).[27] Importantly, we demonstrated that inhibition of Rac1-derived ROS in the RVLM decreased blood pressure, heart rate, and urinary norepinephrine excretion in SHRSP (Figure 3).[27] A similar response occurs after inhibition of Rac1-derived ROS in the nucleus tractus solitarius (NTS).[28]

In addition to the cytosolic production of ROS, mitochondria are the primary source of ROS production in many cells. Ang II increases mitochondrial ROS production in the RVLM, leading to sympathoexcitation.[29] Furthermore, NAD(P)H oxidase-derived ROS might trigger Ca^{2+} accumulation, which leads to mitochondrial ROS production.[29] This suggestion is based on the finding that gene transfer of dominant negative Rac1 attenuated the Ang II-induced increase in reduced Mito-Tracker red fluorescence.[29] In contrast, impairment of mitochondrial electron transport chain complexes in the RVLM might be involved in the neural abnormality underlying hypertension in SHR.[30] This issue was recently discussed by Zimmerman and Zucker.[31] Although we did not detect impairment of brain mitochondrial respiratory complexes in SHRSP, we propose that mitochondria-derived ROS mediate sympathoexcitation via NAD(P)H oxidase activation.[29]

Another possibility for ROS generation is uncoupling nitric oxide synthase (NOS). In the absence of L-arginine or with tetrahydrobiopterin, NO production from inducible NOS (iNOS) causes uncoupling from the oxidation of NADPH, resulting in superoxide generation.[9] iNOS overexpression in the RVLM causes hypertension and sympathoexcitation that is mediated by an increase in oxidative stress.[32] This might be relevant to our observation that iNOS expression levels in the RVLM are greater in SHRSP than in WKY rats.[33] In addition, microinjection of iNOS antagonists into the RVLM reduces blood pressure only in SHR, but not in WKY rats.[33]

ROS-Mediated Activation of Transcriptional Factors

It has been suggested that an Ang II-mediated influx of Ca^{2+} in neurons depends on increased superoxide generation by a Rac1-dependent NAD(P)H oxidase.[34] Ang II also regulates neuronal activity via inhibition of the delayed rectifier potassium current.[35] Ang II-mediated upregulation of L-type Ca^{2+} currents in neurons isolated from the NTS is inhibited by scavenging ROS, indicating a role for NAD(P)H oxidase-derived superoxide in the activation of Ca^{2+} channels in the NTS.[24]

NAD(P)H oxidase-derived superoxide mediates an Ang II-induced pressor effect via the activation of p38 mitogen-activated protein kinase (MAPK) in the RVLM.[36] Recently, we suggested that AT1 receptor-activated caspase-3 acting through the Ras/p38 MAPK/extracellular signal-related protein kinase pathway in the RVLM is involved in sympathoexcitation in SHRSP.[37] These pathways may be downstream effectors of ROS in the RVLM, which in turn plays a crucial role in the pathogenesis of hypertension. Interestingly, the pro-apoptotic proteins Bax and Bad were enhanced and the anti-apoptotic protein Bcl-2 was decreased in the RVLM of SHRSP, and inhibition of caspase-3 normalized these changes in pro- and anti-apoptotic protein levels.[37] These alterations in the RVLM of SHRSP were stimulated by Ang II via activation of the AT1 receptors, which are upregulated in this strain and other hypertensive models.[38] It would be reasonable to consider that different mechanisms may be responsible for sympathoexcitation in different brain sites (influx of Ca^{2+} for RVLM, apoptosis for NTS), and activation of the apoptotic pathway is involved in sympathoexcitation in the RVLM.[37] The exact physiologic implication of these observations requires further evaluation.

Effects of Angiotensin Receptor Blockers on Brain Oxidative Stress

The existence of an independent renin–angiotensin system in the brain is well established. Activation of the brain renin–angiotensin system substantially contributes to the development and maintenance of hypertension through activation of the sympathetic nervous system, vasopressin release, and drinking behavior.[39,40] There is considerable evidence that

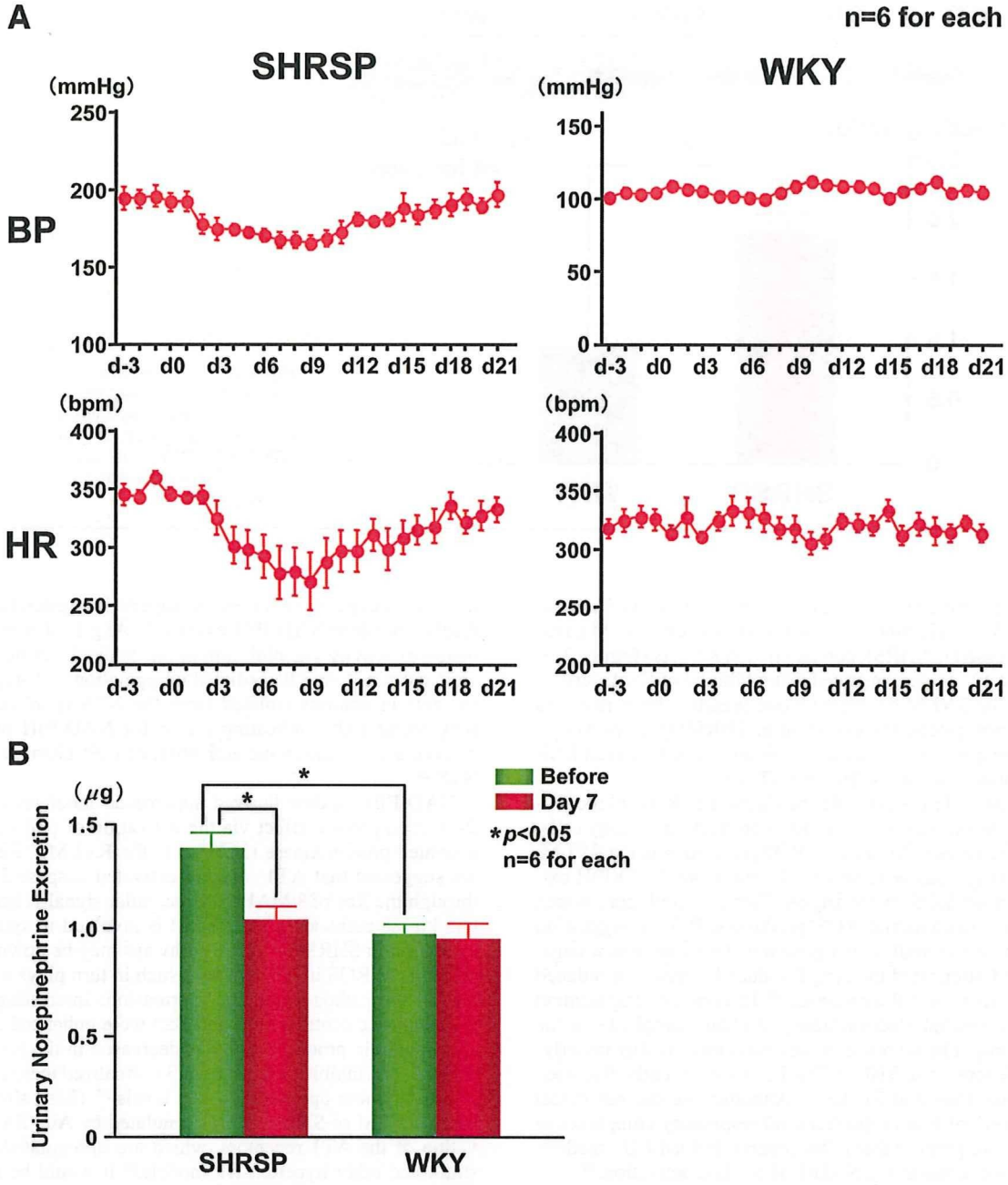

Figure 3. Inhibition of Rac1-dependent superoxide production in the rostral ventrolateral medulla (RVLM) decreases blood pressure, heart rate, and urinary norepinephrine excretion in stroke-prone spontaneously hypertensive rats (SHRSP). (**A**) Time course of mean blood pressure (BP) and heart rate (HR) before and after gene transfer of dominant negative Rac1 (DNRac1). (**B**) Twenty-four-hour urinary norepinephrine excretion before and after gene transfer of DNRac1 into the RVLM. d, day; WKY, Wistar–Kyoto rats.

peripherally administered angiotensin receptor blockers (ARBs) penetrate the blood–brain barrier, although there are some differences among ARBs.[41,42] AT1 receptors are abundant in the circumventricular organs, such as the subfornical organ and the organum vasculosum lamina terminalis, and the area postrema, which lack a blood–brain barrier.[39–42] Therefore, peripherally administered ARBs can also bind to those areas, thereby inhibiting the central actions of Ang II. Oral treatment with the ARB telmisartan appears to inhibit the central responses to Ang II in awake rats.[43] Although other ARBs also inhibit the central actions of Ang II within the brain beyond the blood–brain barrier,[41,42,44] these effects might differ depending on the pharmacokinetics and properties of each drug (ie, lipophilicity etc).[43] We evaluated the

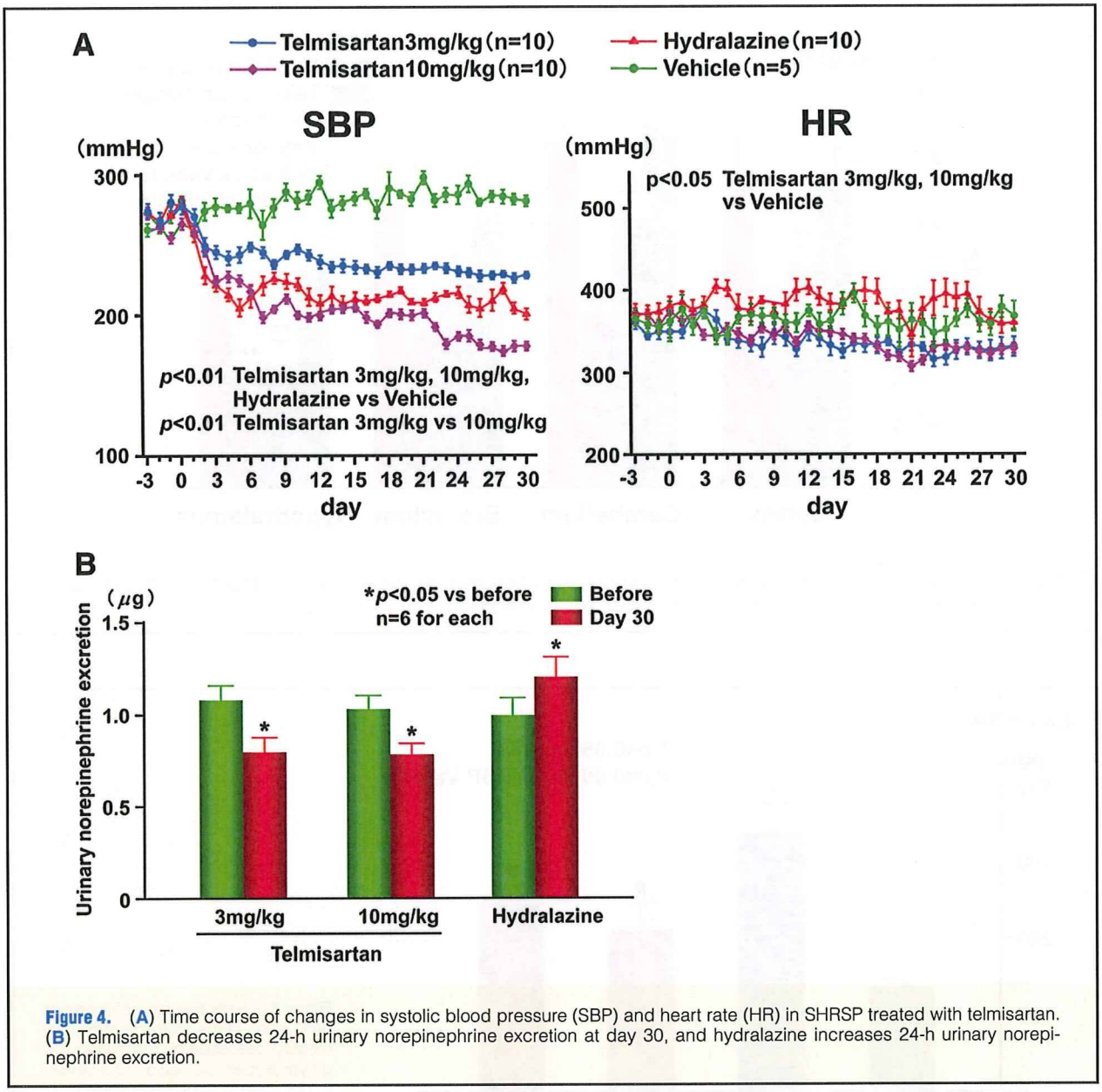

Figure 4. (A) Time course of changes in systolic blood pressure (SBP) and heart rate (HR) in SHRSP treated with telmisartan. (B) Telmisartan decreases 24-h urinary norepinephrine excretion at day 30, and hydralazine increases 24-h urinary norepinephrine excretion.

effect of treatment with telmisartan at either a high dose ($10\,mg \cdot kg^{-1} \cdot day^{-1}$) or a low dose ($3\,mg \cdot kg^{-1} \cdot day^{-1}$), or hydralazine for 30 days on hypertension.[45] Systolic blood pressure (SBP) and heart rate were measured using the tail-cuff method. Urinary norepinephrine excretion was measured as a marker of the sympathetic nervous system activity. We evaluated ROS in the brain (cortex, cerebellum, hypothalamus, and brainstem) of SHRSP on ESR spectroscopy and TBARS. Oral treatment with telmisartan reduced SBP dose-dependently and hydralazine reduced SBP to a similar level to the high dose of telmisartan (Figure 4). Telmisartan reduced, while hydralazine increased, urinary norepinephrine excretion (Figure 4). TBARS levels were significantly increased in each area of the brain of SHRSP compared with WKY rats (Figure 5). Oral treatment with telmisartan reduced the TBARS levels, but hydralazine did not (Figure 5). These findings suggest that (1) anti-hypertensive treatment with telmisartan reduces ROS in the brain of SHRSP; (2) telmisartan decreases blood pressure, at least in part, via a reduction of the sympathetic nervous system activity in SHRSP; and (3) these effects induced by telmisartan might be associated with protection of the brain of SHRSP from oxidative stress. We also measured the concentration of hydroxyl radicals using a modified procedure based on the hydroxylation of sodium salicylate by hydroxyl radicals,[46] leading to the production of 2,3-dihydroxybenzoic acid (2,3-DHBA).[29,47] Inhibition of Rac1 in the RVLM and oral treatment with telmisartan significantly decreased the production of hydroxyl radicals in the RVLM (Figure 6).[47]

Recently, we used in vivo ESR to assess oxidative stress in the brain, and found that oral treatment with another ARB, olmesartan, reduces oxidative stress in the brain of SHRSP without inducing reflex activation of the sympathetic nervous system.[48] In that study we evaluated the in vivo ESR signal

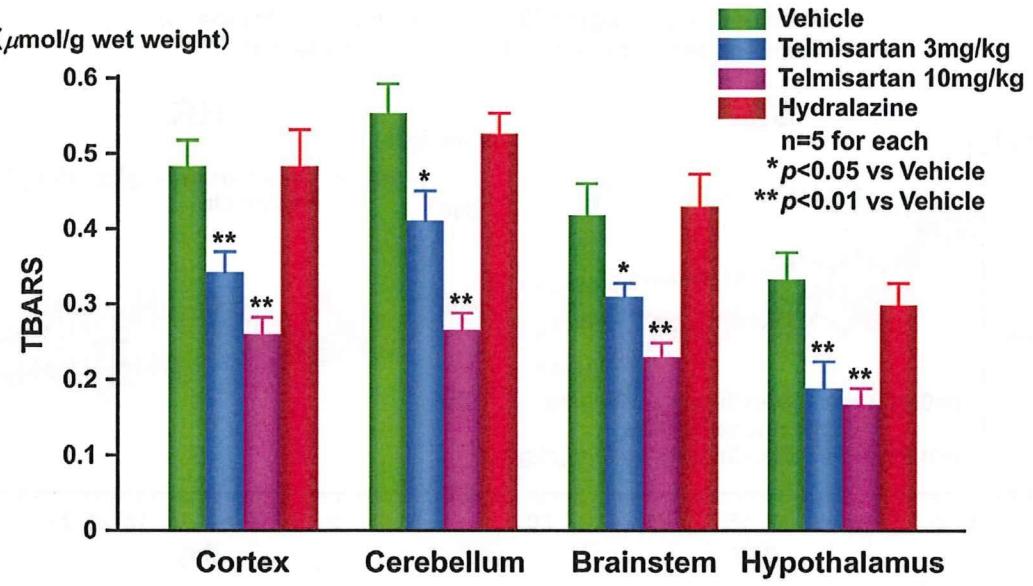

Figure 5. Antioxidant effects of telmisartan in different brain areas. Thiobarbituric acid-reactive substances (TBARS) levels after treatment with telmisartan (3 or 10mg/kg), hydralazine, or vehicle in SHRSP.

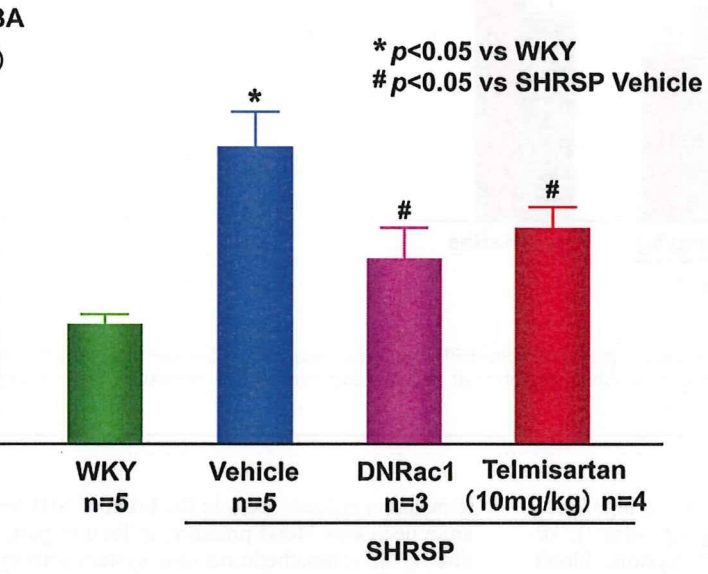

Figure 6. Reactive oxygen species production in the rostral ventrolateral medulla assessed by measuring 2,3-dihydroxybenzoic acid (2,3-DHBA) is decreased after gene transfer of dominant negative Rac1 (DNRac1) or treatment with telmisartan in stroke-prone spontaneously hypersensitive rats (SHRSP). WKY, Wistar–Kyoto rats.

decay rates of the brain using methoxycarbonyl-PROXYL, a nitroxyl radical species, as a blood–brain barrier-permeable spin probe.[49] Oral treatment with olmesartan attenuated the exaggerated pressor response to an excitatory amino acid, L-glutamate, in the RVLM of SHR compared to WKY rats.[50] Further, the pressor response to microinjection of Ang II into the RVLM was diminished in SHR treated with olmesartan.[50] Thus, the importance of oxidative stress in the brain and hypertension is supported by our studies as well as those of others.[11]

Several questions, however, remain to be answered. A recent study suggested that systemic administration of candesartan reduces brain Ang II levels because it attenuates the mRNA expression of both angiotensinogen and angiotensin-converting enzyme in Ang II-infused rats.[51] Whether systemic treatment with ARBs indirectly regulates brain Ang II remains to be determined.[52]

Effects of Other Cardiovascular Drugs on Brain Oxidative Stress

Considering that ARBs act to inhibit NAD(P)H oxidase activ-

ity, it is reasonable that ARBs have an antioxidant effect, although there are some unresolved questions, as mentioned previously. Calcium channel blockers, azelnidipine and amlodipine, but not nicardipine, which also have antioxidant properties, have a sympatho-inhibitory effect on the brain.[53,54] In particular, treatment with azelnidipine reduces oxidative stress in the RVLM associated with a decrease in the activity of NAD(P)H oxidase, Cu/Zn-SOD, and Mn-SOD.[53] These effects might be related to an improvement in NO production,[55] because we also demonstrated that overexpression of endothelial NOS in the NTS or RVLM decreases blood pressure and heart rate via the inhibition of sympathetic nervous system activity.[56–59] Surprisingly, we also found that atorvastatin inhibits the sympathetic nervous system as a result of upregulating NO activity and reducing oxidative stress.[60–63] Further studies are needed to determine if this mechanism is also applicable in humans.

Salt-Sensitive Hypertension and Brain Oxidative Stress

Activation of the sympathetic nervous system, in particular, an increase in central sympathetic outflow, plays an important role in the pathogenesis of salt-sensitive hypertension as well as that of kidney diseases.[64,65] Recent studies suggest that oxidative stress in the brain contributes to blood pressure elevation in salt-sensitive hypertension.[66,67] We demonstrated that high salt intake exacerbates blood pressure elevation and sympathetic nervous system activity during the development of hypertension in SHR, and these responses are mediated by increased ROS generation, probably because of an upregulation of AT1 receptors and NAD(P)H oxidase in the RVLM.[66] The findings of a recent study from Kyushu University Graduate School of Medical Sciences indicate that mice with pressure overload acquired brain salt-sensitivity.[68] This means that high salt intake increases the transport from the blood to the cerebrospinal fluid and the response of the sympathetic nerve activity to salt administered into the brain. These results suggest that pressure overload affects salt sensitivity, thereby enhancing central sympathetic outflow and cardiac function.[68] Left ventricular hypertrophy is an independent risk of cardiovascular event and high salt intake is an important environmental factor of hypertension, both of which increased ROS, and sympathoexcitation may be involved in the pathogenesis of the development of hypertension. A recent clinical trial suggested that left ventricular hypertrophy is related to cardiovascular events in Japanese high-risk hypertensive patients.[69]

Summary and Future Perspectives

Currently in Japan, many patients with hypertension also have metabolic syndrome. Importantly, the prevalence of metabolic syndrome increases linearly with an increase in heart rate among Japanese men and women,[70] suggesting that activation of the sympathetic nervous system is involved in the pathogenesis of hypertension.[71] The prevalence of obstructive sleep apnea has increased as a result of the increase in the number of obese patients with hypertension. Obese patients with sleep apnea have enhanced central sympathetic outflow, which worsens hypertension and leads to cardiovascular events.[72] Further, there is considerable evidence that psychological stress is a major risk factor for cardiovascular diseases and events associated with hypertension.[73] Another therapeutic target for the treatment of hypertension is heart failure with a preserved ejection fraction.[74] As suggested here, salt-sensitivity might also be enhanced in these patients, thereby further enhancing central sympathetic outflow.[68] Oxidative stress in the brain as well as other organs might underlie these mechanisms. Future studies of the effects of oxidative stress in the brain are warranted and will provide useful information for the treatment of hypertension.

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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

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Abstract—In the rostral ventrolateral medulla (RVLM), angiotensin II-derived superoxide anions, which increase sympathetic nerve activity, induce a pressor response by activating the p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal-regulated kinase (ERK) pathway. The small G protein Ras mediates a caspase-3–dependent apoptotic pathway through p38 MAPK, ERK, and c-Jun N-terminal kinase. We hypothesized that angiotensin II type 1 receptors activate caspase-3 through the Ras/p38 MAPK/ERK/c-Jun N-terminal kinase pathway in the RVLM and that this pathway is involved in sympathoexcitation in stroke-prone spontaneously hypertensive rats (SHRSP), a model of human hypertension. The activities of Ras, p38 MAPK, ERK, and caspase-3 in the RVLM were significantly higher in SHRSP (14 to 16 weeks old) than in age-matched Wistar-Kyoto rats (WKY). The mitochondrial apoptotic proteins Bax and Bad in the RVLM were significantly increased in SHRSP compared with WKY. c-Jun N-terminal kinase activity did not differ between SHRSP and WKY. In SHRSP, intracerebroventricular infusion of a Ras inhibitor significantly reduced sympathetic nerve activity and improved baroreflex sensitivity, partially because of inhibition of the Ras/p38 MAPK/ERK, Bax, Bad, and caspase-3 pathway in the RVLM. Intracerebroventricular infusion of a caspase-3 inhibitor also inhibited sympathetic nerve activity and improved baroreflex sensitivity in SHRSP. Intracerebroventricular infusion of an angiotensin II type 1 receptor blocker in SHRSP partially inhibited the Ras/p38 MAPK/ERK, Bax, Bad, and caspase-3 pathway in the RVLM. These findings suggest that in SHRSP, angiotensin II type 1 receptor-activated caspase-3 acting through the Ras/p38 MAPK/ERK pathway in the RVLM might be involved in sympathoexcitation, which in turn plays a crucial role in the pathogenesis of hypertension. (***Hypertension*. 2010;55:291-297.**)

Key Words: angiotensin II ■ apoptosis ■ sympathetic nerve activity ■ brain ■ hypertension

Neuronal apoptosis in the brain is involved in regulating synaptic plasticity and neural function[1–3] and is mainly caused by reactive oxygen species (ROS).[4–8] Ras is a member of a superfamily of related small GTPases implicated in cellular proliferation and transformation, growth arrest, senescence, and apoptosis.[9–13] In cultured tumor cells or endothelial cells, the proapoptotic effects of Ras are mediated by the p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathway through phosphorylation of the proapoptotic proteins Bax and Bad and the antiapoptotic protein Bcl-2, which releases cytochrome *c* in the mitochondria.[14–17] Neuronal apoptosis is characterized by the release of cytochrome *c*, which activates caspase-3, the major executioner caspase in neurons.[18,19] Thus, neuronal apoptosis may be mainly mediated by caspase-3 through the Ras, p38 MAPK, ERK pathway. We previously demonstrated that ROS in a cardiovascular center of the brain stem increase sympathetic nerve activity (SNA) in hypertensive rats.[20] Accumulating evidence suggests that ROS in the brain are involved in the neural mechanisms of hypertension.[21,22] Although ROS are increased in the brain in a hypertensive state, it is not known whether a pivotal signaling pathway (such as the Ras, p38 MAPK, ERK pathway) and caspase-3, activated by ROS in the brain, are chronically activated in the hypertensive state or whether this pathway activates SNA.

The rostral ventrolateral medulla (RVLM) in the brain stem is a major vasomotor center, and it regulates SNA.[23,24] We previously demonstrated that ROS in the RVLM activates SNA and that ROS are increased in the RVLM of stroke-prone spontaneously hypertensive rats (SHRSP), a model of human hypertension,[25] with activation of SNA.[20] In the brain, ROS are produced by activation of the angiotensin II type 1 receptor (AT_1R), which in turn activates nicotinamide-

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adenine dinucleotide phosphate (NAD[P]H) oxidase.[26] NAD(P)H oxidase-derived superoxide anions mediate the angiotensin II-induced pressor effect via the activation of p38 MAPK and ERK in the RVLM.[27] Furthermore, in experimental endotoxemia, the proapoptotic protein Bax and caspase-3–dependent apoptosis in the RVLM mediate cardiovascular responses.[28] The mechanisms by which ROS in the RVLM regulate SNA have not been fully examined, especially the pivotal signaling pathway of ROS.

The aims of the present study were to determine whether stimulation of endogenous AT_1R activates caspase-3 through the Ras/p38 MAPK/ERK/c-Jun N-terminal kinase (JNK) pathway in the RVLM and, if so, to determine whether activation of this pathway is involved in the increased sympathoexcitation in SHRSP. Toward this end, we examined the activity of Ras, p38 MAPK, ERK, JNK, proapoptotic proteins Bax and Bad, antiapoptotic protein Bcl-2, and caspase-3 in the RVLM of SHRSP and normotensive rats. In addition, we performed intracerebroventricular (ICV) injections of a Ras inhibitor, a caspase-3 inhibitor, and an angiotensin receptor blocker (ARB), and examined the changes in blood pressure, heart rate (HR), SNA, and baroreflex sensitivity (BRS). To determine whether ICV injection of a Ras inhibitor, a caspase-3 inhibitor, or an ARB inhibits the pivotal signaling pathway in the RVLM, we also examined the changes in blood pressure, HR, and SNA evoked by microinjection of angiotensin II into the RVLM.

Methods

This study was reviewed and approved by the Committee on the Ethics of Animal Experiments at the Kyushu University Graduate School of Medical Sciences and conducted according to the Guidelines for Animal Experiments of Kyushu University. Details of the methods are available in the online Data Supplement at http://hyper.ahajournals.org.

Animals and General Procedures

Male SHRSP/Izm rats and age-matched Wistar-Kyoto rats (WKY) (14 to 16 weeks old), fed standard feed, were divided into 7 groups (SHRSP treated with Ras inhibitor [S-RI], SHRSP treated with caspase-3 inhibitor [S-CI], SHRSP treated with ARB [S-ARB], SHRSP treated with vehicle [S-Veh], WKY treated with Ras inhibitor [W-RI], WKY treated with caspase-3 inhibitor [W-CI], and WKY with vehicle [W-Veh]; n=5/group). In the S-RI, W-RI, S-CI, W-CI, S-Veh, W-Veh, and S-ARB groups, we measured blood pressure and HR using a radiotelemetry system as described previously.[20] Urinary norepinephrine excretion (uNE) for 24 hours was calculated as an indicator of SNA, as described previously.[20,22] Furthermore, in the S-RI, W-RI, S-CI, W-CI, S-Veh, and W-Veh groups, spectral analysis was performed to provide power spectra for systolic blood pressure.

Activity of Ras, p38 MAPK, ERK, JNK, and Caspase-3 and Expression of Bax, Bad, and Bcl-2 in the RVLM

The activity of Ras, p38 MAPK, ERK, JNK, and caspase-3 and the expression of Bax, Bad, and Bcl-2 in the RVLM were measured as described previously.[29]

ICV Injection of Ras Inhibitor, Caspase-3 Inhibitor, and AT_1R Blocker

S-Farnesylthiosalicylic acid (1 mmol/L), a specific Ras inhibitor[30]; *N*-benzyloxycarbonyl-Asp(OMe)-Glu(OMe)-Val-Asp(OMe) fluoromethyl ketone (Z-DEVD-FMK, 1 μmol/L), a specific caspase-3 inhibitor[31]; candesartan (1 μg/μL); or vehicle was administered by ICV infusion for 14 days with an osmotic minipump (Alzet 1003D). We also determined the changes in blood pressure and HR of SHRSP after terminating the 14-day ICV infusion of the Ras inhibitor (n=4). The candesartan dose was selected as described previously.[32]

Statistical Analysis

Normally distributed variables are expressed as mean±SE. Unpaired *t* and Mann-Whitney U tests were used to compare the differences in normally distributed and nonnormally distributed variables, respectively. Data were also analyzed by a 2-factor repeated-measures analysis of variances. Differences were considered to be statistically significant at $P<0.05$.

Results

Blood Pressure, HR, SNA, and BRS

The Ras inhibitor *S*-farnesylthiosalicylic acid was infused ICV for 14 days. Mean blood pressure (MBP), HR, uNE, and normalized unit of the low-frequency component of systolic blood pressure (LFnuSBP) at day 14 were significantly higher in S-Veh than in W-Veh (Figure 1A through 1D). MBP, HR, and LFnuSBP in SHRSP returned to control levels 4 days after terminating the ICV infusion of *S*-farnesylthiosalicylic acid (data not shown). BRS at day 14 was significantly lower in S-Veh than in W-Veh (Figure 2). At days 2 to 14, MBP and HR were significantly lower in S-RI than in S-Veh (Figure 1A and 1B), and at day 14, uNE and LFnuSBP were significantly lower in S-RI than in S-Veh (Figure 1C and 1D). BRS at day 14 was significantly higher in S-RI than in S-Veh (Figure 2). MBP, HR, LFnuSBP, uNE, and BRS, however, did not differ between W-RI and W-Veh (Figures 1A through 1D and 2).

The caspase-3 inhibitor Z-DEVD-FMK was infused ICV for 14 days. At days 4 to 14, MBP and HR were significantly lower in S-CI than in S-Veh (Figure 1A and 1B), and at day 14, uNE and LFnuSBP were also significantly lower in S-CI than in S-Veh (Figure 1C and 1D). BRS at day 14 was significantly higher in S-CI than in S-Veh (Figure 2). MBP, HR, LFnuSBP, uNE, and BRS did not differ between W-CI and W-Veh (Figures 1A through 1D and 2).

On day 14 of the ICV infusion of candesartan in SHRSP, the systolic blood pressure, HR, uNE, and LFnuSBP were significantly lower in S-ARB than in S-Veh (Figures 1A through 1D).

Ras, p38 MAPK, ERK, and JNK Activity in the RVLM

Ras, p38 MAPK, and ERK activities were significantly higher in S-Veh than in W-Veh and significantly lower in S-RI than in S-Veh (Figure 3A through 3C). Furthermore, Ras, p38 MAPK, and ERK activity was significantly lower in S-ARB than in S-Veh (Figure 3A through 3C). Ras, p38 MAPK, and ERK activity in SHRSP did not differ between S-CI and S-Veh (Figure 3A through 3C) or between W-Veh and W-CI (Figure 3A through 3C). JNK activity did not differ between S-Veh and W-Veh (Figure 3D).

Caspase-3 Activity and Expression of Bax, Bad, and Bcl-2 in the RVLM

Caspase-3 activity in the cytosolic fraction of the RVLM and the expression of Bax and Bad in the mitochondrial fraction of the RVLM were significantly higher in S-Veh than in W-Veh (Figure 4A through 4C) and significantly lower in