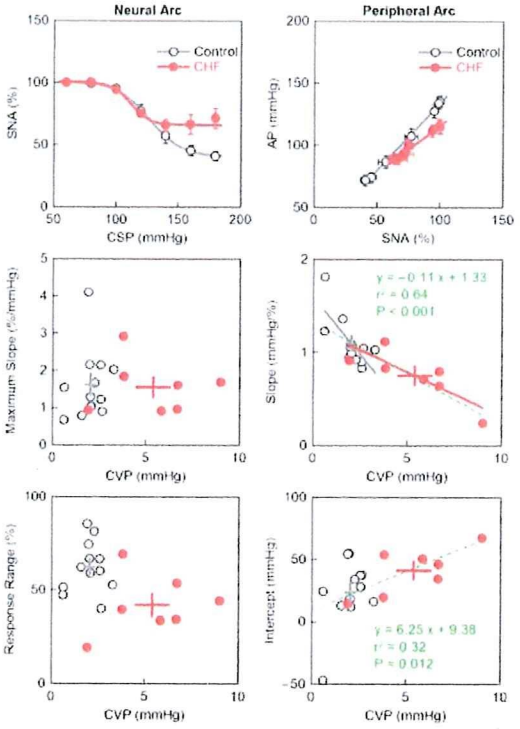
図Ｃ－２－２上段は 12 匹の正常ラットと 7 匹の心不全ラットの圧反射全体の静特性と心拍数制御の静特性をまとめたものである。圧反射全体の静特性では、心不全ラットにおいて血圧の応答範囲の低下が顕著であった（対照群: 64±4 mmHg, 心不全群: 31±6 mmHg, $P<0.01$）。また、心拍数についても応答範囲の低下が観察され（対照群: 49±5 beats/min, 心不全群: 30±6 beats/min, $P<0.05$）、予測に反して心不全ラットにおいて心拍数が低値を示した。

図Ｃ－２－２中段はロジスティック曲線の傾きの最大値（$P_1 \times P_2 \div 4$）と測定された中心静脈圧の関係を示したものである。心不全群では中心静脈圧が高かったが、中心静脈圧と傾きの最大値との間に有意な相関は見られなかった。

図Ｃ－２－２下段はロジスティック曲線の応答範囲（$P_1$）と測定された中心静脈圧との関係を示したものである。心不全群と対照群の全体をまとめて解析すると、中心静脈圧が高いほど応答範囲が小さくなる有意な相関を示した。しかし、心不全群単独の解析では、相関は有意ではなかった。

図Ｃ－２－３上段は圧反射の中枢弓と末梢弓の静特性をまとめたものである。交感神経活動の絶対電位の記録は個体によって大きくばらつくため、心不全群と対照群のいずれにおいても、頚動脈洞内圧が 60 mmHg のときの交感神経活動を 100 %として表示してある。中枢弓の静特性では、心不全群において高い頚動脈洞内圧に対してあまり交感神経活動が抑制されず、応答範囲が縮小した（対照群: 62±4 %, 心不全群: 42±6 %, $P<0.01$）。末梢弓の静特性では、交感神経活動の同じパーセント変化に対して、体血圧の応答が小さく、末梢弓の入出力関係を示す直線の傾きが緩やかであった（対照群: 1.10±0.08, 心不全群: 0.75±0.10, $P<0.05$）。

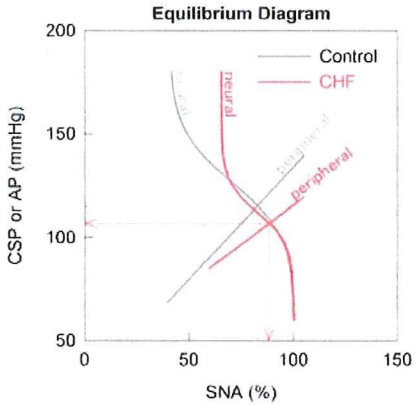



**図Ｃ－２－３．圧反射の中枢弓（Neural Arc）と末梢弓（Peripheral Arc）の静的な入出力関係. CSP: 頚動脈洞内圧, SNA: 交感神経活動, AP: 血圧, CVP: 中心静脈圧, Control: 対照群, CHF: 心不全群.**

図Ｃ－２－３中段左は中枢弓を示すロジスティック曲線の傾きの最大値（$P_1 \times P_2 \div 4$）と中心静脈圧の関係を示したものであるが、両者に有意な相関はなかった。中段右は末梢弓の傾きと中心静脈圧の関係を示したものである。末梢弓の傾きについては、心不全群と対照群をまとめて解析しても個別に解析にしても有意な負の相関が認められた。

図Ｃ－２－３下段左は中枢弓を示すロジスティック曲線の応答範囲（$P_1$）と中心静脈圧の関係を示したものであるが、両者に

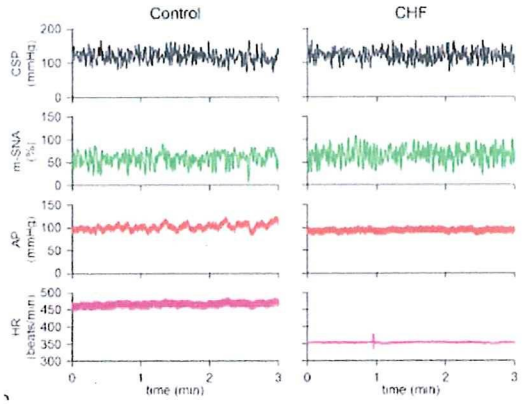
有意な相関はなかった。下段右は末梢弓を直線回帰したときのｙ切片と中心静脈圧の関係を示したものである。心不全群と対照群をまとめて解析すると、有意な正の相関が認められた。しかし、心不全群単独の解析では相関は有意ではなかった。




**図C－2－4. 動脈圧反射の平衡線図(Equilibrium Diagram). CSP: 頚動脈洞内圧, AP: 体血圧, SNA: 交感神経活動, neural: 中枢弓, peripheral: 末梢弓, Control: 対照群, CHF: 心不全群.**

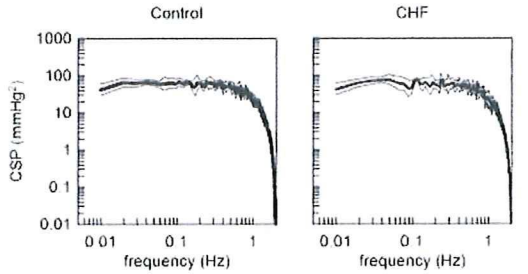
図Ｃ－２－４は頚動脈洞圧反射の平衡線図を示したものである。平衡線図では中枢弓の横軸と縦軸は入れ替えて表示されている。この図における中枢弓と末梢弓の交点から、動脈圧反射を閉ループ状態にしたときの交感神経活動（垂直矢印の先）と血圧（水平矢印の先）が分かる。動作点における交感神経活動に差はなかったが（対照群: 84±3 %, 心不全群: 90±4 %)、血圧は心不全群で有意に低かった（対照群: 116±3 mmHg, 心不全群: 106±3 mmHg, P＜0.05)。一方、中枢弓と末梢弓の傾きの積から計算した動作点における動脈圧反射の開ループゲインには有意差がなかった（対照群: 1.2±0.3, 心不全群: 1.0±0.4)。

## Ｃ－３．心不全ラットにおける頚動脈洞圧反射の動特性




**図C－3－1. 動特性を求める実験例. CSP: 頚動脈洞内圧, m-SNA: 平均交感神経活動, AP: 体血圧, HR: 心拍数, Control: 対照ラット, CHF: 心不全ラット.**

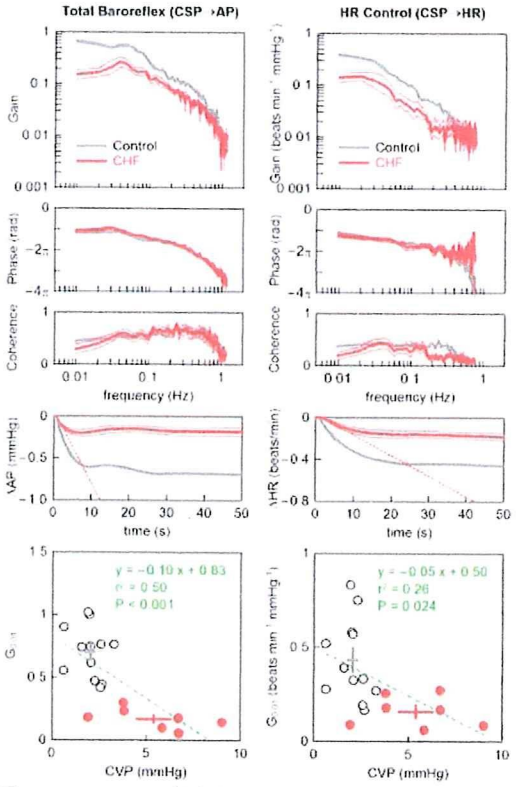
図Ｃ－３－１に実験例を示す。頚動脈洞にガウス白色雑音入力を加えると、それに応じて交感神経活動が変化した。交感神経活動の変動は正常ラットでも心不全ラットでも認められた。一方、正常ラットでは血圧や心拍数に変動がみられたが、心不全ラットではほとんど血圧や心拍数の変動はみられなかった。動脈圧反射の動特性を求める実験においても、予測に反して平均心拍数は心不全ラットで低値を示した。




**図C－3－2. 頚動脈洞圧受容器に印加したガウス白色雑音のパワースペクトル。CSP: 頚動脈洞内圧, Control: 対照群, CHF: 心不全群.**

図Ｃ－３－２に今回の実験で入力したガウス白色雑音のパワースペクトルを示す。

500 ms でコマンドを切り替えているので、それに対応する 2 Hz の周波数で入力パワーは消失する。その半分の約 1 Hz まではほぼ平坦な入力パワーが得られた。伝達関数の計算において、入出力のクロススペクトルを入力のパワースペクトルで除するので、入力パワーが極端に小さくなる周波数帯域では伝達関数の推定は不正確になる。本研究では、頚動脈洞圧反射の伝達関数を約 1 Hz まで推定した。




**図C－3－3．圧反射全体（Total Baroreflex）と心拍数制御（HR Control）の動的な入出力関係を示す伝達関数とコヒーレンスおよびステップ応答を示す。CSP: 頚動脈洞内圧, AP: 体血圧, HR: 心拍数, ΔAP: 血圧変化, ΔHR: 心拍数変化, $G_{0.01}$: 周波数 0.01 Hz における伝達関数のゲイン, CVP: 中心静脈圧, Control: 対照群, CHF: 心不全群.**

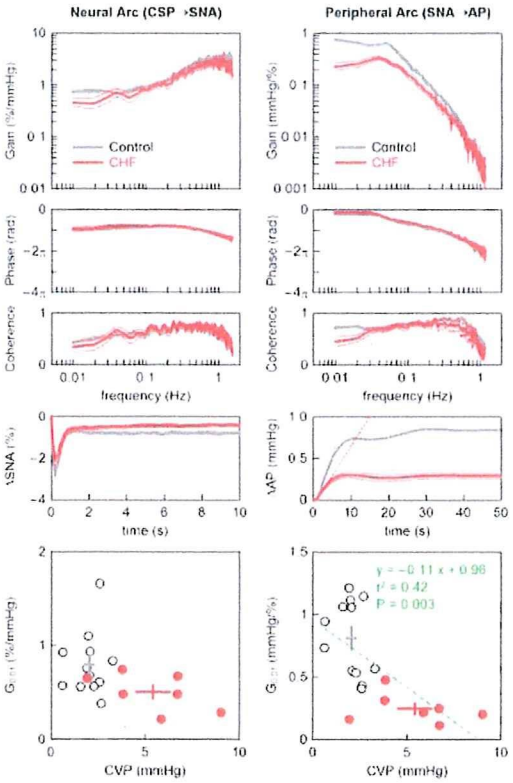
図C－3－3は圧反射全体および心拍数制御の伝達関数を示したものである。上段はゲイン線図、二段目は位相線図、三段目はコヒーレンスを示す。圧反射全体の伝達関数（図C－3－3左）について、対照群と心不全群のいずれにおいても、周波数が高くなるにつれてゲインが小さくなり、位相が遅れるローパスフィルターの性質が観察された。位相線図およびコヒーレンスには大きな差はなかったが、心不全群において動的ゲインが著しく低下した（対照群: 0.70 ± 0.06, 心不全群: 0.17 ± 0.03, $P < 0.01$）。

体血圧のステップ応答においては、応答開始の傾きが心不全群で約 1/2 であり（対照群: −0.166±0.014 mmHg/s, 心不全群: −0.086±0.009 mmHg/s, $P<0.01$）、定常応答は約 1/3 に低下した（対照群: −0.69 ± 0.07 mmHg, 心不全群: −0.18 ± 0.05 mmHg, $P<0.01$）。また、心不全群と対照群をまとめて解析すると、最下段に示すように、周波数 0.01 Hz における動的ゲインと中心静脈圧の間に有意な負の相関がみられた。

心拍数制御の伝達関数（図C－3－3右）においても、周波数が高くなるにつれてゲインが小さくなり、位相が遅れるローパスフィルターの性質が観察された。心不全群では応答が小さいため、もしくは不整脈の発生のために、0.2 Hz 以上でコヒーレンスの低下が起こり、ゲインや位相の推定が悪くなった。心拍数制御の動的ゲインは心不全群で 1/2 以下に低下した（対照群: 0.43± 0.06 (beats/min)/mmHg, 心不全群: 0.16± 0.03 (beats/min)/mmHg, $P<0.01$）。

心拍数のステップ応答では応答開始の傾きが 1/2 以下に（対照群: −0.059±0.009 (beats/min)/s, 心不全群: −0.020 ± 0.004 (beats/min)/s, $P<0.01$）、定常応答も 1/2 以下に低下した（対照群: −0.46 ± 0.08

beats/min, 心不全群：－0.18±0.03 beats/min, P＜0.05)。心不全群と対照群をまとめて解析すると、周波数 0.01 Hz における心拍数制御の動的ゲインと中心静脈圧の間に有意な負の相関がみられた。




**図Ｃ－３－４．圧反射中枢弓（Neural Arc）と末梢弓（Peripheral Arc）の動的な入出力関係を示す伝達関数とコヒーレンスおよびステップ応答を示す。CSP: 頚動脈洞内圧, SNA: 交感神経活動, AP: 体血圧, ΔSNA: 交感神経活動変化. ΔAP: 血圧変化, $G_{0.01}$: 周波数 0.01 Hz における伝達関数のゲイン, CVP: 中心静脈圧, Control: 対照群, CHF: 心不全群.**

図Ｃ－３－４は圧反射の中枢弓および末梢弓の伝達関数を示したものである。上段はゲイン線図、二段目は位相線図、三段目はコヒーレンスを示す。圧反射中枢弓の伝達関数（図Ｃ－３－４左）について、対照群においても心不全群においても、周波数が高くなるほど動的ゲインが大きくなる微分特性が観察された。位相線図は 0.01～1 Hz の範囲でほぼ－πラジアンを示しており、圧入力と交感神経活動の関係が中枢性に反転することを示している。位相線図およびコヒーレンスには大きな差はなかったが、もっとも低い周波数において、心不全群で動的ゲインが低下する傾向がみられた（対照群: 0.80±0.10 %/mmHg. 心不全群: 0.50±0.08, P＝0.051)。

圧反射中枢弓による交感神経活動のステップ応答においては極小応答（対照群: －2.88±0.25%, 心不全群: －2.13±0.30%）および極小応答までの時間（対照群: 0.29±0.01 秒, 心不全群: 0.31±0.01 秒）に、対照群と心不全群とで差はなかった。ただし、交感神経活動のステップ応答における定常応答は心不全群で有意に低下した（対照群: －0.75±0.09%, 心不全群: －0.39±0.06%, P＜0.05)。図最下段に示すように、対照群と心不全群をまとめて解析しても、周波数 0.01 Hz における交感神経制御の動的ゲインと中心静脈圧との間には有意な相関はみられなかった。

圧反射の末梢弓の伝達関数（図Ｃ－３－４右）について、対照群と心不全群のいずれにおいても、周波数が高くなるほど動的ゲインが小さくなるローパスフィルターの性質が観察された。末梢弓の動的ゲインは心不全群で約 1/3 に低下した（対照群: 0.81±0.09 mmHg/%, 心不全群: 0.24±0.05 mmHg/%, P＜0.01)。位相線図に大きな違いは見られなかった。心不全群のコヒーレンスは 0.01 Hz 付近で対照群よりも小さい傾向を示した。

圧反射末梢弓による血圧のステップ応答では、心不全群において応答開始の傾きが約 1/2 に（対照群: 0.134±0.014 mmHg/s,

心不全群：0.071±0.009 mmHg/s, P<0.01)、定常応答が約1/3に低下した（対照群: 0.84±0.08, 心不全群: 0.28±0.03, P<0.01)。図最下段に示すように、心不全群と対照群をまとめて解析すると、周波数0.01 Hzにおける末梢弓の動的ゲインと中心静脈圧との間に有意な負の相関がみられた。

## D．考察

### D－1．心不全ラットにおける頚動脈洞圧反射の静特性

#### D－1－1．開ループ実験について

頚動脈洞圧反射は生理的状態では、血圧が上昇すれば交感神経活動が低下し、交感神経活動が低下すれば血圧が低下するといった閉ループ状態で動作している。閉ループ状態では交感神経活動と血圧のいずれが入力でいずれが出力かを分離できないので、単に交感神経活動や血圧を測定する観察的実験ではシステムの入出力関係を明らかにすることができない。

そこで、本研究では頚動脈洞圧受容器の領域を体循環から分離する開ループ実験法を用いた。頚動脈洞圧受容器を体循環から分離することによって、体血圧が変化しても入力圧が影響を受けることがなくなり、圧反射中枢弓（入力圧→交感神経活動）および圧反射末梢弓（交感神経活動→体血圧）の入出力関係を入力範囲にわたって定量化することができる。

#### D－1－2．心不全における圧反射全体の静特性の変化

本研究では、心不全群において圧反射全体の応答範囲が小さくなり、対照群と心不全群をまとめて解析すると、圧反射ゲインと中心静脈圧との間に有意な負の相関がみられた（図C－2－2左)。また、心拍数応答についても同じで、応答範囲が小さくなり、心拍数制御のゲインと中心静脈圧との間に有意な負の相関がみられた（図C－2－2右)。

#### D－1－3．心不全における中枢弓と末梢弓の静特性の変化

圧反射の中枢処理と心臓血管系の末梢応答のいずれが心不全における圧反射ゲインの低下に関与しているかを調べるために、圧反射中枢弓と末梢弓の入出力関係を定量化した。心不全群において中枢弓の応答範囲の低下がみられたが、シグモイド関数の示す最大ゲインに有意な差はなく、中心静脈圧との有意な相関もみられなかった（図C－2－3左)。これに対して、末梢弓の傾きは心不全群で有意に低下しており、対照群と心不全群を独立に解析しても、まとめて解析しても、末梢弓の傾きは中心静脈圧と有意な負の相関を示した（図C－2－3右)。

以上のことから、交感神経活動を%表示したとき、心不全において中枢弓の最大ゲインは低下しないが応答範囲が縮小する。一方、末梢弓の直線性は保たれるものの、その傾きが低下する。圧反射全体のゲインは中枢弓と末梢弓の積で決まるので、圧反射全体にみられるゲインの低下は末梢弓の傾きの低下、すなわち、交感神経活動の変化に対する血圧応答の低下で説明できる。末梢弓がほぼ直線的であることから、末梢弓の傾きの低下は、同じ交感神経活動の変化に対する体血圧の応答範囲の縮小にも寄与する。一方、中枢弓において交感神経活

動の応答範囲が縮小することから、圧反射全体における応答範囲の縮小には、中枢弓も寄与すると考えられる。

ただし、本研究では交感神経活動を刺激するなどして直接的に変化させたわけではないので、交感神経活動の変化が保たれたとき、心不全群で末梢弓の直線性が失われるかどうかは不明である。

### D－2．心不全ラットにおける頚動脈洞圧反射の動特性

#### D－2－1．ガウス白色雑音入力について

本研究では体循環から分離した頚動脈洞にガウス白色雑音を入力することで頚動脈洞圧反射の動特性を同定した。これまでのウサギを用いた研究から、圧反射の中枢弓は微分特性＋シグモイド状非線形特性で近似できることが分かっている（Kawada et al., *Am J Physiol Heart Circ Physiol* 286: H2272-H2279, 2004)。圧反射中枢弓に存在する非線形特性は動特性の推定に影響を与えうる。たとえば、入力が二値白色雑音であるとき、入力の振幅が大きくなるほど中枢弓の微分特性の性質が失われる（Kawada et al., *Am J Physiol Heart Circ Physiol* 284: H404- H415, 2003)。

しかし、入力がガウス白色雑音であるときはシステムの線形特性は定数倍されて推定されるだけである（Bussgang, *MIT Res Lab Elec Tech Rep* 216: 1-14, 1952)。伝達関数上はゲイン線図が上下に平行移動するが、周波数依存性の変化は影響を受けない。したがって、入力にガウス白色雑音を用いたときは、システムの微分特性あるいはローパスフィルターの特性などの周波数依存的な変化は、非線形特性の存在に関わらず正しく推定できることが期待される。

#### D－2－2．正常ラットにおける中枢弓による圧反射の高速化

正常ラットにおける圧反射中枢弓の動特性の特徴は、入力周波数が高くなるほど動的ゲインが高くなる微分特性を持つことである。この微分特性の存在によって、圧反射末梢弓のローパスフィルターの性質（入力周波数が高くなるほど動的ゲインが小さくなる性質）が補償され、圧反射全体で捉えたときは、高周波数領域における動的ゲインの低下が緩和される。これを時間軸で表現すると、圧反射中枢弓の微分特性は圧反射全体の応答の高速化に寄与していると言える（Ikeda et al., *Am J Physiol* 271: H882-H890, 1996)。しかしながら、心不全の状態において、そのような中枢弓の微分特性による圧反射特性の補償が保たれているかどうかは不明であった。

#### D－2－3．心不全ラットにおける中枢弓による圧反射の高速化

本研究において、心不全群においても対照群と同様に圧反射中枢弓の動特性は、入力周波数が高くなるほど動的ゲインが大きくなる微分特性を示した（図C－3－4左)。これによって、ステップ応答でみた場合、末梢弓による血圧変化の応答開始の傾きよりも、圧反射全体による血圧変化の応答開始の傾きの絶対値が大きくなり、血圧応答が高速化されていることが判明した。したがって、心不全においても中枢弓は微分特性を維持しており、圧反射全体の応答を高速化するのに役立っていると言える。しかしながら、応答開始の傾きは心不全群にお

いて対照群よりも小さく、中枢弓による補償が存在しても、依然として血圧応答は正常群に比べて遅いことが示唆された。

### D－3．実験に関する制限事項など

#### D－3－1．迷走神経について

本研究では他の反射系の影響をできるだけ除外して、頚動脈洞圧反射の静特性および動特性を同定することを目的としたので、急性実験では迷走神経を切断した。これによって、心肺受容器領域に存在する低圧受容器からの迷走神経を介する反射を除くことができる。しかしながら、心不全増悪の一因は迷走神経の活動消失にあると考えられるため、迷走神経制御を含めた圧反射特性の推定が必要になってくる。特に、迷走神経は心拍数調節に深く関わるため、今回の実験で観察した心拍数制御の応答範囲の縮小やゲインの低下が、迷走神経の存在下でも観察できるかどうかは今後の研究課題である。

#### D－3－2．交感神経活動の定量化について

交感神経活動の絶対電位は記録する神経の太さや記録電極と神経との接触の程度によって個体ごとに大きく異なるので、本研究では対照群と心不全群の交感神経活動を絶対電位で比較しても両者に差はみられなかった。そこで、頚動脈洞内圧を 60 mmHg に保ったときの交感神経活動を 100%、神経節遮断薬を投与した後のノイズレベルを0%として交感神経活動を記述した。そのため、圧反射の静特性において交感神経活動の最大値は、対照群も心不全群もほぼ100%になっている。

血中カテコラミンの高値などから心不全においては交感神経が過剰に活動していると考えられているが、本研究の平衡線図（図C－2－4）では、そのことが図に反映されていない。交感神経活動を絶対電位で記述できたとすると、心不全群において絶対電位が大きいことが期待されるので、心不全群の平衡線図は横軸方向に拡大されると考えられる。その場合、心不全群において、圧反射中枢弓のゲインは現在の方法よりも大きく評価され、圧反射末梢弓の傾きは現在の方法よりも小さく評価されることになる。今後、交感神経活動の解析についてはより良い正規化法の開発が必要である。

#### D－3－3．麻酔の影響

本研究では慢性心不全ラットを用いたが、頚動脈洞圧反射の静特性および動特性を求める急性実験は麻酔下で行った。麻酔下において、予測に反して心不全群において対照群よりも平均心拍数が低値を示した。実験では体重当たり同量の麻酔薬を用いたが、心不全群では循環不全によって麻酔薬の代謝が遅延し、麻酔のレベルが相対的に深くなった可能性は否定できない。しかし、心不全群においても、血圧や心拍数が経時的に低下していくことはなかった。

### E．結論

本年度は心筋梗塞後ラット慢性心不全モデルを作成し、頚動脈洞圧反射の静特性および動特性を定量化した。これにより、圧反射静特性について、①心不全群における血圧応答範囲の縮小は圧反射中枢弓と末梢弓の両方で生じること、②交感神経活動を%表示したとき、圧反射中枢弓の最大ゲ

インは保たれていること、③圧反射末梢弓において交感神経活動の変化に対する血圧応答が低下していること、が明らかになった。また、圧反射動特性については、①心不全群においても中枢弓の微分特性は維持されていること、②末梢弓の動的ゲインが低下していること、③中枢弓の補償があっても圧反射全体としては、正常群に比べて血圧応答の速さも大きさも小さくなっていること、が判明した。

頚動脈洞マイクロマシンを用いた自律神経制御において、圧反射中枢の機能が維持されていることが必要となる。本研究において、圧反射中枢弓の応答範囲の縮小がみられたが、シグモイド曲線の最大ゲインおよび微分特性は維持されており、圧反射を用いて自律神経活動に介入することが可能であることが確認された。一方、本研究では圧反射中枢弓の機能を圧入力に対する交感神経活動の応答として評価しており、応答範囲の縮小が圧受容器レベルで生じたのか、求心神経から交感神経遠心路に至る血管運動中枢で生じたのかは不明である。今後、頚動脈洞神経活動の記録あるいは刺激を用いて両者を分けて評価することが必要になると考えられる。

## F．健康危険情報

なし

## G．研究発表

### G－1．論文


27. Role of $Ca^{2+}$-activated $K^+$ channels in catecholamine release from in vivo rat adrenal medulla. Akiyama T, Yamazaki T, Kawada T, Shimizu S, Sugimachi M, Shirai M. *Nurochem Int* 2009 (in press).
28. In vivo direct monitoring of interstitial norepinephrine levels at the sinoatrial node. Shimizu S, Akiyama T, Kawada T, Shishido T, Mizuno M, Kamiya A, Yamazaki T, Sano S, Sugimachi M. *Auton Neurosci* 2009 (in press).
29. Detection of endogenous acetylcholine release during brief ischemia in the rabbit ventricle: a possible trigger for ischemic preconditioning. Kawada T, Akiyama T, Shimizu S, Kamiya A, Uemura K, Li M, Shirai M, Sugimachi M. *Life Sci* 85: 597-601, 2009.
30. High levels of circulating angiotensin II shift the open-loop baroreflex control of splanchnic sympathetic nerve activity, heart rate and arterial pressure in anesthetized rats. Kawada T, Kamiya A, Li M, Shimizu S, Uemura K, Yamamoto H, Sugimachi M. *J Physiol Sci* 59: 447-455, 2009.
31. Coronary artery volume non-invasively measured with multislice computed tomography. Definition, accuracy and implication. Sugimachi M, Kawada T. *Circ J* 73: 1395-1396, 2009.
32. Slow head-up tilt causes lower activation of muscle sympathetic nerve activity: loading speed dependence of orthostatic

Kawada T, Shimizu S, Iwase S, Sugimachi M, Mano T. *Am J Physiol Heart Circ Physiol* 297: H53-H58, 2009.

33. Artificial neural interfaces for bionic cardiovascular treatments. Kawada T, Sugimachi M. *J Artif Organs* 12: 17-22, 2009 (review).
34. Servo-controlled hind-limb electrical stimulation for short-term arterial pressure control. Kawada T, Shimizu S, Yamamoto H, Shishido T, Kamiya A, Miyamoto T, Sunagawa K, Sugimachi M. *Circ J* 73: 851-859, 2009.
35. In vivo direct monitoring of vagal acetylcholine release to the sinoatrial node. Shimizu S, Akiyama T, Kawada T, Shishido T, Yamazaki T, Kamiya A, Mizuno M, Sano S, Sugimachi M. *Auton Neurosci* 148: 44-49, 2009.
36. Angiotensin II disproportionally attenuates dynamic vagal and sympathetic heart rate controls. Kawada T, Mizuno M, Shimizu S, Uemura K, Kamiya A, Sugimachi M. *Am J Physiol Heart Circ Physiol* 296: H1666-H1674, 2009.

## G－2．学会発表

25. アンジオテンシンⅡが動脈圧反射系の開ループ静特性に及ぼす影響．川田徹、清水　秀二、水野　正樹、宍戸　稔聡、杉町　勝．第 48 回日本生体医工学会大会．
26. 心臓マイクロダイアリシス法による洞房結節における交感・副交感神経活動の定量化．清水　秀二、秋山　剛、川田　徹、宍戸　稔聡、神谷　厚範、杉町　勝．第 48 回日本生体医工学会大会．
27. 心筋梗塞後心不全ラットにおける開ループ動脈圧反射特性．川田　徹、李　梅花、上村　和紀、宍戸　稔聡、杉町　勝．第 30 回日本循環制御医学会総会．
28. 上村　和紀、鄭　燦、李　梅花、川田徹、杉町　勝．迷走神経を、冠虚血再灌流後の 3 日間電気刺激するだけで心筋梗塞後左心室リモデリングを劇的に改善することができる．第 30 回日本循環制御医学会総会．
29. アセチルコリンエステラーゼ阻害薬（ドネペジル）投与による心筋梗塞後重症心不全治療におけるα7-ニコチン性アセチルコリン受容体（α7 nAChR）の関与．李　梅花、川田　徹、上村　和紀、宍戸　稔聡、杉町　勝．第 30 回日本循環制御医学会総会．
30. Effects of angiotensin II on open-loop carotid sinus baroreflex function. Kawada T, Shimizu S, Uemura K, Shishido T, Sugimachi M. The 36th International Congress of Physiological Sciences.
31. 重症心不全に対する迷走神経刺激療法．宍戸　稔聡、李　梅花、鄭　燦、上村　和紀、川田　徹、杉町　勝．第 24 回生体・生理工学シンポジウム．
32. ロサルタンとドネペジルの併用投与による心筋梗塞後重症心不全ラットの新

川田　徹、稲垣　正司、杉町　勝．第7回交感神経とアンジオテンシンII研究会．

33. ストレスを軽減する鎮静剤α2アゴニストは薬理学的にも副交感神経を賦活化するか？　清水　秀二、秋山　剛、川田　徹、宍戸　稔聡、神谷　厚範、稲垣　正司、杉町　勝．第10回Neurocardiology Workshop．

34. 静水圧要因による中心循環動態の変化は呼吸化学調節負帰還システムの静特性を変化させる．宮本　忠吉、三木屋良輔、中原　英博、川田　徹、杉町　勝．第10回Neurocardiology Workshop．

35. ラットにおける頚動脈洞圧反射の定常応答の推定に必要とされるステップ状入力時間の検討．川田　徹、神谷　厚範．第55回日本航空宇宙環境医学会大会．

36. システム生理学によって、自律神経系および循環器系の動態を予測する試み．神谷　厚範、川田　徹、杉町　勝．第55回日本航空宇宙環境医学会大会．

## 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

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| Akiyama T, Yamazaki T, Kawada T, Shimizu S, Sugimachi M, Shirai M. | Role of Ca(2+)-activated K(+) channels in catecholamine release from in vivo rat adrenal medulla. | Neurochem Int | | | 2009 |
| Kamiya A, Kawada T, Shimizu S, Iwase S, Sugimachi M, Mano T. | Slow head-up tilt causes lower activation of muscle sympathetic nerve activity: loading speed dependence of orthostatic sympathetic activation in humans. | Am J Physiol Heart Circ Physiol | 297 | H53-58 | 2009 |
| Kashihara K, Kawada T, Sugimachi M, Sunagawa K. | Wavelet-based system identification of short-term dynamic characteristics of arterial baroreflex. | Ann Biomed Eng | 37 | 112-128 | 2009 |
| Kawada T, Mizuno M, Shimizu S, Uemura K, Kamiya A, Sugimachi M. | Angiotensin II disproportionally attenuates dynamic vagal and sympathetic heart rate controls. | Am J Physiol Heart Circ Physiol | 296 | H1666-1674 | 2009 |
| Kawada T, Shimizu S, Yamamoto H, Shishido T, Kamiya A, Miyamoto T, Sunagawa K, Sugimachi M. | Servo-controlled hind-limb electrical stimulation for short-term arterial pressure control. | Circ J | 73 | 851-859 | 2009 |
| Kawada T, Sugimachi M. | Artificial neural interfaces for bionic cardiovascular treatments. | J Artif Organs | 12 | 17-22 | 2009 |

| | | | | | |
|---|---|---|---|---|---|
| Kawada T, Kamiya A, Li M, Shimizu S, Uemura K, Yamamoto H, Sugimachi M. | High levels of circulating angiotensin II shift the open-loop baroreflex control of splanchnic sympathetic nerve activity, heart rate and arterial pressure in anesthetized rats. | J Physiol Sci | 59 | 447-455 | 2009 |
| Kawada T, Akiyama T, Shimizu S, Kamiya A, Uemura K, Li M, Shirai M, Sugimachi M. | Detection of endogenous acetylcholine release during brief ischemia in the rabbit ventricle: a possible trigger for ischemic preconditioning. | Life Sci | 85 | 597-601 | 2009 |
| Shimizu S, Akiyama T, Kawada T, Shishido T, Yamazaki T, Kamiya A, Mizuno M, Sano S, Sugimachi M. | In vivo direct monitoring of vagal acetylcholine release to the sinoatrial node. | Auton Neurosci | 148 | 44-49 | 2009 |
| Shimizu S, Akiyama T, Kawada T, Shishido T, Mizuno M, Kamiya A, Yamazaki T, Sano S, Sugimachi M. | In vivo direct monitoring of interstitial norepinephrine levels at the sinoatrial node. | Auton Neurosci | | | 2009 |
| Sugimachi M, Kawada T. | Coronary artery volume noninvasively measured with multislice computed tomography. Definition, accuracy and implication. | Circ J | 73 | 1395-1396 | 2009 |
| Sugimachi M, Sunagawa K, Uemura K, Kamiya A, Shimizu S, Inagaki M, Shishido T. | Feedback control of multiple hemodynamic variables with multiple cardiovascular drugs. | Conf Proc IEEE Eng Med Biol Soc | 1 | 2030-2032 | 2009 |
| Sugimachi M, Sunagawa K, Uemura K, Kamiya A, Shimizu S, Inagaki M, Shishido T. | Macroscopic two-pump two-vasculature cardiovascular model to support treatment of acute heart failure. | Conf Proc IEEE Eng Med Biol Soc | 1 | 2365-2368 | 2009 |

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# Role of $Ca^{2+}$-activated $K^+$ channels in catecholamine release from *in vivo* rat adrenal medulla

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ABSTRACT

To elucidate the role of $Ca^{2+}$-activated $K^+$ ($K_{Ca}$) channels in the presynaptic acetylcholine (ACh) release from splanchnic nerve endings and the postsynaptic catecholamine release from chromaffin cells, we applied microdialysis technique to the left adrenal medulla of anesthetized rats and investigated the effects of local administration of $K_{Ca}$ channel antagonists through dialysis probes on the release of ACh and/or catecholamine, induced by electrical stimulation of splanchnic nerves or local administration of ACh through the dialysis probes. *Nerve stimulation-induced release*: in the presence of a cholinesterase inhibitor, neostigmine, large-conductance $K_{Ca}$ (BK) channel antagonists, iberiotoxin and paxilline enhanced the presynaptic ACh release and postsynaptic norepinephrine (NE) and epinephrine (Epi) release. Small-conductance $K_{Ca}$ (SK) channel antagonists, apamin and scyllatoxin enhanced the Epi release without any changes in ACh or NE release. In the absence of neostigmine, ACh release was not detected. Iberiotoxin and paxilline enhanced NE and Epi release. Apamin and scyllatoxin had no effect on NE or Epi release. *Exogenous ACh-induced release*: iberiotoxin and paxilline enhanced the Epi release, but had no effect on the NE release. Apamin and scyllatoxin enhanced both NE and Epi release. In conclusion, BK channels on splanchnic nerve endings play an inhibitory role in the physiological catecholamine release from adrenal medulla by limiting presynaptic ACh release while SK channels do not. BK channels on Epi-storing cells may play an inhibitory role in nerve stimulation-induced Epi release. SK channels on NE- and Epi-storing cells play a minor role in nerve stimulation-induced catecholamine release.


## 1. Introduction

The physiological release of catecholamine from adrenal medulla is controlled by central sympathetic neurons through splanchnic nerves. Splanchnic nerve endings make synaptic-like contacts with chromaffin cells (Coupland, 1965). Activation of splanchnic nerve endings causes $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels, which evokes exocytotic acetylcholine (ACh) release. This ACh release activates cholinergic receptors on chromaffin cells, which causes $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels and evokes exocytotic catecholamine release from chromaffin cells (García et al., 2006).

$Ca^{2+}$-activated $K^+$ ($K_{Ca}$) currents are consistently found at neuronal cells or nerve terminals (Meir et al., 1999). $K_{Ca}$ channels are located in the vicinity of voltage-dependent $Ca^{2+}$ channels and activated by $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels. Activation of the $K_{Ca}$ channels induces outward efflux of $K^+$, causes hyperpolarization of the membrane, and subsequently limits $Ca^{2+}$ entry through voltage-dependent $Ca^{2+}$channels. Thus, $K_{Ca}$ channels may be present at two different sites in the adrenal medulla: splanchnic nerve endings and chromaffin cells, and are then involved in the physiological regulation of presynaptic ACh release and/or postsynaptic catecholamine release. In fact, it has been reported that $K_{Ca}$ channels on chromaffin cells play an important role in catecholamine release (Montiel et al., 1995; Uceda et al., 1992; Wada et al., 1995). Little information is, however, available on the role of $K_{Ca}$ channels in the presynaptic ACh release from splanchnic nerve endings.

We have recently developed a dialysis technique to simultaneously monitor the release of presynaptic ACh and postsynaptic catecholamine in the *in vivo* adrenal medulla (Akiyama et al., 2004a). This method makes it possible to investigate the functional roles of $K_{Ca}$ channels in the ACh release from splanchnic nerve endings and the catecholamine release from adrenal medulla in the *in vivo* state. In the present study, we applied the microdialysis technique to the adrenal medulla of anesthetized rats and investigated the effects of $K_{Ca}$ channel antagonists on the release of presynaptic ACh and postsynaptic catecholamine.

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In electrophysiological studies, $K_{Ca}$ channels can be divided into two types based on their single channel conductance: large-conductance (BK) and small-conductance $K_{Ca}$ (SK) channels (Blatz and Magleby, 1987). We tested two types of BK channel antagonists: the selective peptidergic BK channel antagonist, iberiotoxin (Candia et al., 1992) and the non-peptidergic BK channel antagonist, paxilline (Kanus et al., 1994). Similarly we tested two types of SK channel antagonists: the selective peptidergic SK channel antagonist, apamin (Blatz and Magleby, 1986) and the selective peptidergic SK channel antagonist different in amino acid sequence, scyllatoxin (Auguste et al., 1990).

## 2. Materials and methods

### 2.1. Animal preparation

Animal care was provided in strict accordance with the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences* approved by the Physiological Society of Japan. All protocols were approved by the Animal Subject Committee of the National Cardiovascular Center. Adult male Wistar rats weighing 380–460 g were anesthetized with pentobarbital sodium (50–55 mg/kg, i.p.). A cervical midline incision was made to expose the trachea, which was then cannulated. The rats were ventilated with a constant-volume respirator using room air mixed with oxygen. The left femoral artery and vein were cannulated for monitoring arterial blood pressure and administration of anesthetic, respectively. The level of anesthesia was maintained with a continuous intravenous infusion of pentobarbital sodium (15–25 mg/kg/h, i.v.). The electrocardiogram was monitored to record the heart rate. A thermostatic heating pad was used to keep the esophageal temperature within a range of 37–38 °C. With the animal in the lateral position, the left adrenal gland and left splanchnic nerve were exposed by a subcostal flank incision, and the left splanchnic nerve was transected. In protocols requiring nerve stimulation, shielded bipolar stainless steel electrodes were applied to the distal end of the nerve, which was then stimulated with a digital stimulator (SEN-7203, Nihon Kohden, Japan) with a rectangular pulse (10 V and 1 ms in duration).

### 2.2. Dialysis technique

Dialysis probe construction was the same as that used in our previous dialysis experiments (Akiyama et al., 2003, 2004a,b). Each end of a dialysis fiber (0.32 mm OD, and 0.25 mm ID; PAN-DX 100,000 mol wt 100% cutoff, Asahi Chemical, Japan) was inserted into a polyethylene tube (25 cm length, 0.5 mm OD, and 0.2 mm ID; SP-8) and glued. The length of the dialysis fiber exposed was 3 mm. At a perfusion speed of 10 μl/min, *in vitro* recovery rates of ACh, norepinephrine (NE) and epinephrine (Epi) were (%): 3.21 ± 0.07, 2.68 ± 0.03, and 2.80 ± 0.03, respectively (number of dialysis probes: 3).

The left adrenal gland was gently lifted, and the dialysis probe was implanted in the medulla of the left adrenal gland along the long axis using a fine guiding needle. The dialysis probe was perfused with Ringer's solution or Ringer's solution containing pharmacological agents at a speed of 10 μl/min using a microinjection pump (CMA/100, Carnegie Medicin, Sweden). Ringer's solution consisted of (in mM) 147.0 NaCl, 4.0 KCl, 2.25 $CaCl_2$. All $K_{Ca}$ channel antagonists tested were locally administered by perfusion through the dialysis probe after being dissolved in Ringer's solution. We started the protocols followed by a stabilization period of 3–4 h and sampled dialysate taking the dead space volume between the dialysis membrane and sample tube into account. Dialysate ACh and catecholamine concentrations were separately measured using each high-performance liquid chromatography with electrochemical detection as previously described (Akiyama et al., 2004a,b).

### 2.3. Experimental protocols

The experiment was performed based on the previous experiment showing that dialysate ACh and/or catecholamine responses were reproducible on repetition of the pharmacological or electrical stimulation (Akiyama et al., 2004a,b). At the end of the experiment, the rats were sacrificed with pentobarbital sodium, and the implant sites were examined. The dialysis probes were confirmed to have been implanted in the adrenal medulla, and no bleeding or necrosis was found macroscopically.

### 2.4. Protocol 1

We perfused the dialysis probe with Ringer's solution containing a cholinesterase inhibitor, neostigmine (10 μM) and investigated the effects of BK and SK channel antagonists on the nerve stimulation-induced responses of dialysate ACh and catecholamine concentration. The left splanchnic nerves were firstly electrically stimulated for 2 min at 2 Hz. Then, after a 30-min interval, nerves were subjected to a second stimulation for 2 min at 4 Hz. After these control stimulations, local administration of iberiotoxin (1 μM, $n = 7$), paxilline (100 μM, $n = 7$), apamin (10 μM, $n = 7$) or scyllatoxin (2 μM, $n = 7$) was started. Thirty minutes after local administration of $K_{Ca}$ channel antagonists, nerves were stimulated for 2 min at 2 Hz. Next, after a 30-min interval, nerves were stimulated again for 2 min at 4 Hz. Phosphate buffer (pH 3.5, 4 μl) was transferred into each sample tube before dialysate sampling. Two dialysate samples were continuously collected per nerve stimulation: one before and one during stimulation. One sampling period was 2 min (1 sample volume = 20 μl). Half of the dialysate sample was used for the measurement of ACh, and the remaining half for the measurement of NE and Epi.

### 2.5. Protocol 2

We investigated the effects of $K_{Ca}$ channel antagonists on the nerve stimulation-induced catecholamine release in the absence of neostigmine. Like in protocol 1, the left splanchnic nerves were stimulated before and 30 min after administration of iberiotoxin ($n = 7$), paxilline ($n = 7$), apamin ($n = 7$) or scyllatoxin ($n = 7$) and two dialysate samples were collected per nerve stimulation. The dialysate sample was used for the measurement of NE and Epi.

### 2.6. Protocol 3

We investigated the effects of $K_{Ca}$ channel antagonists on exogenous ACh-induced catecholamine release. The dialysis probe was perfused with Ringer's solution. ACh (1 mM) was locally administered to the adrenal medulla through the dialysis probe for 1 min. After first administration of ACh, local administration of iberiotoxin (1 μM, $n = 7$), paxilline (100 μM, $n = 7$), apamin (10 μM, $n = 7$) or scyllatoxin (2 μM, $n = 7$) was started. Thirty minutes after local administration of $K_{Ca}$ channel antagonists, ACh (1 mM) was locally administered again for 1 min. Phosphate buffer (pH 3.5, 2 μl) was transferred into each sample tube before dialysate sampling. Two dialysate samples were continuously collected per local administration of ACh: one before and one during administration. One sampling period was 1 min (1 sample volume = 10 μl). The dialysate sample was used for the measurement of NE and Epi.

### 2.7. Drugs

Drugs were mixed fresh for each experiment. Neostigmine methylsulfate (Shionogi, Japan), iberiotoxin (Peptide Institute, Japan), apamin (Peptide Institute) and scyllatoxin (Peptide Institute) were dissolved and diluted in Ringer's solution. Paxilline (Sigma Chemical, USA) was dissolved in DMSO and diluted in Ringer's solution. The final concentration of DMSO in the working solution was 0.5% (v/v).

### 2.8. Statistical methods

To examine the effects of nerve stimulation, local administration of ACh, and $K_{Ca}$ channel antagonists, we analyzed heart rate and mean arterial pressure, basal dialysate NE and Epi content, and dialysate ACh, NE and Epi responses, by using one-way analysis of variance with repeated measures. When statistical significance was detected, the Newman–Keuls test was applied (Winer, 1971). Statistical significance was defined as $P < 0.05$. Values are presented as means ± SE.

## 3. Results

### 3.1. Changes in heart rate and mean arterial pressure

Local administration of neostigmine, $K_{Ca}$ channel antagonists, and ACh through the dialysis probe did not change basal heart rate or mean arterial pressure. In protocol 1, nerve stimulation increased mean arterial pressure from 113 ± 3 mmHg in control to 131 ± 2 mmHg at 2 Hz ($n = 28$, $P < 0.05$) and 132 ± 2 mmHg at 4 Hz ($n = 28$, $P < 0.05$), and decreased heart rate from 436 ± 4 beats/min in control to 424 ± 4 beats/min at 2 Hz ($n = 28$, $P < 0.05$) and 420 ± 4 beats/min at 4 Hz ($n = 28$, $P < 0.05$). In protocol 2, nerve stimulation increased mean arterial pressure from 115 ± 4 mmHg in control to 129 ± 3 mmHg at 2 Hz ($n = 28$, $P < 0.05$) and 131 ± 3 mmHg at 4 Hz ($n = 28$, $P < 0.05$), and decreased heart rate from 423 ± 3 beats/min in control to 410 ± 4 beats/min at 2 Hz ($n = 28$, $P < 0.05$) and 404 ± 3 beats/min at 4 Hz ($n = 28$, $P < 0.05$). Heart rate and mean arterial pressure recovered to basal levels after nerve stimulation. After administration of $K_{Ca}$ channel antagonists, nerve stimulation evoked the same responses of heart rate and mean arterial pressure.

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**Table 1**
Basal NE and Epi release before and after local administration of $K_{Ca}$ channel antagonists.

| | NE (nM) | Epi (nM) |
|---|---|---|
| Iberiotoxin ($n=21$) | | |
| Before administration | 4.8 ± 0.3 | 16.7 ± 1.0 |
| After administration | 5.0 ± 0.4 | 21.7 ± 1.6* |
| Paxilline ($n=21$) | | |
| Before administration | 4.7 ± 0.3 | 15.7 ± 1.1 |
| After administration | 4.8 ± 0.4 | 22.0 ± 1.9* |
| Apamin ($n=21$) | | |
| Before administration | 4.9 ± 0.4 | 17.1 ± 1.1 |
| After administration | 4.6 ± 0.5 | 21.2 ± 1.6* |
| Scyllatoxin ($n=21$) | | |
| Before administration | 4.9 ± 0.3 | 15.3 ± 0.7 |
| After administration | 5.1 ± 0.4 | 20.6 ± 0.9* |

Values are means ± SE. *n*, no. of rats; NE, norepinephrine; Epi, epinephrine. *$P < 0.05$ vs. values before administration.

### 3.2. Basal ACh and catecholamine release

ACh could not be detected in dialysate before nerve stimulation even in the presence of neostigmine. In contrast, substantial amounts of NE and Epi were observed in dialysate before nerve stimulation or ACh administration. Local administration of neostigmine did not influence this basal catecholamine release. BK channel antagonists, iberiotoxin and paxilline did not change basal NE release but increased basal Epi release. Similarly, the SK channel antagonists, apamin and scyllatoxin did not change basal NE release, but increased basal Epi release (Table 1).

ACh was detected in dialysate only during nerve stimulation in the presence of neostigmine. Thus, we expressed this detected dialysate ACh concentration as an index of ACh release induced by nerve stimulation. In contrast, we subtracted basal dialysate NE and Epi content before nerve stimulation or ACh administration from those during stimulation or ACh administration, and expressed these subtracted values as indices of NE and Epi release induced by nerve stimulation or ACh administration.

### 3.3. Effects of $K_{Ca}$ channel antagonists on the nerve stimulation-induced ACh and catecholamine release in the presence of neostigmine

Iberiotoxin enhanced the nerve stimulation-induced release of presynaptic ACh and postsynaptic catecholamine (Fig. 1A). ACh release increased from 4.5 ± 0.8 to 7.4 ± 0.7 nM at 2 Hz and from 9.4 ± 1.0 to 14.0 ± 1.0 nM at 4 Hz. NE release increased from 7 ± 0.5 to 32 ± 3 nM at 2 Hz and from 27 ± 3 to 74 ± 9 nM at 4 Hz. Epi release increased from 39 ± 5 to 78 ± 5 nM at 2 Hz, and from 105 ± 8 to 193 ± 15 nM at 4 Hz. Similarly, paxilline enhanced the nerve stimulation-induced release of ACh and catecholamine (Fig. 1B). ACh release increased from 4.1 ± 0.4 to 5.9 ± 0.5 nM at 2 Hz and from 9.4 ± 0.7 to 13.7 ± 0.9 nM at 4 Hz. NE release increased from 11 ± 2 to 26 ± 4 nM at 2 Hz, from 31 ± 5 to 58 ± 8 nM at 4 Hz. Epi release increased from 41 ± 7 to 77 ± 14 nM at 2 Hz and from 108 ± 14 to 195 ± 17 nM at 4 Hz.

Apamin had no effect on the nerve stimulation-induced release of ACh and NE, but enhanced the nerve stimulation-induced Epi release (Fig. 2A). Epi release increased from 45 ± 3 to 59 ± 4 nM at 2 Hz and from 108 ± 7 to 139 ± 17 nM at 4 Hz. Scyllatoxin had no effect on the nerve stimulation-induced release of ACh and NE either, but enhanced the nerve stimulation-induced Epi release (Fig. 2B). Epi release increased from 37 ± 4 to 50 ± 3 nM at 2 Hz and from 122 ± 5 to 152 ± 12 nM at 4 Hz.

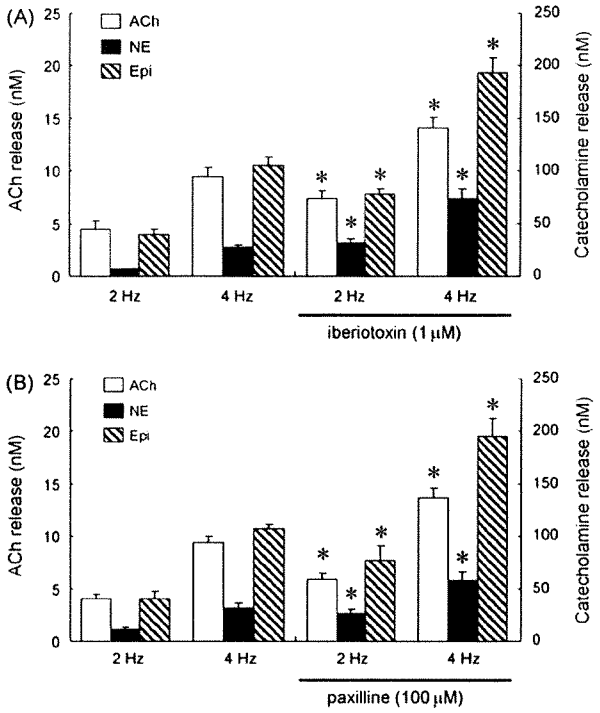


**Fig. 1.** Effects of BK channel antagonists on the nerve stimulation-induced release of acetylcholine (ACh), norepinephrine (NE) and epinephrine (Epi) in the presence of neostigmine (10 μM): iberiotoxin (A) and paxilline (B) enhanced the release of ACh, NE and Epi at 2 and 4 Hz. Values are means ± SE from seven rats. *$P < 0.05$ vs. ACh, NE or Epi release at the same frequency as before administration of BK channel antagonists.

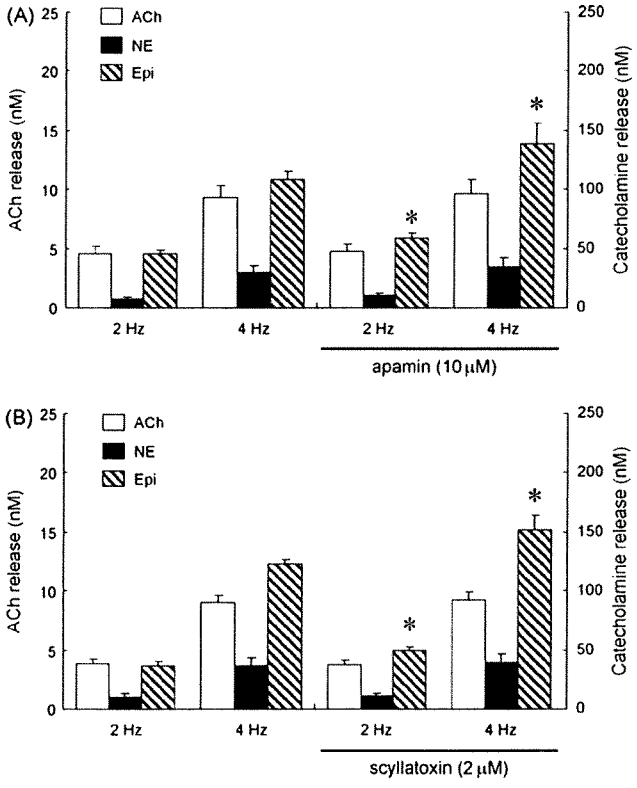


**Fig. 2.** Effects of SK channel antagonists on the nerve stimulation-induced release of ACh, NE and Epi in the presence of neostigmine (10 μM): apamin (A) and scyllatoxin (B) had no effect on the release of ACh or NE, but enhanced the Epi release at 2 and 4 Hz. Values are means ± SE from seven rats. *$P < 0.05$ vs. ACh, NE or Epi release at the same frequency as before administration of SK channel antagonists.

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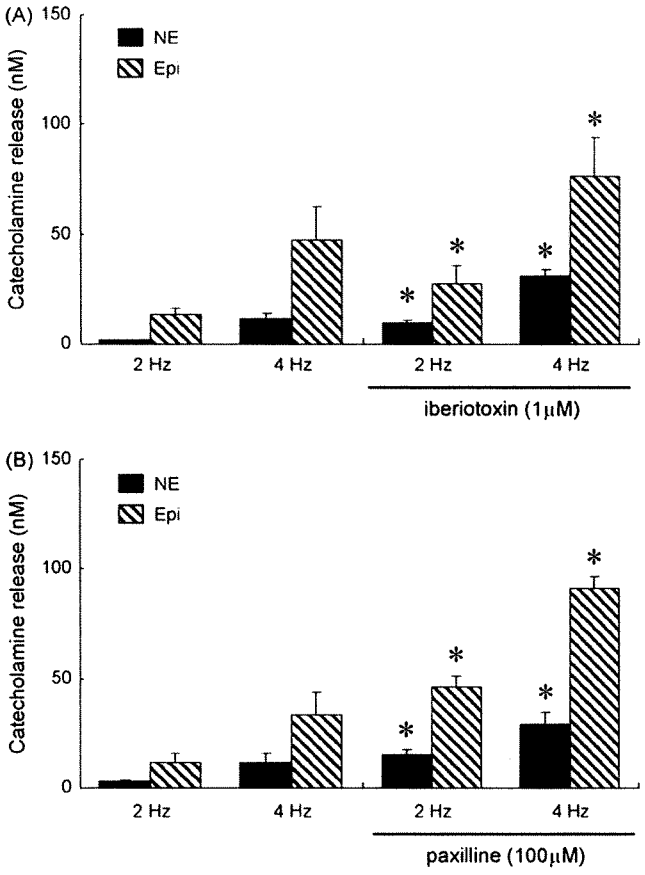


**Fig. 3.** Effects of BK channel antagonists on the nerve stimulation-induced release of NE and Epi in the absence of neostigmine: iberiotoxin (A) and paxilline (B) enhanced the release of NE and Epi at 2 and 4 Hz. Values are means ± SE from seven rats. *$P < 0.05$ vs. NE or Epi release at the same frequency as before administration of BK channel antagonists.

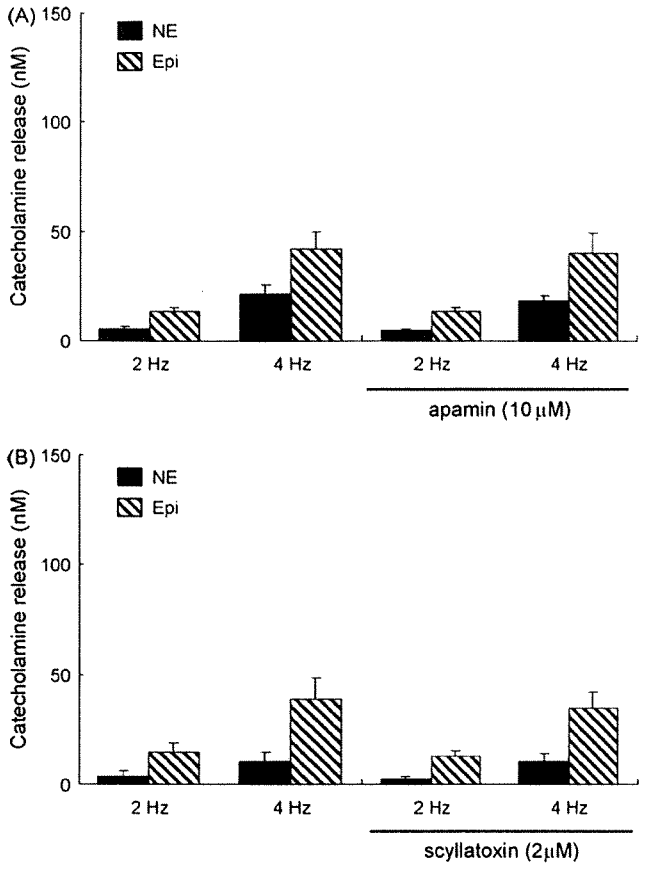


**Fig. 4.** Effects of SK channel antagonists on the nerve stimulation-induced release of NE and Epi in the absence of neostigmine: apamin (A) and scyllatoxin (B) had no effect on the release of NE or Epi at 2 or 4 Hz. Values are means ± SE from seven rats.

### *3.4. Effects of $K_{Ca}$ channel antagonists on the nerve stimulation-induced catecholamine release in the absence of neostigmine*

Iberiotoxin enhanced the nerve stimulation-induced catecholamine release at both 2 and 4 Hz (Fig. 3A). NE release increased from 2 ± 0.3 to 10 ± 2 nM at 2 Hz and from 12 ± 3 to 31 ± 3 nM at 4 Hz. Epi release increased from 13 ± 3 to 27 ± 9 nM at 2 Hz and from 47 ± 15 to 76 ± 18 nM at 4 Hz. Similarly, paxilline enhanced the nerve stimulation-induced catecholamine release (Fig. 3B). NE release increased from 3 ± 0.6 to 15 ± 2 nM at 2 Hz and from 12 ± 4 to 29 ± 5 nM at 4 Hz. Epi release increased from 12 ± 4 to 46 ± 5 nM at 2 Hz and from 34 ± 10 to 91 ± 6 nM at 4 Hz. Apamin and scyllatoxin had no effect on the nerve stimulation-induced catecholamine release at 2 or 4 Hz (Fig. 4A and B).

### *3.5. Effects of $K_{Ca}$ channel antagonists on the exogenous ACh-induced catecholamine release*

Iberiotoxin had no effect on the exogenous ACh-induced NE release, but enhanced the exogenous ACh-induced Epi release. Epi release increased from 108 ± 11 to 127 ± 10 nM (Fig. 5A). Similarly, paxilline had no effect on the exogenous ACh-induced NE release but enhanced the exogenous ACh-induced Epi release. Epi release increased from 93 ± 5 to 137 ± 13 nM (Fig. 5B). Apamin enhanced the exogenous ACh-induced catecholamine release (Fig. 6A). NE release increased from 37 ± 4 to 49 ± 4 nM and Epi release from 103 ± 8 to 122 ± 9 nM. Similarly scyllatoxin enhanced the exogenous ACh-induced catecholamine release (Fig. 6B). NE release increased from 32 ± 3 to 47 ± 3 nM and Epi release from 108 ± 6 to 140 ± 11 nM.

## 4. Discussion

### *4.1. Roles of $K_{Ca}$ channels on splanchnic nerve endings in presynaptic ACh release*

We found that, in the *in vivo* adrenal medulla, both iberiotoxin and paxilline enhanced the nerve stimulation-induced release of presynaptic ACh at 2 and 4 Hz by ~50% in the presence of neostigmine (Fig. 1). BK channels currents have been confirmed on cholinergic nerve endings including motor nerves in the neuromuscular junction (Flink and Atchison, 2003), presynaptic nerves in the chick ciliary ganglion (Sun et al., 1999) and tracheal parasympathetic nerves (Zhang et al., 1998). Activation of the $K_{Ca}$ conductance is considered to limit $Ca^{2+}$ entry through voltage-dependent $Ca^{2+}$channels, and subsequently reduce transmitter release (Meir et al., 1999). Our results strongly suggest that BK channels are present on the splanchnic nerve endings and involved in the control of ACh release. In the perfused cat adrenal gland, charybdotoxin, a BK channel antagonist, enhanced catecholamine release when transmural electrical stimulation was applied at low external $Ca^{2+}$ concentrations, but not when exogenous ACh was administered (Montiel et al., 1995). In the perfused rat adrenal gland, charybdotoxin enhanced the release of Epi and NE induced by transmural electrical stimulation, but not the release induced by administration of ACh (Nagayama et al., 2000b). These indirect studies suggested that BK channels may be involved in the control

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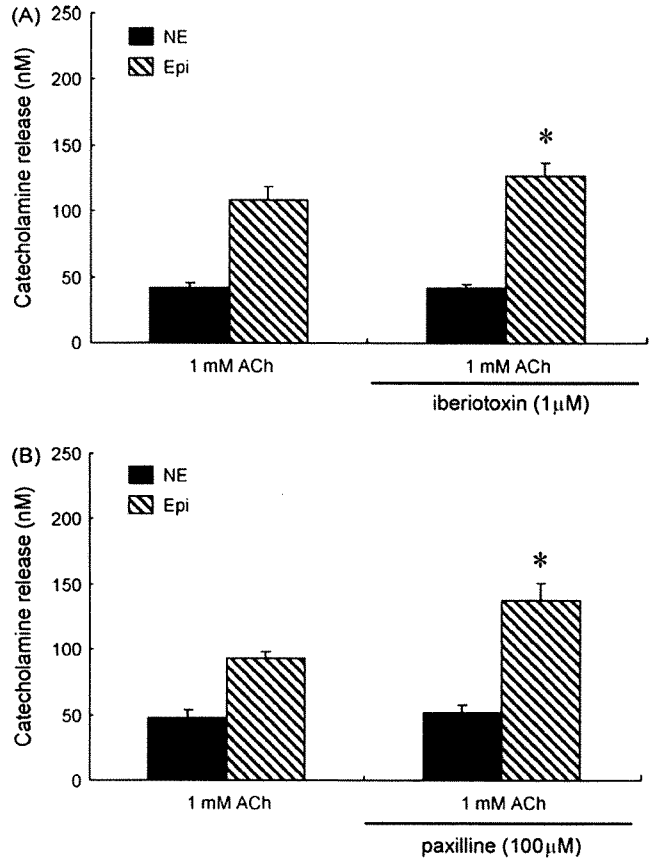


**Fig. 5.** Effects of BK channel antagonists on the exogenous ACh-induced release of NE and Epi: iberiotoxin (A) and paxilline (B) had no effect on NE release, but enhanced Epi release. Values are means ± SE from seven rats. $^*P < 0.05$ vs. NE or Epi release before administration of BK channel antagonists.

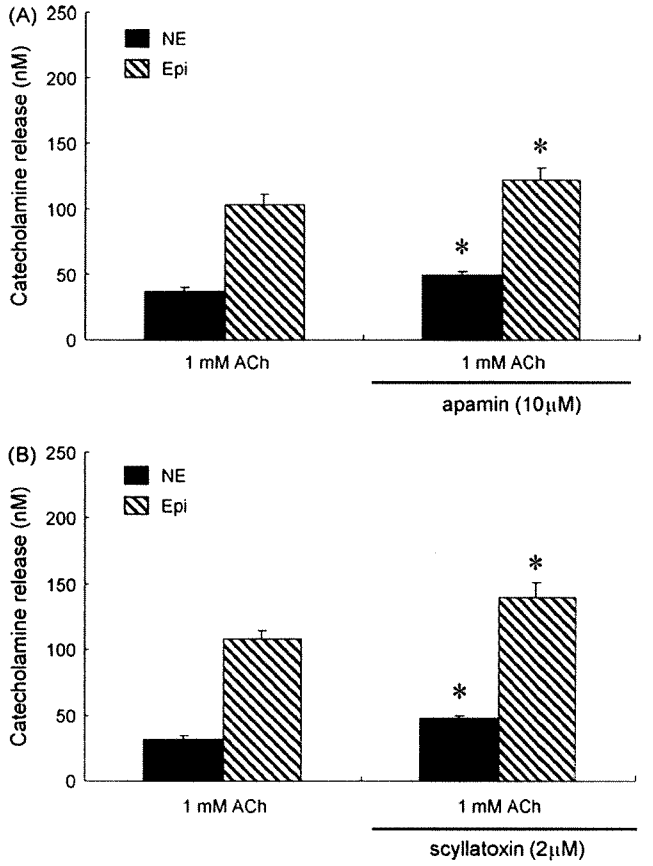


**Fig. 6.** Effects of SK channel antagonists on the exogenous ACh-induced release of NE and Epi: apamin (A) and scyllatoxin (B) enhanced the release of NE and Epi. Values are means ± SE from seven rats. $^*P < 0.05$ vs. NE or Epi release before administration of SK channel antagonists.

of catecholamine release at the presynaptic site. But there has been no direct study investigating the effect of BK channel antagonists on ACh release from splanchnic nerve endings. This is the first direct study to demonstrate that BK channels are involved in the control of ACh release from splanchnic nerve endings. In the *in vivo* adrenal medulla, we observed a substantial enhancement of ACh release by BK channel antagonists at a frequency of 2 Hz with this degree of enhancement being similar to that at a frequency of 4 Hz (Fig. 1). BK channels on splanchnic nerve endings could be functional under physiological conditions. In our previous study, the nerve stimulation-induced catecholamine release was in large part cholinergic in the presence or absence of neostigmine (Akiyama et al., 2003). Thus, BK channels play an inhibitory role in the physiological catecholamine release from adrenal medulla by limiting presynaptic ACh release.

In contrast to BK channel antagonists, apamin and scyllatoxin had no effect on the nerve stimulation-induced ACh release at 2 or 4 Hz (Fig. 2). In perfused cat adrenal glands preloaded with [$^3$H]-choline, apamin did not modify the efflux of [$^3$H]-labeled compound evoked by transmural electrical stimulation (Montiel et al., 1995). SK channels seem to be absent on splanchnic nerve endings or play a minor role in the ACh release from splanchnic nerve endings.

### 4.2. Role of $K_{Ca}$ channels on chromaffin cells in catecholamine release

Iberiotoxin and paxilline had no effect on the exogenous ACh-induced NE release, but enhanced exogenous ACh-induced Epi release (Fig. 5). Adrenal chromaffin cells are divided into two populations: NE- and Epi-storing cells (Coupland, 1984). While BK channels seem to be absent on NE-storing cells or play a minor role in the nerve stimulation-induced NE release, BK channels seem to be present on Epi-storing cells. It has been reported that BK channels present at rat chromaffin cells are activated by $Ca^{2+}$ influx and contribute to the rapid termination of action potentials (Prakriya and Lingle, 1999), while iberiotoxin augments the nicotinic receptor-mediated catecholamine secretion from bovine adrenal chromaffin cells (Wada et al., 1995). The enhancement by BK channel antagonists of nerve stimulation-induced Epi release may be in part ascribed to their direct effects on Epi-storing cells. BK channels on Epi-storing cells may be involved in the control of nerve stimulation-induced Epi release. In perfused rat and cat adrenal glands, charybdotoxin, a BK channel antagonist, does not affect the exogenous ACh-induced catecholamine release (Montiel et al., 1995; Nagayama et al., 2000b). Our results of Epi release were inconsistent with these studies, possibly due to differences in the BK channel antagonists used and/or in methodology because charybdotoxin is pharmacologically less selective than iberiotoxin for BK channels (Garcia et al., 1991).

Both apamin and scyllatoxin enhanced the nerve stimulation-induced Epi release in the presence of neostigmine without changes in ACh release (Fig. 2), and the exogenous ACh-induced release of NE and Epi (Fig. 6). These results suggest that SK channels are present on both NE- and Epi-storing cells and that such enhancement is due to the direct effects of SK channel antagonists on chromaffin cells. Neither apamin nor scyllatoxin, however, had any effect on the nerve stimulation-induced NE release in the presence or absence of neostigmine, and the nerve

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stimulation-induced Epi release in the absence of neostigmine (Figs. 2 and 4). SK channels on chromaffin cells may play a minor role in the nerve stimulation-induced catecholamine release. It has been reported that SK channels on chromaffin cells are activated by muscarinic receptor stimulation (Nagayama et al., 2000a; Uceda et al., 1992). In our previous study of the same preparation, we demonstrated that muscarinic receptors are present on NE- and Epi-storing cells but play a minor role in the nerve stimulation-induced release of NE and Epi, and that cholinesterase inhibitor elicited muscarinic receptor-mediated Epi release when splanchnic nerve was stimulated (Akiyama et al., 2003). Therefore, SK channels on NE- and Epi-storing cells play an important role in the catecholamine release induced by activation of muscarinic or non-cholinergic receptors including PACAP receptor (Fukushima et al., 2002).

In the perfused rat adrenal gland, apamin enhanced NE release induced by transmural electrical stimulation and a nicotinic receptor agonist (Nagayama et al., 2000b). Therefore, SK channels on NE-storing cells could be activated by nicotinic as well as muscarinic receptors. But, our results of NE release induced by nerve stimulation were inconsistent with this study. In anesthetized dogs, scyllatoxin enhanced catecholamine release induced by a nicotinic receptor agonist but did not affect catecholamine release induced by splanchnic nerve stimulation (Nagayama et al., 1998). Thus, this inconsistency may be due to the difference in the method of nerve stimulation and SK channels on NE-storing cells may be activated by nicotinic receptors in the extrasynaptic region.

### 4.3. Roles of $K_{Ca}$ channels in basal NE and Epi release

In the present study, substantial basal release of NE and Epi was observed in dialysate before nerve stimulation or ACh administration. Both BK and SK channel antagonists enhanced the basal Epi release but not the basal NE release. In our preparation, splanchnic nerves had been transected before control sampling and basal catecholamine release was not enhanced by a cholinesterase inhibitor, neostigmine. Furthermore, using the same preparation we demonstrated that basal catecholamine release is resistant to not only cholinergic antagonists, but also N-, P/Q-, and L-type $Ca^{2+}$ channel antagonists (Akiyama et al., 2004b). Basal catecholamine release seems to be non-cholinergic and independent of $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels. $Ca^{2+}$ release from intracellular $Ca^{2+}$ stores may be involved in this basal catecholamine release. It has been suggested in chromaffin cells that $K_{Ca}$ channels on the cell surface are activated by $Ca^{2+}$ release from intracellular $Ca^{2+}$ stores (Ohta et al., 1998). On Epi-storing cells, BK and SK channels may play a role in the Epi release induced by $Ca^{2+}$ release from intracellular $Ca^{2+}$ stores.

### 4.4. Methodological considerations

Because previous results suggested that distribution across the dialysis membrane is required (Akiyama et al., 2003, 2004a), we used the $K_{Ca}$ channel antagonists at a concentration 10 times higher than that required for complete channel blockade in experimental settings *in vitro*. Then, we tested two different types of selective BK and SK channel antagonists in the present study because higher concentrations of $K_{Ca}$ channel antagonists might induce other pharmacological effects.

Cholinesterase inhibitor was necessary to monitor endogenous ACh even during the splanchnic nerve stimulation because released ACh is rapidly degraded by acetylcholinesterase before reaching the dialysis fiber. Then, we examined the effects of $K_{Ca}$ channel antagonists in the presence or absence of neostigmine because neostigmine may influence the effects of $K_{Ca}$ channel antagonists. Local administration of neostigmine enhanced the nerve stimulation-induced catecholamine release to about 2-fold before and after administration of $K_{Ca}$ channel antagonists (Figs. 1 and 3). This enhancement could be due to the elevation of synaptic ACh levels by inhibition of acetylcholinesterase.

## 5. Conclusion

We applied dialysis technique to the adrenal medulla of anesthetized rats and investigated the effects of $K_{Ca}$ channel antagonists on the presynaptic ACh release from splanchnic nerve endings and the postsynaptic catecholamine release from chromaffin cells. BK channels on presynaptic splanchnic nerve endings play an inhibitory role in the physiological catecholamine release from adrenal medulla by limiting presynaptic ACh release while SK channels do not. BK channels on Epi-storing cells may play an inhibitory role in the nerve stimulation-induced Epi release. SK channels are present on NE- and Epi-storing cells, but play a minor role in the nerve stimulation-induced catecholamine release.

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## References

Akiyama, T., Yamazaki, T., Mori, H., Sunagawa, K., 2003. Inhibition of cholinesterase elicits muscarinic receptor-mediated synaptic transmission in the rat adrenal medulla. Auton. Neurosci. 107, 65–73.
Akiyama, T., Yamazaki, T., Mori, H., Sunagawa, K., 2004a. Simultaneous monitoring of acetylcholine and catecholamine release in the in vivo rat adrenal medulla. Neurochem. Int. 44, 497–503.
Akiyama, T., Yamazaki, T., Mori, H., Sunagawa, K., 2004b. Effects of $Ca^{2+}$ channel antagonists on acetylcholine and catecholamine releases in the in vivo rat adrenal medulla. Am. J. Physiol. 287, R161–R166.
Auguste, P., Hugues, M., Gravé, B., Gesquière, J.C., Maes, P., Tartar, A., Romey, G., Schweitz, H., Lazdunski, M., 1990. Leiurotoxin I (scyllatoxin), a peptide ligand for $Ca^{2+}$-activated $K^+$ channels. J. Biol. Chem. 265, 4753–4759.
Blatz, A., Magleby, K.L., 1986. Single apamin-blocked Ca-activated $K^+$ channels of small conductance in cultured rat skeletal muscle. Nature 323, 718–720.
Blatz, A., Magleby, K.L., 1987. Calcium-activated potassium channels. Trends. Neurosci. 10, 463–467.
Candia, S., Garcia, M.L., Latorre, R., 1992. Mode of action of iberiotoxin, a potent blocker of the large conductance $Ca^{2+}$-activated $K^+$ channel. Biophys. J. 63, 583–590.
Coupland, R.E., 1965. The Natural History of the Chromaffin Cell. Longmans, London.
Coupland, R.E., 1984. Ultrastructural features of the mammalian adrenal medulla. In: Motta, P.M. (Ed.), Ultrastructure of Endocrine Cells and Tissues. Nijhoff, Boston, MA, pp. 168–179.
Flink, M.T., Atchison, W.D., 2003. Iberiotoxin-induced block of $Ca^{2+}$-activated $K^+$ channels induces dihydropyridine sensitivity of ACh release from mammalian motor nerve terminals. J. Pharmacol. Exp. Ther. 305, 646–652.
Fukushima, Y., Nagayama, T., Hikichi, H., Mizukami, K., Yoshida, M., Suzuki-Kusaba, M., Hisa, H., Kimura, T., Satoh, S., 2002. Role of $K^+$ channels in the PACAP-induced catecholamine secretion from the rat adrenal gland. Eur. J. Pharmacol. 437, 69–72.
García, A.G., García-De-Diego, A.M., Gandía, L., Borges, R., García-Sancho, J., 2006. Calcium signaling and exocytosis in adrenal chromaffin cells. Physiol. Rev. 86, 1093–1131.
Garcia, M.L., Galvez, A., Garcia-Calvo, M., King, V.F., Vazquez, J., Kaczorowski, G.J., 1991. Use of toxins to study potassium channels. J. Bioenerg. Biomembr. 23, 615–646.
Kanus, H.G., McManus, O.B., Lee, S.H., Schmalhofer, W.A., Galcia-Calvo, M., Helms, L.M., Sanchez, M., Giangiacomo, K., Reuben, J.P., Smith, A.B., 1994. Tremorgenic indole alkaloids potently inhibit smooth muscle high-conductance calcium-activated potassium channels. Biochemistry 33, 5819–5828.
Meir, A., Ginsburg, S., Butkevich, A., Kachalsky, S.G., Kaiserman, I., Ahdut, R., Demirgoren, S., Rahamimoff, R., 1999. Ion channels in presynaptic nerve terminals and control of transmitter release. Physiol. Rev. 79, 1019–1088.
Montiel, C., López, M.G., Sánchez-García, P., Maroto, R., Zapater, P., García, A.G., 1995. Contribution of SK and BK channels in the control of catecholamine release by electrical stimulation of the cat adrenal gland. J. Physiol. 486, 427–437.

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Nagayama, T., Fukushima, Y., Hikichi, H., Yoshida, M., Suzuki-Kusaba, M., Hisa, H., Kimura, T., Satoh, S., 2000a. Interaction of $SK_{Ca}$ channels and L-type $Ca^{2+}$ channels in catecholamine secretion in the rat adrenal gland. Am. J. Physiol. 279, R1731–R1736.

Nagayama, T., Fukushima, Y., Yoshida, M., Suzuki-Kusaba, M., Hisa, H., Kimura, T., Satoh, S., 2000b. Role of potassium channels in catecholamine secretion in the rat adrenal gland. Am. J. Physiol. 279, R448–R454.

Nagayama, T., Masada, K., Yoshida, M., Suzuki-Kusaba, M., Hisa, H., Kimura, T., Satoh, S., 1998. Role of $K^+$ channels in adrenal catecholamine secretion in anesthetized dogs. Am. J. Physiol. 274, R1125–R1130.

Ohta, T., Ito, S., Nakazato, Y., 1998. $Ca^{2+}$-dependent $K^+$ currents induced by muscarinic receptor activation in guinea pig adrenal chromaffin cells. J. Neurochem. 70, 1280–1288.

Prakriya, M., Lingle, C.J., 1999. BK channel activation by brief deporalizations requires $Ca^{2+}$ influx through L- and Q-type $Ca^{2+}$ channels in rat chromaffin cells. J. Neurophysiol. 81, 2267–2278.

Sun, X.P., Schlichter, L.C., Stanley, E.F., 1999. Single-channel properties of BK-type calcium-activated potassium channels at a cholinergic presynaptic nerve terminal. J. Physiol. 518, 639–651.

Uceda, G., Artalejo, A.R., López, M.G., Abad, F., Neher, E., García, A.G., 1992. $Ca^{2+}$-activated $K^+$ channels modulate muscarinic secretion in cat chromaffin cells. J. Physiol. 454, 213–230.

Wada, A., Urabe, M., Yuhi, T., Yamamoto, R., Yanagita, T., Niina, H., Kobayashi, H., 1995. Large- and small-conductance $Ca^{2+}$-activated $K^+$ channels: their role in the nicotinic receptor-mediated catecholamine secretion in bovine adrenal medulla. Naunyn Schmiedeberg's Arch. Pharmacol. 352, 545–549.

Winer, B.J., 1971. Statistical Principles in Experimental Design, 2nd ed. McGraw-Hill, New York.

Zhang, X.Y., Zhu, F.X., Robinson, N.E., 1998. Role of cAMP and neuronal $K^+$ channels on $\alpha_2$-AR-induced inhibition of ACh release in equine trachea. Am. J. Physiol. 274, L827–L832.

Please cite this article in press as: Akiyama, T., et al., Role of $Ca^{2+}$-activated $K^+$ channels in catecholamine release from *in vivo* rat adrenal medulla. Neurochem. Int. (2009), doi:10.1016/j.neuint.2009.10.010

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# Slow head-up tilt causes lower activation of muscle sympathetic nerve activity: loading speed dependence of orthostatic sympathetic activation in humans

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**Kamiya A, Kawada T, Shimizu S, Iwase S, Sugimachi M, Mano T.** Slow head-up tilt causes lower activation of muscle sympathetic nerve activity: loading speed dependence of orthostatic sympathetic activation in humans. *Am J Physiol Heart Circ Physiol* 297: H53–H58, 2009. First published May 15, 2009; doi:10.1152/ajpheart.00260.2009.—Many earlier human studies have reported that increasing the tilt angle of head-up tilt (HUT) results in greater muscle sympathetic nerve activity (MSNA) response, indicating the amplitude dependence of sympathetic activation in response to orthostatic stress. However, little is known about whether and how the inclining speed of HUT influences the MSNA response to HUT, independent of the magnitude of HUT. Twelve healthy subjects participated in passive 30° HUT tests at inclining speeds of 1° (control), 0.1° (slow), and 0.0167° (very slow) per second. We recorded MSNA (tibial nerve) by microneurography and assessed nonstationary time-dependent changes of R-R interval variability using a complex demodulation technique. MSNA averaged over every 10° tilt angle increased during inclination from 0° to 30°, with smaller increases in the slow and very slow tests than in the control test. Although a 3-min MSNA overshoot after reaching 30° HUT was observed in the control test, no overshoot was detected in the slow and very slow tests. In contrast with MSNA, increases in heart rate during the inclination and after reaching 30° were similar in these tests, probably because when compared with the control test, greater increases in plasma epinephrine counteracted smaller autonomic responses in the very slow test. These results indicate that slower HUT results in lower activation of MSNA, suggesting that HUT-induced sympathetic activation depends partially on the speed of inclination during HUT in humans.

autonomic nervous system; baroreflex; heart rate variability; microneurography

HUMANS HAVE BEEN SUBJECTED to ceaseless orthostatic stresses since they first evolved and assume an orthostatic posture most of their lives. Thus the maintenance of arterial pressure (AP) under orthostatic stress against gravity-driven fluid shift is of great importance. During standing, gravitational fluid shift toward the lower part of the body (i.e., abdominal vascular bed, lower limbs) would cause severe orthostatic hypotension if not counteracted by compensatory mechanisms (27). Orthostatic sympathetic activation mediated by arterial baroreflex has been considered to be the major compensatory mechanism (2, 26, 27) since denervation of baroreceptor afferents causes profound postural hypotension (30). Therefore, many earlier human studies have recorded muscle sympathetic nerve activity (MSNA) by microneurographic technique and investigated MSNA response to various orthostatic stresses such as head-up tilt (HUT) and lower body negative pressure (LBNP) (1, 5, 24). One of the important findings is that stronger orthostatic stress results in greater MSNA response during incremental HUT (3, 13, 14, 28) and LBNP (17), indicating the amplitude dependence of orthostatic MSNA activation. However, less attention has been paid to the effects of loading speed of orthostatic stress on orthostatic sympathetic activation in humans. Although earlier studies reported that rapid HUT causes dynamic and transient hemodynamic response (33, 34, 36), they did not investigate MSNA. Thus it remains unclear whether and how the inclining speed of HUT affects HUT-induced activation of MSNA (loading speed dependence of orthostatic MSNA activation), independent of the magnitude of HUT. This is an important clinical issue because the speed of upright tilting of each patient's bed would influence his/her autonomic nervous and hemodynamic conditions.

Orthostatic sympathetic activation is mainly mediated by arterial baroreflex control of MSNA, which exhibits high-pass filter dynamic transfer characteristics at least in anesthetized animals such as rabbits (15) and rats (29), indicating that more rapid change of AP results in greater response of MSNA to pressure change (15). Accordingly, we hypothesized that a lower speed of HUT results in less MSNA activation in humans. To test the hypothesis, we performed passive 30° HUT tests at three inclining speeds (1°, 0.1°, and 0.0167°/s) in 12 healthy volunteers. We compared the responses of MSNA measured by microneurography and hemodynamics during these tests.

## METHODS

### *Subjects*

The subjects were 12 healthy volunteers (10 males and 2 females) with a mean age (±SE) of 24 ± 5 yr, mean height of 164 ± 11 cm, and mean weight of 58 ± 9 kg. They were carefully screened by medical history, physical examination, complete blood count, blood chemistry analyses, electrocardiogram, and psychological testing. Candidates were excluded if they had evidence of cardiovascular or other disease, smoked tobacco products, took medications, or were obese (body mass index >30 kg/m$^2$). None of the subjects had experienced spontaneous syncope within the past 5 yr. All had a sedentary lifestyle and were not athletes. All subjects gave informed consent to participate in this study, which was approved by the

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