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1. 画像診断装置

2. 機能代行機器

3. 人 工 臓 器

(percutaneous cardiopulmonary support system, **PCPS**) が応用されるようになり，救命に威力を発揮している。大腿動脈と静脈に針を刺すだけで簡単に用いることができるので，市場としても増大しているところである。

救急に携わる医療従事者であれば，PCPS の基本知識くらいは今後知っておく必要があろう。

3.3 循環器系人工臓器

循環器系人工臓器は，主に血液を体に循環させるための循環器あるいは心臓血管系の働きを代行するための人工臓器である。循環器系人工臓器は大きく分けて，不整脈の治療に用いる心臓ペースメーカ，血液の流れを保ったり，流れを遮断したりする人工血管・人工弁，主に心臓手術中の血液循環とガス交換を保つための人工心肺，および比較的長期にわたって心臓のポンプ機能を代行するための人工心臓がある。

3.3.1 心臓ペースメーカ

〔1〕 使用目的

心臓のリズミカルな収縮は右心房の洞結節から発した電気刺激が心筋まで伝わることで保たれる。心臓内のこの特殊な電気的経路を**刺激伝導系** (excitation conducting system) という。刺激伝導系の異常は房室ブロック，徐脈性不整脈，頻脈性不整脈などの疾患をもたらす。**心臓ペースメーカ** (pacemaker，以下では単に**ペースメーカ**と略す) は，心臓に人工的な電気刺激を与えることによってこれらの治療を行うシステムである。能動的機能を有した人工物を体内に長期に埋め込んで使用する人工臓器として，他の人工臓器と比較し，ペースメーカは最も早く実用化され世界中で数多く使用されている[4]。

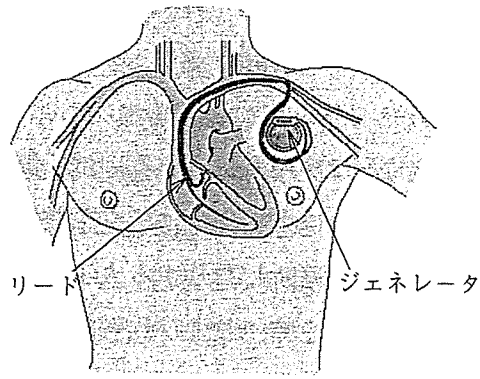
ペースメーカを用いた治療には，**体外式一時的ペーシング法** (temporary pacing) と**永久的ペーシング法** (permanent pacing) の 2 種類がある。

体外式一時的ペーシング法は，開心術後や心筋梗塞などによって一過性に生じた房室ブロックや洞房ブロックを消失させるために行うものであり，体外にある本体から伸びるカテーテル電極を上肢あるいは下肢の末梢血管から心臓内まで挿入してペーシングを行い，治療終了後は電極を抜き去るものである。

一方，洞不全症候群や房室ブロックによって刺激伝導の異常が続く患者は，心拍出量が減少し日常的動作も困難となる。これを治療するためには体内にペースメーカ植込永久的ペーシング法を施すことになる。

〔2〕 植込型ペースメーカの種類と機能

図3.1のように，植込型ペースメーカは，電気刺激を発生する本体であるジェネレータとそれを心腔内に伝える電極であるリードから構成されている。ジェネレータは左または右の鎖骨下の皮下に埋め込む。リードは鎖骨下静脈から挿入し心室または心房内にリードの先端が達するように留置する。



この型の場合，刺激すべき部位が心室と心房の両方(双極)，感知部位が心室と心房の両方，反応様式が心房同期・心室抑制であることを意味する。

図3.1　植込型ペースメーカ（DDD の例）

ペースメーカの種類は，例えばVVI，AAI，あるいはDDDのような複数の文字列で表すことになっている。初めの文字は刺激すべき部位が**心室**（ventricle，**V**）か，**心房**（atrium，**A**）か，あるいは**両方**（dual，**D**）であるかを表す。Dが付くものはリードが2本（双極）であり，付かないものは1本（単極）である。2番目の文字は心臓の電気的興奮を感知する部位が心室（V）か，心房（A）か，両方（D）か，あるいはその機能がないか（O）であるかを表す。3番目の文字は反応様式を表すものであり，**同期**（triggered，**T**），**抑制**（inhibited，**I**），**心房同期・心室抑制**（dual，**D**），**機能なし**（**O**）を表す。病態の違いによって反応様式を適切に選定する必要がある。

最近では，**レート応答**（rate-response）**機能**を有するペースメーカも市販されている。レート応答機能とは，体動などを感知すると自動的にその動きの度合いに応じて適切なペーシングレートに変わっていく機能である。

〔3〕 植込型ペースメーカの電池の寿命

現在のペースメーカの寿命は6年程度であるため，通常，最初の手術後6年目以降に電池交換の手術を行う必要がある。実際には，定期的なペースメーカの機能チェックが行われ，ペーシングレートや電池電圧の低下に基づいて交換時期が推定できる。

〔4〕 ペースメーカ植込み後の注意事項

ペースメーカを植え込んだ患者にとって日常生活における特別な制限はない。ただし，超短波治療器，低周波治療器，磁気共鳴装置（MRI），電気メスなど，体内に高エネルギーが直接達するものは使用できない。電気信号や電磁波による干渉を受けた場合，それを自己心拍と間違い電気刺激を出さなくなってしまうことがあるからである。また，国内で販売して

いる一般的な携帯電話については，ペースメーカから 22 cm 以上離して使用するというガイドライン[5]がある。PHS は携帯電話に比べて発生する電磁エネルギーが微弱であるため，影響は少ないと報告されている。

3.3.2 人工血管・人工弁

〔1〕 人 工 血 管

人工血管（artificial blood vessel）は，動脈瘤などの血管の異常拡張や閉塞性動脈硬化症などによる血管の狭窄・閉塞の治療に用いる。動脈瘤の場合には，その部分を切除して人工血管で置き換え，動脈閉塞の場合には，その部分を人工血管でバイパスし末梢への血液循環を再建する。現在の人工血管は，**図 3.2** のような大動脈などの太い血管を対象とする場合において臨床応用が進んでいるが，直径 5 mm 以下の細い動脈あるいは血流速の遅い静脈に対する実用化は遅れている。その理由は，後者の場合には人工血管の内側に血栓が蓄積しやすく，すぐに閉塞するからである[5]。

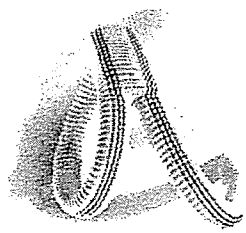


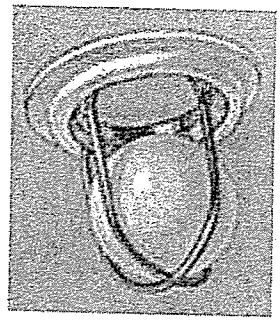
図 3.2 腹部大動脈用の人工血管の例
（日本ライフライン株式会社）

人工血管の材料と構造はこの血栓形成による閉塞の防止を考慮してつくられている。人工血管は，ダクロンやテフロンの繊維を管状に編んだり織り込んだものと，テフロンの管を伸展して無数の亀裂を生じさせてつくった微細な穴を多数有するものの 2 種類に大別できる。どちらの場合も，繊維の隙間や微細な穴の存在により，血栓形成を防止するのとは逆に，かえってこの部分に血栓が付きやすい。しかし，人工血管の内側の隙間や穴に付いた血栓は，その後フィブリンが主体のものに置き換わっていき，さらに繊維芽細胞や平滑筋細胞が現れ，偽内膜が形成されていくとともに，人工血管との吻合部では生体の血管から内皮細胞が成長し偽内膜を被うようになる。これらの作用により，それ以上の血栓の蓄積が防止される。

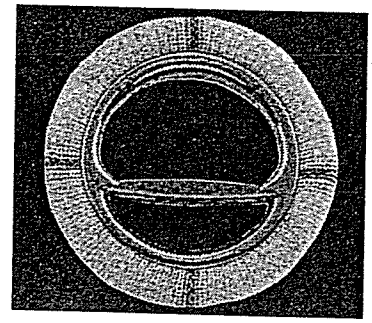
〔2〕 人 工 弁

心臓には四つの弁（左心房と左心室の間の僧帽弁，左心室と大動脈の間の大動脈弁，右心房と右心室の間の三尖弁，右心房と肺動脈の間の肺動脈弁）がある。心臓弁は心房から心室へまたは心室から動脈へ血液を一方向にだけ流す（逆流を防止する）働きをする。病変によりまたは先天的に心臓弁に狭窄や閉鎖不全のような異常がある場合に，これが**人工弁**（artificial valve）で置換される。

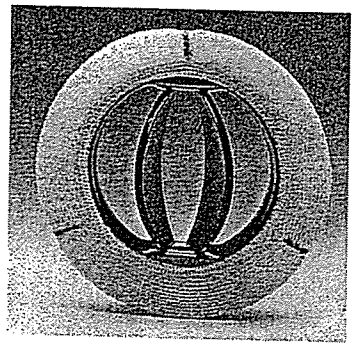
現在臨床で用いられている人工弁は機械弁と生体弁に大別される（図**3.3**）。機械弁は完全に人工物でつくられた弁であり，ボール弁，ディスク弁，傾斜型ディスク弁，二葉弁などの種類がある。このほか，人工心臓用に開発された外観がクラゲに似ているジェリーフィッシュ弁もある。生体弁はブタの大動脈弁やウシの心膜を加工してつくられるものと，ヒトの死体から採取した弁を凍結保存して使用するものがある。



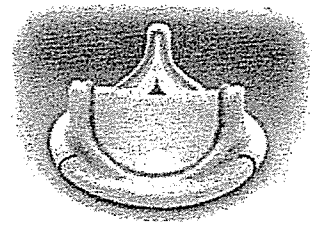
(a) ボール弁(機械弁)



(b) 傾斜型ディスク弁(機械弁)
((株)グッドマン)



(c) 二葉弁(機械弁)(エドワーズライフサイエンス(株))



(d) 生体弁(エドワーズライフサイエンス(株))

図3.3 人　工　弁

機械弁と生体弁のどちらを使用すべきかについては，人工弁がもつべき性質に関係し，それぞれの長所と短所を考慮する必要がある[6]。すなわち，人工弁がもつべき性質は，容易に開閉し，開放時の流れが中心流に近く，流路抵抗や逆流が少なく，耐久性に優れ（20年程度），抗血栓性があることである。現在の機械弁では，抗血栓性を除くこれらの性質を満たす二葉弁が主流となりつつあり，生体弁に比べて流路抵抗が少なく，耐久性に優れている。しかしその半面，抗血栓性が低いため，置換手術後の患者は生涯にわたり抗凝固療法を受けなければならない。これに対して，生体弁は抗血栓性が高いため，通常術後2，3箇月で抗凝固療法を終了でき，高齢者，妊娠希望女性，出血性の潰瘍や血液凝固傾向を有する患者にも用いることができる。しかし，生体弁は流路抵抗が大きく耐久性が低いばかりでなく，カルシウム代謝の高い慢性腎不全患者や小児では石灰沈着を起こす欠点をもつので注意が必要である。

3.3.3 人工心肺・体外循環装置

人工心肺（cardiopulmonary bypass）は体外循環装置の一つであり，心臓手術を，心臓を停止し無血化して行う必要があるときに用いられ，この装置で患者の心臓に代わって血液を循環させ，血液中の二酸化炭素を除去し，酸素を付加することを目的としている[6]。血液のガス交換（呼吸）機能だけを行う部分を人工肺と呼び，人工肺と血液ポンプ部分を合わせたシステムを人工心肺（**図3.4**）と呼ぶ。人工肺についてはすでに3.2.2項で述べた。人工心肺装置に付随する最大の問題点は血栓形成である。このため最近のシステムでは，人工心肺装置の血液と接する部分の多くを抗凝固剤のヘパリンでコーティングして抗血栓性を高めているものがある。

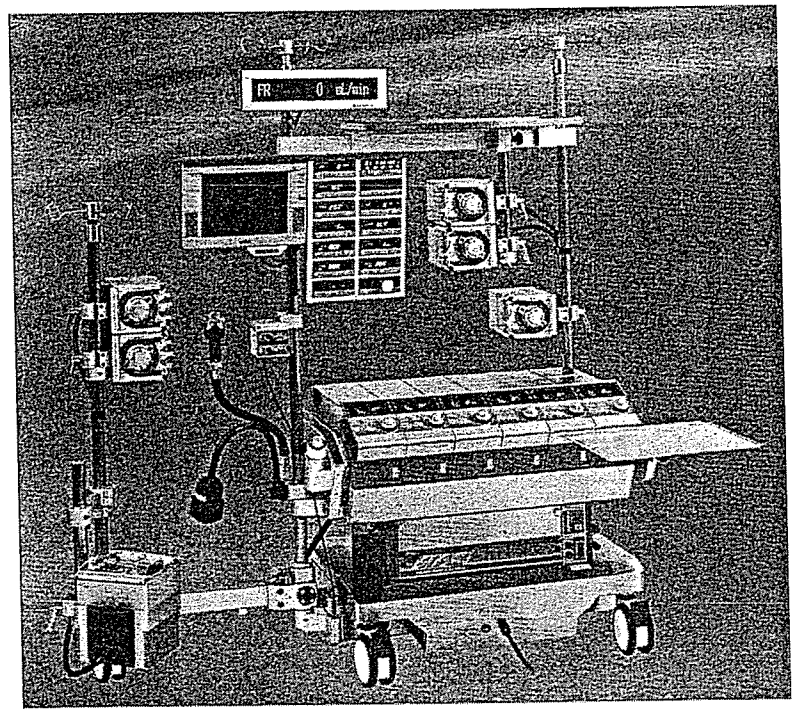


図3.4 人工心肺装置の例（泉工医科工業株式会社）

3.3.4 人 工 心 臓

〔1〕 使 用 目 的

人工心肺は長くて数時間の短期間の血液循環を維持するシステムであるが，**人工心臓**（artificial heart）は数週間から可能であれば数年の長期にわたって心臓のポンプ機能を代行することを目的とするものである[6), 8) ~ 10)]。人工心臓は，心筋梗塞などでダメージを受けた自分の心臓（自己心）は切除せずにそのポンプ機能を補助する**図3.5**のような**補助人工心臓**（ventricular assist device）と，拡張型心筋症などのように回復の見込みのない心臓を完全に切除して人工心臓に置きかえる**図3.6**のような**完全人工心臓**（total artificial heart，**完全置換型人工心臓**あるいは**全人工心臓**とも呼ぶ）に大別される。

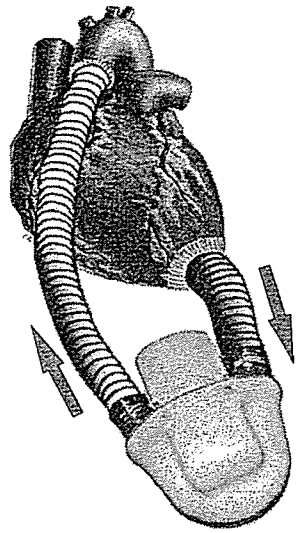
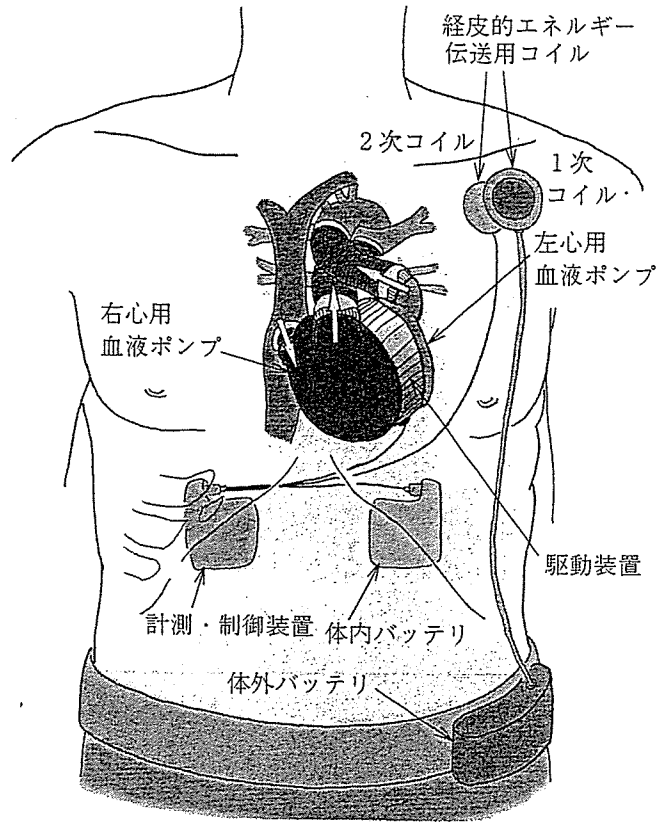


図 3.5　補助人工心臓



体内には，人工心臓本体(左右心用血液ポンプと駆動装置)，計測·制御装置，体内バッテリ，および2次コイルを装着する。体外には，1次コイルと体外バッテリのほか，監視装置を装着する場合もある

図 3.6　体内埋込型完全人工心臓の装着模式図

重症の心臓疾患の根本的治療法として，人工心臓以外に心臓移植がある。わが国でも1997年に臓器移植法ができ，脳死体からの臓器移植についての法的な整備が進んだが，いまのところ心臓移植の実施数は多くない。欧米でも臓器提供者数の慢性的な不足に悩んでいる。心臓移植の根本的な問題は，移植対象患者と免疫適合性のある臓器提供者が偶然現れない限り実施できないところにある。これに対して人工心臓は，技術的問題が解決し安価であれば，どんな患者でもいつでも利用可能である。また，特に補助人工心臓は，臓器提供者が出現するまでのつなぎ（橋渡し）として心臓移植を希望する患者に現在すでに多く使用されている。

〔2〕 構　　造

人工心臓は，図3.6に示したように，血液ポンプ，駆動装置，エネルギー源（バッテリなど），計測・制御装置などからなる。

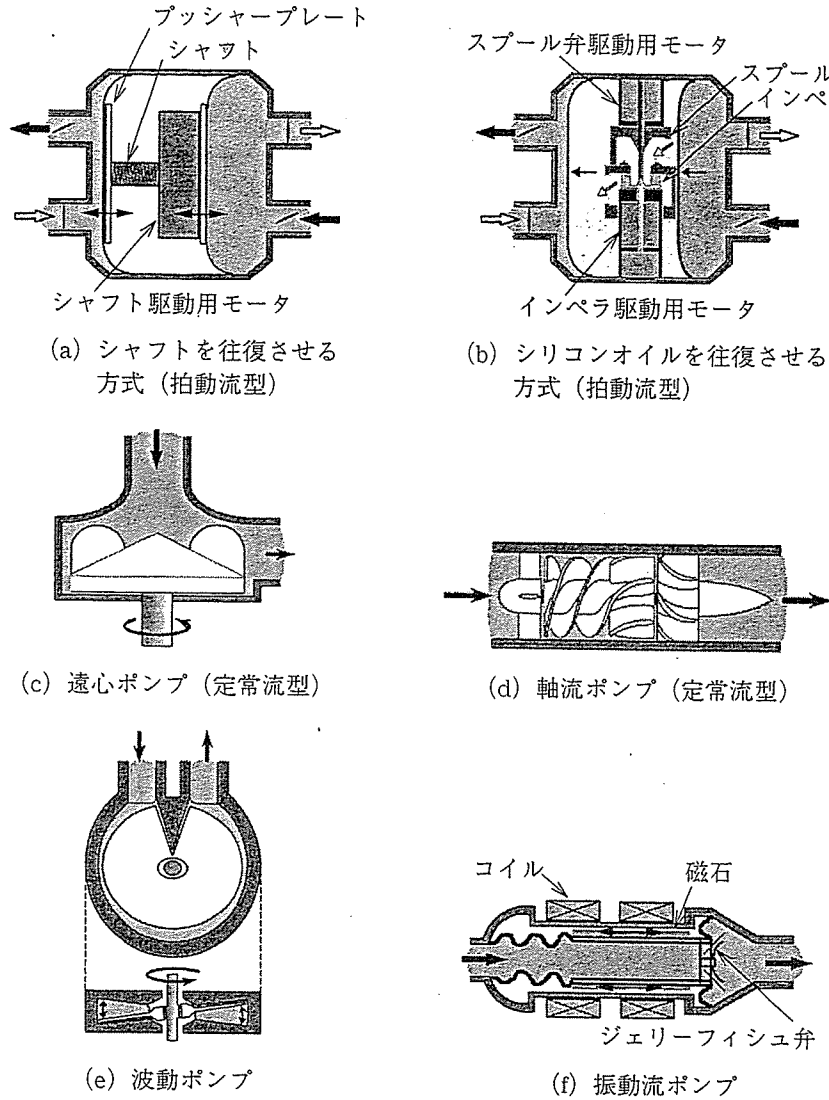
(a) 血液ポンプと駆動装置 人工心臓の中心的構成要素は血液ポンプである。人間の心臓（自然心臓）は，普通の大人で安静時に毎分5リットル，運動時に毎分10リットル程度の血液を体に循環させる。この程度の流量を流すだけであれば，家庭用の風呂水汲み上げ用小型ポンプでも十分対応できる。しかし，人工心臓用の血液ポンプには非常に厳しい条件が課せられている。すなわち，血栓形成・カルシウム沈着・血球破壊（溶血）が生じないようにするという血液適合性があることである。また，心臓が1日に約10万回の拍動するため，1年で4000万回程度の拍動に耐えられる耐久性をもたなければならない。したがって風呂水汲み上げ用ポンプは適さない。

現在臨床で使用されている血液ポンプは，拍動流型と定常流型の二つに大別できる。拍動流型は，自然心臓と同様に心室に相当する部分が収縮拡張運動をして血液が間欠的に流れるものである。これには逆流防止のための人工弁が必要である。定常流型は，人工心肺装置の血液ポンプとしてもよく使われるものであり，脈動がほとんどなく一定に近い流速になるものである。定常流型は生体の血流パターンと異なるが，補助人工心臓としての使用であれば自己心の拍動成分がある程度重畳するので問題がないとみなされている。

従来の人工心臓は空気圧駆動装置で駆動されるものが多かった。しかし最近では，電気モータで駆動される人工心臓が主流となりつつある。人工心臓には，**図3.7**のように，駆動方式としてさまざまなタイプのものが考案されているが，一長一短があり，それぞれで独自に開発が進められている。どのようなタイプの血液ポンプであっても，最も重要な問題は，やはり血栓形成をできる限り防止することである。そのために，多くの血液ポンプでは血液に触れる要素がチタンなどの血栓形成が少ない物質でつくられていたり，ヘパリンなどの抗凝固性物質でコーティングされている。また，血液の動きが少なくなり淀みができると血栓が形成されやすい。このため，淀みができるだけ起こらないように，流体力学的な設計を緻密に行って血液ポンプやカニューレの形を決めたり，ポンプの動作を適切に制御するような試みがなされている。

(b) エネルギー源と計測・制御装置 血液ポンプ，駆動装置，計測・制御装置のすべてを体内に埋め込むことを目指す電磁駆動式の人工心臓は，これらを動作させるための電気エネルギーを体外から内部のポンプへ供給する必要がある。現在では，電源トランスの仕組みと同様な方法で，皮膚を貫通することなく電力と情報を体内に送る装置が実用化しつつある。また，人工心臓にはその動作を制御し監視するための計測・制御装置も必要である。これはシングルチップマイクロコンピュータを中心とした電子回路で構成され，体内に埋め込んで使用できるようなものが開発されている。

(c) 循環制御 補助人工心臓の場合，自己心による循環制御機能が働くが，完全人工心臓では循環制御をすべて人為的に行わなければならない。循環制御には，左心と右心の拍



(a) シャフトを往復させる方式（拍動流型）

(b) シリコンオイルを往復させる方式（拍動流型）

(c) 遠心ポンプ（定常流型）

(d) 軸流ポンプ（定常流型）

(e) 波動ポンプ

(f) 振動流ポンプ

図 3.7　人工心臓血液ポンプの種々の駆動方式

出量のバランスをとるためのバランス制御と，生体が要求する循環量を保つための心拍出量制御の二つがある。バランス制御については左右の心房圧の差を一定に保つように右心の流量を操作する方式が成功している。しかし，ある時点で体全体にどのくらいの血液を拍出したらよいかという心拍出量制御の問題はまだ完全には解決していない[11]。

現在，完全人工心臓の場合も補助人工心臓の場合も，完全に体内に埋め込むことができる電磁駆動方式のものが臨床応用されつつある。2004 年 10 月現在，米国において電気モータ駆動方式完全人工心臓を体内に埋め込んだ患者が 512 日間の生存を記録している。わが国でも，例えば**図 3.8** のような，小柄な日本人向けの人工心臓が独自に開発されており[12]，近い将来，臨床応用が進むと予測される。

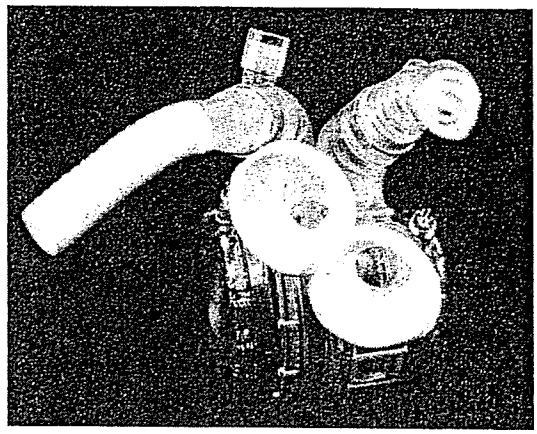


図3.8 小型化が容易な体内埋込型完全人工心臓の例(東京大学)[12]

3.4 代謝系人工臓器

3.4.1 人工透析・人工腎臓

テレビドラマなどを見ていると，白髪の恰幅(かっぷく)のよいお医者さんが，「今晩が山です」とか，「会わせておきたい方がいたら…」などと，患者さんの命が危ないことをムンテラ（患者さんやご家族への病状説明）している場面がよく出てくる。一見，このお医者さんは患者さんの病気の状態を完璧に把握しているようにも見える演出だが，現実には，そんなにたいしたことをしているわけではない。癌にせよ重症心不全にせよ，末期的な病態では多臓器不全を来して，急性腎不全のためにおしっこ（尿）が出なくなる。したがって，医者は重症の患者さんの尿の量さえ見ていれば，ある程度の病態を把握することができるわけである。人間は，尿が出ないと体に毒素が貯まっていくので，通常2～3日しか生命を長らえることはできない。他に重症の病気をもっている状態の悪い患者さんでは，尿が出なくなったときには，ほぼ1日前後の余命と推測できるわけであり，ほぼこの予測は外れることは少ない。このように腎不全の患者さんは，人工透析のような他の手段がなければ，生命を維持することができない。

現在，わが国だけでも約16万人強が人工透析の治療を受けているといわれており，その数は毎年約1万人ずつ増加している。平均すれば日本では，1時間に一人以上の割合で人工透析を受ける患者さんが増え続けており，つぎの1時間にはあなたの友人，家族，さらにはあなた自身が透析を受ける患者さんの仲間入りをする可能性がある。

腎臓にはいくつかの働きがあることが知られている。最も大事な一つは，もちろん，おしっこを出すことによって老廃物の除去する機能である。また，血圧の調節作用も重要であり，高血圧患者のかなりの部分が腎臓に原因があることが知られている。この他にも，赤血球の産出，ビタミンDの活性化など，さまざまな機能をもっており，生命現象の重要な役

編者・執筆者一覧

編　者

山形　仁（東芝メディカルシステムズ（株））

執　筆　者（五十音順）

	執筆担当
泉　隆（北海道東海大学）	2.5節，2.6.1項，2.6.2項，2.7.3項，2.8.1項
井野　秀一（東京大学）	2.6.3項，2.7.1項，2.7.2項，2.8.2項，2.9節
伊福部　達（東京大学）	2.1～2.4節
大川井宏明（岩手大学）	1.5節
仁田　新一（東北大学）	3.1節，3.5.3項
本村　信篤（東芝メディカルシステムズ(株)）	1.6節
森　一生（東北大学）	1.3節
山形　仁（東芝メディカルシステムズ(株)）	1.1節，1.2節，1.4節
山家　智之（東北大学）	3.2節，3.4節，3.5.1項，3.5.2項
吉澤　誠（東北大学）	3.3節

（所属は初版第1刷発行当時）

J Appl Physiol 102: 622–627, 2007.
First published November 2, 2006; doi:10.1152/japplphysiol.00622.2006.

Hypothermia reduces ischemia- and stimulation-induced myocardial interstitial norepinephrine and acetylcholine releases

Toru Kawada,[1] Hirotoshi Kitagawa,[2] Toji Yamazaki,[2] Tsuyoshi Akiyama,[2] Atsunori Kamiya,[1] Kazunori Uemura,[1] Hidezo Mori,[2] and Masaru Sugimachi[1]

[1]*Department of Cardiovascular Dynamics, Advanced Medical Engineering Center, and*
[2]*Department of Cardiac Physiology, National Cardiovascular Center Research Institute, Osaka, Japan*

Submitted 4 June 2006; accepted in final form 1 November 2006

Kawada T, Kitagawa H, Yamazaki T, Akiyama T, Kamiya A, Uemura K, Mori H, Sugimachi M. Hypothermia reduces ischemia- and stimulation-induced myocardial interstitial norepinephrine and acetylcholine releases. *J Appl Physiol* 102: 622–627, 2007. First published November 2, 2006; doi:10.1152/japplphysiol.00622.2006.—Although hypothermia is one of the most powerful modulators that can reduce ischemic injury, the effects of hypothermia on the function of the cardiac autonomic nerves in vivo are not well understood. We examined the effects of hypothermia on the myocardial interstitial norepinephrine (NE) and ACh releases in response to acute myocardial ischemia and to efferent sympathetic or vagal nerve stimulation in anesthetized cats. We induced acute myocardial ischemia by coronary artery occlusion. Compared with normothermia ($n = 8$), hypothermia at 33°C ($n = 6$) suppressed the ischemia-induced NE release [63 nM (SD 39) vs. 18 nM (SD 25), $P < 0.01$] and ACh release [11.6 nM (SD 7.6) vs. 2.4 nM (SD 1.3), $P < 0.01$] in the ischemic region. Under hypothermia, the coronary occlusion increased the ACh level from 0.67 nM (SD 0.44) to 6.0 nM (SD 6.0) ($P < 0.05$) and decreased the NE level from 0.63 nM (SD 0.19) to 0.40 nM (SD 0.25) ($P < 0.05$) in the nonischemic region. Hypothermia attenuated the nerve stimulation-induced NE release from 1.05 nM (SD 0.85) to 0.73 nM (SD 0.73) ($P < 0.05$, $n = 6$) and ACh release from 10.2 nM (SD 5.1) to 7.1 nM (SD 3.4) ($P < 0.05$, $n = 5$). In conclusion, hypothermia attenuated the ischemia-induced NE and ACh releases in the ischemic region. Moreover, hypothermia also attenuated the nerve stimulation-induced NE and ACh releases. The Bezold-Jarisch reflex evoked by the left anterior descending coronary artery occlusion, however, did not appear to be affected under hypothermia.

vagal nerve; sympathetic nerve; cardiac microdialysis; cats

HYPOTHERMIA IS ONE OF THE most powerful modulators that can reduce ischemic injury in the central nervous system, heart, and other organs. The general consensus is that hypothermia induces a hypometabolic state in tissues and balances energy supply and demand (25). With respect to the myocardial ischemia, the size of a myocardial infarction correlates with temperature (6), and mild hypothermia can protect the myocardium against acute ischemic injury (9). The effects of hypothermia on the function of the cardiac autonomic nerves in terms of neurotransmitter releases, however, are not fully understood. Because autonomic neurotransmitters such as norepinephrine (NE) and ACh directly impinge on the myocardium, they would be implicated in the cardioprotection by hypothermia.

In previous studies from our laboratory, Kitagawa et al. (16) demonstrated that hypothermia attenuated the nonexocytotic NE release induced pharmacologically by ouabain, tyramine, or cyanide. Kitagawa et al. (15) also demonstrated that hypothermia attenuated the exocytotic NE release in response to vena cava occlusion or to local administration of high K^+. The effects of hypothermia on the ischemia-induced myocardial interstitial NE release, however, were not examined in those studies. In addition, the effects of hypothermia on the ischemia-induced myocardial interstitial ACh release have never been examined. Because both sympathetic and parasympathetic nerves control the heart, simultaneous monitoring of the myocardial interstitial releases of NE and ACh (14, 31) would help integrative understanding of the autonomic nerve terminal function under hypothermia in conjunction with acute myocardial ischemia.

In the present study, the effects of hypothermia on the ischemia-induced and nerve stimulation-induced myocardial interstitial neurotransmitter releases were examined. We implanted a dialysis probe into the left ventricular free wall of anesthetized cats and measured dialysate NE and ACh levels as indexes of neurotransmitter outputs from the cardiac sympathetic and vagal nerve terminals, respectively. Based on our laboratory's previous results (15, 16), we hypothesized that hypothermia would attenuate the neurotransmitter releases in response to acute myocardial ischemia and to electrical nerve stimulation.

MATERIALS AND METHODS

Surgical Preparation and Protocols

Animals were cared for in 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 reviewed and approved by the Animal Subjects Committee of National Cardiovascular Center. Adult cats were anesthetized via an intraperitoneal injection of pentobarbital sodium (30–35 mg/kg) and ventilated mechanically through an endotracheal tube with oxygen-enriched room air. The level of anesthesia was maintained with a continuous intravenous infusion of pentobarbital sodium (1–2 $mg \cdot kg^{-1} \cdot h^{-1}$) through a catheter inserted from the right femoral vein. Mean arterial pressure (MAP) was measured using a pressure transducer connected to a catheter inserted from the right femoral artery. Heart rate (HR) was determined from an electrocardiogram.

Protocol 1: acute myocardial ischemia. We examined the effects of hypothermia on the ischemia-induced myocardial interstitial releases of NE and ACh. The heart was exposed by partially removing the left fifth and/or sixth rib. A dialysis probe was implanted transversely into

Address for reprint requests and other correspondence: T. Kawada, Dept. of Cardiovascular Dynamics, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan (e-mail: torukawa@res.ncvc.go.jp).

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the anterolateral free wall of the left ventricle perfused by the left anterior descending coronary artery (LAD) to monitor myocardial interstitial NE and ACh levels in the ischemic region during occlusion of the LAD (13). Another dialysis probe was implanted transversely into the posterior free wall of the left ventricle perfused by the left circumflex coronary artery to monitor myocardial interstitial NE and ACh levels in a nonischemic region. Heparin sodium (100 U/kg) was administered intravenously to prevent blood coagulation. Animals were divided into a normothermic group (n = 8) and a hypothermic group (n = 6). In the hypothermic group, surface cooling with ice bags was performed until the esophageal temperature decreased to 33°C (15, 16). A stable hypothermic condition was obtained within ~2 h. In each group, we occluded the LAD for 60 min and examined changes in the myocardial interstitial NE and ACh levels in the ischemic region (i.e., the LAD region) and nonischemic region (i.e., the left circumflex coronary artery region). Fifteen-minute dialysate samples were obtained during the preocclusion baseline condition and during the periods of 0–15, 15–30, 30–45, and 45–60 min of the LAD occlusion.

Protocol 2: sympathetic stimulation. We examined the effects of hypothermia on the sympathetic nerve stimulation-induced myocardial interstitial NE release (n = 6). A dialysis probe was implanted transversely into the anterolateral free wall of the left ventricle. The bilateral cardiac sympathetic nerves originating from the stellate ganglia were exposed through a second intercostal space and sectioned. The cardiac end of each sectioned nerve was placed on a bipolar platinum electrode for sympathetic stimulation (5 Hz, 10 V, 1-ms pulse duration). The electrodes and nerves were covered with mineral oil to provide insulation and prevent desiccation. A 4-min dialysate sample was obtained during the sympathetic stimulation under the normothermic condition. Thereafter, hypothermia was introduced using the same cooling procedure as in *protocol 1*, and a second 4-min dialysate sample was obtained during the sympathetic stimulation.

Protocol 3: vagal stimulation. We examined the effects of hypothermia on the vagal nerve stimulation-induced ACh release (n = 5). A dialysis probe was implanted transversely into the anterolateral free wall of the left ventricle. The bilateral vagi were exposed through a midline cervical incision and sectioned at the neck. The cardiac end of each sectioned nerve was placed on a bipolar platinum electrode for vagal stimulation (20 Hz, 10 V, 1-ms pulse duration). To prevent severe bradycardia and cardiac arrest, which can be induced by the vagal stimulation, the heart was paced at 200 beats/min using pacing wires attached to the apex of the heart during the stimulation period. A 4-min dialysate sample was obtained during the vagal stimulation under the normothermic condition. Thereafter, hypothermia was introduced using the same cooling procedure as in *protocol 1*, and a second 4-min dialysate sample was obtained during the vagal stimulation.

Because of the relatively intense stimulation of the sympathetic or vagal nerve, the stimulation period in *protocols 2* and *3* was limited to 4 min to minimize gradual waning of the stimulation effects. At the end of the experiment, the animals were killed by increasing the depth of anesthesia with an overdose of pentobarbital sodium. We then confirmed that the dialysis probes had been threaded in the middle layer of the left ventricular myocardium.

Dialysis Technique

The dialysate NE and ACh concentrations were measured as indexes of myocardial interstitial NE and ACh levels, respectively. The materials and properties of the dialysis probe have been described previously (2, 3). Briefly, we designed a transverse dialysis probe. A dialysis fiber (13-mm length, 310-μm outer diameter, 200-μm inner diameter; PAN-1200, 50,000 molecular weight cutoff; Asahi Chemical) was connected at both ends to polyethylene tubes (25-cm length, 500-μm outer diameter, 200-μm inner diameter). The dialysis probe was perfused with Ringer solution containing a cholinesterase inhibitor eserine (10^{-4} M) at a rate of 2 μl/min. We started dialysate sampling from 2 h after the implantation of the dialysis probe(s), when the dialysate NE and ACh concentrations had reached steady states. The actual dialysate sampling was delayed by 5 min from the collection period to account for the dead space volume between the semipermeable membrane and the sample tube. Each sample was collected in a microtube containing 3 μl of HCl to prevent amine oxidation. The dialysate ACh concentration was measured directly by HPLC with electrochemical detection (Eicom). The in vitro recovery rate of ACh was ~70%. With the use of a criterion of signal-to-noise ratio of higher than three, the detection limit for ACh was 3 pg per injection. The dialysate NE concentration was measured by another HPLC-electrochemical detection system after the removal of interfering compounds by an alumina procedure. The in vitro recovery rate of NE was ~55%. With the use of a criterion of signal-to-noise ratio of higher than three, the detection limit for NE was 200 fg per injection.

Statistical Analysis

All data are presented as means and SD values. For *protocol 1*, we performed two-way repeated-measures ANOVA using hypothermia as one factor and the dialysate sampling periods (the effects of ischemia) as the other factor. For *protocols 2* and *3*, we compared stimulation-induced releases of NE and ACh before and during hypothermia using a paired t-test. For all of the statistics, the difference was considered significant when $P < 0.05$.

RESULTS

Figure 1*A* illustrates changes in myocardial interstitial NE levels in the ischemic region during LAD occlusion obtained from *protocol 1*. The *inset* shows the magnified ordinate for the

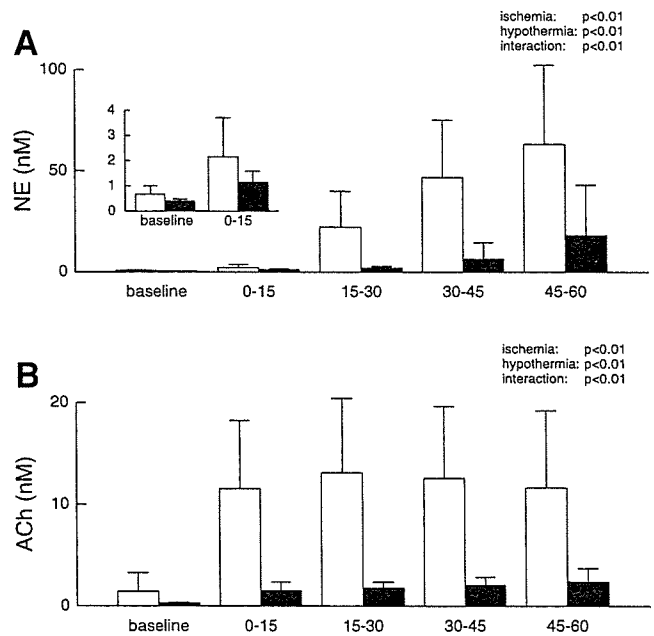

Fig. 1. *A*: ischemia-induced myocardial interstitial norepinephrine (NE) release in the ischemic region. Acute myocardial ischemia caused a progressive increase in the level of myocardial interstitial NE. Hypothermia attenuated the ischemia-induced NE release. *Inset*: magnified ordinate for the baseline and the 0- to 15-min period of ischemia. *B*: ischemia-induced myocardial interstitial ACh release in the ischemic region. Acute myocardial ischemia increased the myocardial interstitial ACh levels. Hypothermia attenuated the ischemia-induced ACh release. Open bars: normothermia; solid bars: hypothermia.

baseline and the 0- to 15-min period of ischemia. In the normothermic group (open bars), the LAD occlusion caused an ~94-fold increase in the NE level during the 45- to 60-min interval. In the hypothermic group (solid bars), the LAD occlusion caused an ~45-fold increase in the NE level during the 45- to 60-min interval. Compared with normothermia, hypothermia suppressed the baseline NE level to ~59% and the NE level during the 45- to 60-min period to ~29%. Statistical analysis indicated that the effects of both hypothermia and ischemia on the NE release were significant, and the interaction between hypothermia and ischemia was also significant.

Figure 1*B* illustrates changes in myocardial interstitial ACh levels in the ischemic region during the LAD occlusion. In both the normothermic (open bars) and hypothermic (solid bars) groups, the LAD occlusion caused an approximately eightfold increase in the ACh level during the 45- to 60-min interval. Compared with normothermia, however, hypothermia suppressed both the baseline ACh level and the ACh level during the 45- to 60-min period of ischemia to ~20%. Statistical analysis indicated that the effects of both hypothermia and ischemia on the ACh release were significant, and the interaction between hypothermia and ischemia was also significant.

Figure 2*A* illustrates changes in myocardial interstitial NE levels in the nonischemic region during the LAD occlusion. Note that scale of the ordinate is only one-hundredth of that in Fig. 1*A*. The LAD occlusion decreased the NE level in the normothermic group (open bars); the NE level during the 45- to 60-min interval was ~59% of the baseline level. The LAD occlusion also decreased the NE level in the hypothermic group (solid bars); the NE level during the 45- to 60-min interval was ~64% of the baseline level. Although the LAD occlusion resulted in a decrease in the NE level under both conditions, the NE level under hypothermia was nearly twice that measured under normothermia. The statistical analysis indicated that the effects of both hypothermia and ischemia on the NE release were significant, whereas the interaction between hypothermia and ischemia was not significant.

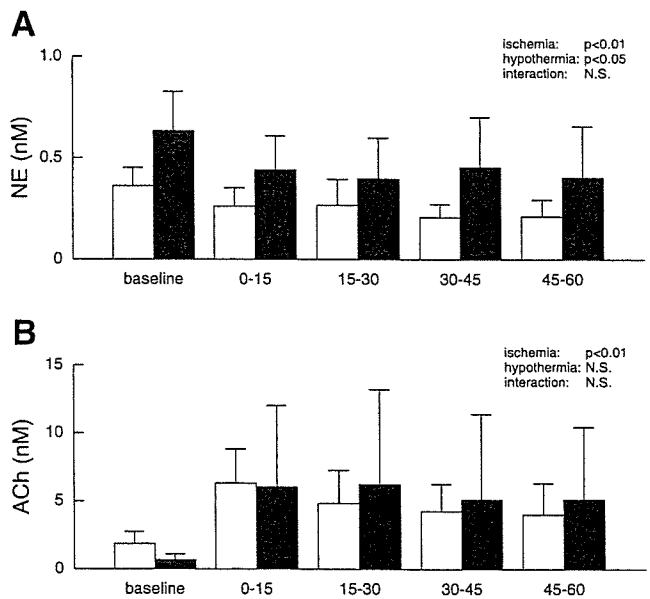

Fig. 2. *A*: changes in the myocardial interstitial NE levels in the nonischemic region. Acute myocardial ischemia decreased the level of myocardial interstitial NE from the baseline level. Hypothermia increased the myocardial interstitial NE levels in the nonischemic region. *B*: changes in the myocardial interstitial ACh levels in the nonischemic region. Acute myocardial ischemia increased the myocardial interstitial ACh level. Hypothermia did not attenuate the increasing response of ACh to the left anterior descending coronary artery occlusion. Open bars: normothermia; solid bars: hypothermia. NS, not significant.

Table 1. *Mean arterial pressure during acute myocardial ischemia obtained in protocol 1*

	Baseline	5 min	15 min	30 min	45 min	60 min
Normothermia	108 (23)	102 (28)	101 (24)	101 (20)	102 (21)	102 (21)
Hypothermia	108 (11)	80 (17)	87 (10)	85 (10)	86 (10)	91 (11)

Values are means (SD) (in mmHg) obtained during preocclusion baseline period and 5-, 15-, 30-, 45-, and 60-min periods of coronary artery occlusion. Ischemia: $P < 0.01$; hypothermia: not significant; interaction: $P < 0.01$.

Figure 2*B* illustrates changes in myocardial interstitial ACh levels in the nonischemic region during the LAD occlusion. The LAD occlusion caused an ~3.4-fold increase in the ACh level during the 0- to 15-min interval in the normothermic group (open bars). The LAD occlusion caused an approximately ninefold increase in the ACh level during the 0- to 15-min interval in the hypothermic group (solid bars). These effects of ischemia on the ACh release were statistically significant. Although hypothermia seemed to attenuate the baseline ACh level, the overall effects of hypothermia on the ACh level were insignificant.

Tables 1 and 2 summarize the MAP and HR data, respectively, obtained in *protocol 1*. Acute myocardial ischemia significantly reduced MAP ($P < 0.01$) and HR ($P < 0.01$). Hypothermia did not affect MAP but did decrease HR ($P < 0.01$). The interaction between ischemia and hypothermia was significant for MAP but not for HR by the two-way repeated-measures ANOVA.

For *protocol 2*, hypothermia significantly attenuated the sympathetic stimulation-induced NE release to ~70% of the level observed during normothermia (Fig. 3*A*). Under normothermia, the sympathetic stimulation increased MAP from 114 mmHg (SD 27) to 134 mmHg (SD 33) ($P < 0.01$) and HR from 147 beats/min (SD 9) to 207 beats/min (SD 5) ($P < 0.01$). Under hypothermia, the sympathetic stimulation increased MAP from 117 mmHg (SD 11) to 136 mmHg (SD 22) ($P < 0.05$) and HR from 125 beats/min (SD 16) to 164 beats/min (SD 10) ($P < 0.01$).

For *protocol 3*, hypothermia significantly attenuated the vagal stimulation-induced ACh release to ~70% of the level observed during normothermia (Fig. 3*B*). Hypothermia did not change MAP [117 mmHg (SD 18) vs. 118 mmHg (SD 27)] but

Table 2. *Heart rate during acute myocardial ischemia obtained in protocol 1*

	Baseline	5 min	15 min	30 min	45 min	60 min
Normothermia	183 (26)	160 (18)	163 (16)	163 (18)	166 (20)	165 (21)
Hypothermia	146 (25)	116 (19)	113 (19)	126 (39)	112 (20)	97 (31)

Values are means (SD) (in beats/min) obtained during preocclusion baseline period and 5-, 15-, 30-, 45-, and 60-min periods of coronary artery occlusion. Ischemia: $P < 0.01$; hypothermia: $P < 0.01$; interaction: not significant.

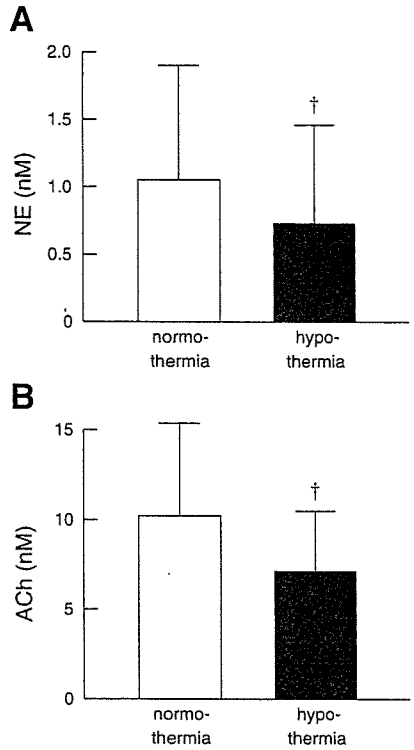

Fig. 3. *A*: efferent sympathetic nerve stimulation-induced release of myocardial interstitial NE before and during hypothermia. †Hypothermia significantly attenuated the stimulation-induced NE release. *B*: efferent vagal nerve stimulation-induced release of myocardial interstitial ACh before and during hypothermia. †Hypothermia significantly attenuated the stimulation-induced ACh release.

did decrease HR from 202 beats/min (SD 24) to 179 beats/min (SD 15) ($P < 0.05$) during the prestimulation, unpaced condition. MAP during the stimulation was 105 mmHg (SD 19) under normothermia and 93 mmHg (SD 33) under hypothermia.

DISCUSSION

A cardiac microdialysis is a powerful tool to estimate neurotransmitter levels in the myocardial interstitium in vivo (2, 3, 14, 19, 20, 31). The present study demonstrated that hypothermia significantly attenuated the myocardial interstitial releases of NE and ACh in the ischemic region during the LAD occlusion. In contrast, the increasing response in the ACh level from its baseline level and the decreasing response in the NE level from its baseline level observed in the nonischemic region were maintained under hypothermia. To our knowledge, this is the first report showing the effects of hypothermia on the myocardial interstitial releases of NE and ACh during acute myocardial ischemia in vivo. In addition, the present study showed that hypothermia significantly attenuated nerve stimulation-induced myocardial interstitial NE and ACh releases in vivo.

Effects of Hypothermia on Ischemia-induced NE and ACh Releases in the Ischemic Region

Acute myocardial ischemia causes energy depletion, which leads to myocardial interstitial NE release in the ischemic region (Fig. 1*A*). The NE release can be classified as exocytotic or nonexocytotic (18, 24). Exocytotic release indicates NE release from synaptic vesicles, which normally occurs in response to nerve discharge and subsequent Ca^{2+} influx through voltage-dependent Ca^{2+} channels. On the other hand, nonexocytotic release indicates NE release from the axoplasm, such as that mediated by a reverse transport through the NE transporter. A neuronal uptake blocker, desipramine, can suppress the ischemia-induced NE release (19, 24). Whereas exocytotic release contributes to the ischemia-induced NE release in the initial phase of ischemia (within ~20 min), carrier-mediated nonexocytotic release becomes predominant as the ischemic period is prolonged (1). Hypothermia significantly attenuated the ischemia-induced NE release (Fig. 1*A*). The NE level during the 45- to 60-min period of ischemia under hypothermia was ~20% of that obtained under normothermia. The NE uptake transporter is driven by the Na^+ gradient across the cell membrane (23). The loss of the Na^+ gradient due to ischemia causes NE to be transported out of the cell by reversing the action of the NE transporter. Hypothermia inhibits the action of the NE transporter and also suppresses the intracellular Na^+ accumulation (8), thereby reducing nonexocytotic NE release during ischemia. The present results are in line with an in vitro study that showed hypothermia suppressed nonexocytotic NE release induced by deprivation of oxygen and glucose (30). The present results are also consistent with a previous study from our laboratory that showed hypothermia attenuated the nonexocytotic NE release induced by ouabain, tyramine, or cyanide (16).

Acute myocardial ischemia increases myocardial interstitial ACh level in the ischemic region, as reported previously (Fig. 1*B*) (13). The level of ischemia-induced ACh release during 0- to 15-, 15- to 30-, 30- to 45-, or 45- to 60-min period of ischemia is comparable to that evoked by 4-min electrical stimulation of the bilateral vagi (Fig. 3*B*). Compared with the normothermic condition, hypothermia significantly attenuated the ischemia-induced myocardial interstitial release of ACh in the ischemic region. Our laboratory's previous study indicated that intracellular Ca^{2+} mobilization is essential for the ischemia-induced release of ACh (13). Hypothermia may have prevented the Ca^{2+} overload, thereby reducing the ischemia-induced ACh release. Alternatively, hypothermia may reduce the extent of the ischemic injury, which in turn suppressed the ischemia-induced ACh release. Because ACh has protective effects on the cardiomyocytes against ischemia (11), the suppression of ischemia-induced ACh release during hypothermia itself may be unfavorable for cardioprotection.

There is considerable controversy regarding the cardioprotective effects of β-adrenergic blockade during severe ischemia, with studies demonstrating a reduction of infarct size (10, 17) or no effects (7, 27). The β-adrenergic blockade seems effective to protect the heart only when the heart is reperfused within a certain period after the coronary occlusion. The β-adrenergic blockade would reduce the myocardial oxygen consumption through the reduction of HR and ventricular contractility and delay the progression of ischemic injury. Hence the infarct size might be reduced when the heart is reperfused before the ischemic damage becomes irreversible. The ischemia-induced NE release reached nearly 100 times the baseline NE level under normothermia (Fig. 1*A*), which by far exceeded the NE level attained by electrical stimulation of the

bilateral stellate ganglia (Fig. 3*A*). Because high NE levels have cardiotoxic effects (22), ischemia-induced NE release might aggravate the ischemic injury. However, catecholamine depletion by a reserpine treatment fails to reduce the infarct size (26, 29), throwing a doubt on the involvement of catecholamine toxicity in the progression of myocardial damage during ischemia. It is, therefore, most likely that the hypothermia-induced reductions in NE and ACh are the result of reduced myocardial damage or a direct effect on nerve endings.

Van den Doel et al. (28) showed that hypothermia does not abolish necrosis, but rather delays necrosis during sustained ischemia, so that hypothermia protected against infarction produced by a 30-min occlusion but not against infarction produced by a 60-min occlusion in the rat heart. At the same time, they mentioned that hypothermia was able to reduce the infarct size after a 60-min coronary occlusion in the dog, possibly because of the significant collateral flow in the canine hearts. Because the feline hearts are similar to the canine hearts in that they have considerable collateral flow compared with the rat hearts (21), hypothermia should have protected the feline heart against the 60-min coronary occlusion in the present study.

Effects of Hypothermia on the NE and ACh Releases in the Nonischemic Region and on the Electrical Stimulation-induced NE and ACh Releases

The NE and ACh levels in the nonischemic region may reflect the sympathetic and parasympathetic drives to this region. As an example, myocardial interstitial ACh levels increase during activations of the arterial baroreflex and the Bezold-Jarisch reflex (14). In the present study, acute myocardial ischemia decreased the NE level from its baseline level, whereas it increased the ACh level from its baseline level (Fig. 2). Ischemia also decreased MAP and HR (Tables 1 and 2), suggesting that the Bezold-Jarisch reflex was induced by the LAD occlusion under both normothermia and hypothermia. Taking into account the fact that electrical stimulation-induced ACh release was attenuated to ~70% (Fig. 3), similar ACh levels during ischemia imply the enhancement of the parasympathetic outflow via the Bezold-Jarisch reflex under hypothermia. These results are in line with the study by Zheng et al. (32), where pulmonary chemoreflex-induced bradycardia was maintained under hypothermia. Hypothermia increased the NE level in the nonischemic region, suggesting that sympathetic drive to this region also increased. Hypothermic stress is known to cause sympathetic activation, accompanying increases in MAP, HR, plasma NE, and epinephrine levels (4). In the present study, because the effect of hypothermia on MAP was insignificant (Table 1) and HR decreased under hypothermia (Table 2), the sympathetic activation observed in the nonischemic region might have been regional and not systemic.

Hypothermia attenuated the releases of NE and ACh in response to respective nerve stimulation to ~70% of that observed under normothermia (Fig. 3). The suppression of the exocytotic NE release by hypothermia is consistent with a previous study from our laboratory, where hypothermia attenuated the myocardial interstitial NE release in response to vena cava occlusion or to a local high K^+ administration (15). The suppression of NE release by hypothermia is consistent with an in vitro study by Kao and Westhead (12) in which catecholamine secretion from adrenal chromaffin cells induced by elevated K^+ levels increased as the temperature increased from 4 to 37°C. On the other hand, because hypothermia inhibits the neuronal NE uptake, the NE concentration at the synaptic cleft is expected to be increased if the level of NE release remains unchanged. Actually, Vizi (30) demonstrated that hypothermia increased NE release in response to field stimulation in vitro. In the present study, however, the suppression of NE release might have canceled the potential accumulation of NE due to NE uptake inhibition. The present study also demonstrated that the ACh release was suppressed by hypothermia. In the rat striatum, hypothermia decreases the extracellular ACh concentration and increases the choline concentration (5). Hypothermia may inhibit a choline uptake transporter in the same manner as it inhibits a NE uptake transporter. The inhibition of the choline transporter by hypothermia may have hampered the replenishment of the available pool of ACh and thereby contributed to the suppression of the stimulation-induced ACh release.

Limitations

In *protocol 1*, because we did not measure the infarct size in the present study, the degree of myocardial protection by hypothermia was undetermined. Whether the reduction of ischemia-induced neurotransmitter release correlates with the reduction of infact size requires further investigations. In *protocols 2* and *3*, baseline NE and ACh levels were not measured. The reduction of stimulation-induced NE and ACh release by hypothermia might be partly due to the reduction of baseline NE and ACh levels. However, because transection of the stellate ganglia (31) or vagi (3) reduces the baseline NE and ACh levels, changes in the baseline NE and ACh levels by hypothermia in *protocols 2* and *3* could not be as large as those observed under innervated conditions in *protocol 1* (Figs. 1 and 2).

In conclusion, hypothermia attenuated the ischemia-induced releases of NE and ACh in the ischemic region to ~30 and 20% of those observed under normothermia, respectively. Hypothermia also attenuated the nerve stimulation-induced releases of NE and ACh to ~70% of those observed during normothermia. In contrast, hypothermia did not affect the decreasing response in the NE level and the increasing response in the ACh level in the nonischemic region, suggesting that the Bezold-Jarisch reflex evoked by the LAD occlusion was maintained.

GRANTS

This study was supported by Health and Labour Sciences Research Grant for Research on Advanced Medical Technology, Health and Labour Sciences Research Grant for Research on Medical Devices for Analyzing, Supporting and Substituting the Function of Human Body, and Health and Labour Sciences Research Grant H18-Iryo-Ippan-023 from the Ministry of Health, Labour and Welfare of Japan; Program for Promotion of Fundamental Studies in Health Science from the National Institute of Biomedical Innovation; a grant provided by the Ichiro Kanehara Foundation; Ground-based Research Announcement for Space Utilization promoted by the Japan Space Forum; and Industrial Technology Research Grant Program 03A47075 from the New Energy and Industrial Technology Development Organization of Japan.

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Am J Physiol Heart Circ Physiol 292: H921–H927, 2007.
First published September 29, 2006; doi:10.1152/ajpheart.00019.2006.

Evaluation of transmural distribution of viable muscle by myocardial strain profile and dobutamine stress echocardiography

Takeshi Maruo,[1] Satoshi Nakatani,[1] Yintie Jin,[2] Kazunori Uemura,[2] Masaru Sugimachi,[2] Hatsue Ueda-Ishibashi,[3] Masafumi Kitakaze,[1] Tohru Ohe,[4] Kenji Sunagawa,[2] and Kunio Miyatake[1]

Departments of [1]Cardiology and [3]Pathology, National Cardiovascular Center, and [2]Department of Cardiovascular Dynamics, National Cardiovascular Center Research Institute, Osaka, Japan; and [4]Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Okayama, Japan

Submitted 5 January 2006; accepted in final form 27 September 2006

Maruo T, Nakatani S, Jin Y, Uemura K, Sugimachi M, Ueda-Ishibashi H, Kitakaze M, Ohe T, Sunagawa K, Miyatake K. Evaluation of transmural distribution of viable muscle by myocardial strain profile and dobutamine stress echocardiography. *Am J Physiol Heart Circ Physiol* 292: H921–H927, 2007. First published September 29, 2006; doi:10.1152/ajpheart.00019.2006.—Transmural distribution of viable myocardium in the ischemic myocardium has not been quantified and fully elucidated. To address this issue, we evaluated transmural myocardial strain profile (TMSP) in dogs with myocardial infarction using a newly developed tissue strain imaging. TMSP was obtained from the posterior wall at the epicardial left ventricular short-axis view in 13 anesthetized open-chest dogs. After control measurements, the left circumflex coronary artery was occluded for 90 min to induce subendocardial infarction (SMI). Subsequently, latex microbeads (90 μm) were injected in the same artery to create transmural infarction (TMI). In each stage, measurements were done before and after dobutamine challenge (10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 10 min) to estimate transmural myocardial viability. Strain in the subendocardium in the control stage increased by dobutamine (from 53.6 ± 17.1 to 73.3 ± 21.8%, $P < 0.001$), whereas that in SMI and TMI stages was almost zero at baseline and did not increase significantly by dobutamine [from 0.8 ± 8.8 to 1.3 ± 7.0%, P = not significant (NS) for SMI, from −3.9 ± 5.6 to −1.9 ± 6.0%, P = NS for TMI]. Strain in the subepicardium increased by dobutamine in the control stage (from 23.9 ± 6.1 to 26.3 ± 6.4%, $P < 0.05$) and in the SMI stage (from 12.4 ± 7.3 to 27.1 ± 8.8%, $P < 0.005$), whereas that in the TMI stage did not change (from −1.0 ± 7.8 to −0.7 ± 8.3%, P = NS). In SMI, the subendocardial contraction was lost, but the subepicardium showed a significant increase in contraction with dobutamine. However, in TMI, even the subepicardial increase was not seen. Assessment of transmural strain profile using tissue strain imaging was a new and useful method to estimate transmural distribution of the viable myocardium in myocardial infarction.

myocardial infarction; strain; viability; echocardiography

IT IS WELL KNOWN that myocardial contraction has transmural heterogeneity. Several experimental studies confirmed that the subendocardium contributes greater to overall myocardial thickening than the subepicardium (6, 25). On the other hand, when a reduction of coronary blood flow occurs, a severe reduction of perfusion and kinesis occurs in the subendocardium, but only a trivial reduction can be detected in the subepicardium (5, 31). After a long period of ischemia, myocardial necrosis progresses from the endocardium to the epicardium (8, 13).

Myocardial strain reflects regional myocardial function. With the recent advancement of tissue Doppler echocardiography, myocardial strain can be obtained noninvasively (3, 33) and has been reported to be useful to quantify regional myocardial systolic function in ischemic heart disease (9, 11, 24, 29, 36). The recently developed myocardial strain imaging system provides us myocardial strain in each wall layer and shows its distribution in a form of transmural myocardial strain profile (TMSP; see Ref. 1). Thus combination of TMSP and dobutamine stress echocardiography (DSE), which has been used for the assessment of myocardial viability (18), is expected to demonstrate transmural distribution of viability. There have been no methods to visualize distribution of myocardial viability over the ventricular wall in myocardial infarction, and such method would provide important information in the clinical situation.

In the present study, to assess the transmural extent of myocardial infarction, we investigated TMSP in subendocardial and transmural myocardial infarction dog models and quantified the transmural heterogeneity of myocardial viability using myocardial strain imaging with DSE.

MATERIALS AND METHODS

Experimental subjects and settings. We used 13 mongrel dogs (weighing 27.3 ± 2.2 kg). After induction with intravenous pentobarbital sodium (25 mg/kg body wt), they were anesthetized with 2% isoflurane with oxygen. A median sternotomy was performed, the pericardium was split from apex to base, and, after the instrumentation, the edges of the pericardial incision were loosely resutured. A 5-Fr. micromanometer-tipped catheter (model MPC-500; Millar Instruments, Houston, TX) was positioned in the left ventricle through the apex to obtain peak systolic left ventricular pressure and peak positive and negative dP/dt. Electrocardiogram (ECG) was monitored from limb leads. Left ventricular pressure signals and ECG were digitized online. The care and use of animals was in strict accordance with the guiding principles of the American Physiological Society, and the experimental protocol was approved by the National Cardiovascular Center Committees on Animal Experiments.

Experimental protocol. A 6-Fr. sheath was placed in the right femoral artery, and an angioplasty balloon catheter was positioned in the proximal segment of the left circumflex coronary artery by the standard catheterization technique. DSE (dobutamine infusion at 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 10 min) was used to assess myocardial viability. At the control stage, echocardiographic and hemodynamic measurements were done before and after DSE. A subendocardial myocardial infarction was created by inflating the balloon for 90 min

Address for reprint requests and other correspondence: S. Nakatani, Dept. of Cardiology, National Cardiovascular Center, 5-7-1, Fujishiro-dai, Suita, Osaka 565-8565, Japan (e-mail: nakatas@hsp.ncvc.go.jp).

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