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Preconditioning by Sevoflurane Decreases Biochemical Markers for Myocardial and Renal Dysfunction in Coronary Artery Bypass Graft Surgery: A Double-blinded, Placebo-controlled, Multicenter Study

Karine Julier, M.D., Rafaela da Silva, M.S.,† Carlos Garcia, M.D.,‡ Lukas Bestmann, Ph.D.,§ Philippe Frascarolo, Ph.D.,‖ Andreas Zollinger, M.D.,# Pierre-Guy Chassot, M.D.,** Edith R. Schmid, M.D.,†† Marko I. Turina, M.D.,‡‡ Ludwig K. von Segesser, M.D.,§§ Thomas Pasch, M.D.,‖‖ Donat R. Spahn, M.D.,## Michael Zaugg, M.D., D.E.A.A.****

Background: **Preconditioning by volatile anesthetics is a promising therapeutic strategy to render myocardial tissue resistant to perioperative ischemia. It was hypothesized that sevoflurane preconditioning would decrease postoperative release of brain natriuretic peptide, a biochemical marker for myocardial dysfunction. In addition, several variables associated with the protective effects of preconditioning were evaluated.**

Methods: **Seventy-two patients scheduled for coronary artery bypass graft surgery under cardioplegic arrest were randomly assigned to preconditioning during the first 10 min of complete cardiopulmonary bypass with either placebo (oxygen–air mixture only) or sevoflurane 4 vol% (2 minimum alveolar concentration). No other volatile anesthetics were administered at any time during the study. Treatment was strictly blinded to anesthesiologists, perfusionists, and surgeons. Biochemical markers of myocardial dysfunction and injury (brain natriuretic peptide, creatine kinase–MB activity, and cardiac troponin T), and renal dysfunction (cystatin C) were determined. Results of Holter electrocardiography were recorded perioperatively. Translocation of protein kinase C was assessed by immunohistochemical analysis of atrial samples.**

Results: **Sevoflurane preconditioning significantly decreased postoperative release of brain natriuretic peptide, a sensitive biochemical marker of myocardial contractile dysfunction. Pronounced protein kinase C δ and ϵ translocation was observed in sevoflurane-preconditioned myocardium. In addition, postoperative plasma cystatin C concentrations increased significantly less in sevoflurane-preconditioned patients. No differences between groups were found for perioperative ST-segment changes, arrhythmias, or creatine kinase–MB and cardiac troponin T release.**

Conclusions: **Sevoflurane preconditioning preserves myocardial and renal function as assessed by biochemical markers in patients undergoing coronary artery bypass graft surgery under cardioplegic arrest. This study demonstrated for the first time translocation of protein kinase C isoforms δ and ϵ in human myocardium in response to sevoflurane.**

VOLATILE anesthetics exert significant protection against myocardial ischemia[1] and excitotoxic cardiomyocyte death.[2] One of the mechanisms by which volatile anesthetics induce protection in myocytes is pharmacological preconditioning,[3,4] the activation of a potent endogenous protective mechanism in cardiac tissue against a variety of important stressors. Recent work from our laboratory and others unraveled the multiple complex signaling pathways involved in anesthetic-induced preconditioning in cardiomyocytes.[5,6] To date, laboratory investigations further stress the concept that volatile anesthetics may precondition endothelial and smooth muscle cells,[7] implying that anesthetic preconditioning might beneficially affect a much wider variety of tissues including the brain, spinal cord, liver, and kidneys.

In contrast, only a few small clinical studies have investigated the preconditioning effects of volatile anesthetics in human myocardium.[8–11] The results of these studies are encouraging, but they are hampered by the small number of patients and the fact that significant bias might have occurred by their mostly unblinded study design. Cardioplegic arrest in patients undergoing coronary artery bypass graft (CABG) surgery is one of the few controlled models of human myocardial ischemia. We therefore conducted a double-blinded, placebo-controlled trial including these patients. We hypothesized that sevoflurane preconditioning would decrease postoperative plasma concentrations of N-terminal pro brain natriuretic peptide (NT-proBNP). NT-proBNP was chosen because of its pivotal role as a sensitive correlate of myocardial dysfunction[12–16] and its prognostic value for predicting the short- and long-term risk of myocardial infarction, heart failure, and cardiac death.[12,14,17,18] The diagnostic and prognostic value of BNP was previously well established by angiography, echocardiography, concomitant measurements of hemodynamic parameters, and radionuclide ventriculography in a variety of clinical settings including the specific situation of cardiac recov-

* Research Fellow, ‖ Research Associate, ** Head, Cardiac Anesthesia, ## Professor and Chairman, Department of Anesthesiology, §§ Professor and Chairman, Department of Cardiovascular Surgery, University Hospital Lausanne, Lausanne. † Ph.D. Student, Institute of Pharmacology and Toxicology, ‡ Research Fellow, †† Professor and Head, Division of Cardiovascular Anesthesia, ‖‖ Professor and Chairman, *** Head, Cardiovascular Anesthesia Laboratory, Institute of Anesthesiology Triemli, § Head, General Analytics Laboratory, Institute of Clinical Chemistry, ‡‡ Professor and Chairman, Department of Cardiovascular Surgery, University Hospital Zurich, Zurich. # Chief, Institute of Anesthesia, City Hospital and Institute of Phamacology and Toxicology, Zurich, Switzerland.

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Address reprint requests to Dr. Zaugg: Institute of Anesthesiology, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland. Address electronic mail to: Michael.zaugg@ifa.usz.ch.

ery following CABG surgery.[15,16,19–23] Although not primary outcome measures in this study, several biochemical markers for myocardial damage (creatine kinase–MB [CK-MB] activity and cardiac troponin T [cTnT]) were measured perioperatively, and the incidence of perioperative myocardial ischemic and arrhythmic events was assessed by Holter electrocardiography. In addition, translocation of protein kinase C (PKC) isoforms to subcellular targets was visualized in atrial samples by immunohistochemical methods and served to determine the occurrence of an effective preconditioning process at the cellular level. Finally, cardiopulmonary bypass (CPB)–associated renal dysfunction was assessed in all patients by measuring the postoperative plasma concentrations of cystatin C (CysC), a more sensitive marker of subtle changes in the renal glomerular filtration rate than plasma creatinine concentrations.[24–26]

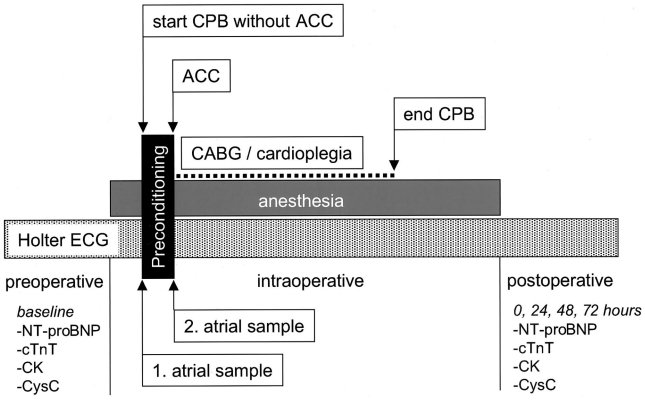

Fig. 1. Schematic diagram of the preconditioning protocol and perioperative data collection. ACC = aortic cross clamping; CABG = coronary artery bypass graft surgery; CPB = cardiopulmonary bypass; CK = creatine kinase; cTnT = cardiac troponin T; CysC = cystatin C; ECG = electrocardiography; NT-proBNP = N-terminal pro brain natriuretic peptide.

Patients and Methods

The local ethics committees of the three hospital centers approved this study, and written informed consent was obtained from all patients. Seventy-two patients scheduled for elective CABG surgery were enrolled at three hospital centers in Switzerland, the University Hospital Zurich, the City Hospital Triemli Zurich, and the University Hospital Lausanne.

Study Criteria

Inclusion criteria were being scheduled for elective CABG surgery on CPB circuit with cardiac arrest and age of 40–80 yr. Exclusion criteria were as follows: concomitant aortic or valvular surgery, elevated cardiac enzyme concentrations within 24 h before surgery, unstable angina, angina within 24 h before surgery, left ventricular bundle branch block or marked resting ST–T-segment abnormalities precluding electrocardiography interpretation, cardiac pacemaker dependency, hemodynamic instability with the need for medical or mechanical inotropic support, anesthesia or surgery within 24 h before CABG surgery, and administration of adenosine triphosphate–sensitive potassium channel agonists or antagonists such as diazoxide, nicorandil, sulfonylurea, or theophylline.

Anesthetic and Surgical Management and Preconditioning Protocol

The immediate study period included the preoperative period with a duration of at least 12 h before CABG surgery through 72 h after surgery (fig. 1). On the preoperative day, monitoring with Holter electrocardiography was started until 72 h after CABG surgery. On the same day, patients were randomly allocated (by opening of an envelope) to preconditioning with placebo or sevoflurane. All patients received midazolam or flunitrazepam for premedication. Anesthesia was induced in all patients with propofol or etomidate, opioids including fentanyl or remifentanil, and the muscle relaxants pancuronium or vecuronium. All necessary monitoring lines were then inserted, and anesthesia was maintained with propofol infusion, continuous infusion or repeated doses of opioids, and pancuronium or vecuronium administration as required. Median sternotomy and pericardiotomy were performed, and the right atrium and the ascending aorta were cannulated. After administration of heparin (300 U/kg), standard CPB with a disposable hollow fiber oxygenator and aprotinin (2×10^6 U) administered to the priming volume was started. With completely established CPB ($2.4\ l \cdot min^{-1} \cdot m^{-2}$ body surface area) and with the heart totally decompressed, the vaporizer was set to 4 vol% for exactly 10 min. To apply the preconditioning stimulus, a sevoflurane vaporizer Sevotec 5 (Abbott, Baar, Switzerland) was integrated into the CPB machine between the fresh gas flow inlet and the membrane oxygenator. Preconditioning with placebo was achieved by administration of an oxygen–air gas mixture without sevoflurane. Patients assigned to preconditioning with sevoflurane received sevoflurane 4 vol% corresponding to 2 minimum alveolar concentration. Sevoflurane concentrations were also measured by gas chromatography (Perkin–Elmer, Norwalk, CT) in selected patients ($n = 10$); these concentrations reached 1.4 ± 0.4 mM in the venous reservoir of the CPB circuit at the end of the preconditioning procedure. Blinding of the treatment to surgeons, anesthesiologists, and perfusionists was guaranteed by covering the anesthetic liquid label of the vaporizer, which was either completely filled with sevoflurane or totally empty depending on the randomization. Phenylephrine was administered to maintain aortic blood pressure above 50 mmHg. Immediately after this preconditioning period, the aorta was cross clamped

and the cold (4°C) cardioplegic solution was administered to achieve cardiac arrest. No volatile anesthetics were administered during the study except for the preischemic period in patients randomized to the sevoflurane group. Depending on the anatomic circumstances and the surgeons' preferences, cardioplegic solution with or without blood was administered anterogradely, retrogradely, or both every 10 min intermittently. Distal anastomoses were performed during a single period of aortic cross clamping. After completion of the distal anastomoses, cold cardioplegic solution was administered through the vein grafts. The proximal anastomoses were conducted during side clamping. In all patients, at least one internal mammary artery graft was used. Core temperature was actively cooled to 32°C or was allowed to drop spontaneously with α-stat regulation of blood pH. Atrial samples were taken before preconditioning at the time of cannulation and at the end of the preconditioning stimulus immediately before induction of cardioplegic arrest (fig. 1). After termination of CPB, heparin was antagonized by protamine. Hemoglobin concentrations were kept above 7 g/dl during CPB and above 9 g/dl postoperatively. After surgery, all patients were admitted to the intensive care unit. Patients from both treatment groups received the same routine postoperative care as determined by the caring physicians.

Determination of Biochemical Markers

Blood samples were obtained preoperatively, at arrival in the intensive care unit, and 24, 48, and 72 h after surgery; they were stored at −20°C until analysis. The following parameters were determined using the Roche/Hitachi 917 or the Roche Elecsys 2010 (Roche Diagnostics, Mannheim, Germany): NT-proBNP (electrochemiluminescence sandwich immunoassay)—sensitivity, >5 ng/l; intra- and interassay coefficients of variance, <3%; normal value (97.5th percentile, age of older than 50 yr) for men, <334 ng/l; normal value for women, <227 ng/l; total creatine kinase (enzymatic reaction with NADPH formation)—sensitivity, 2 U/l; intra- and interassay coefficients of variance, <2%; normal value for men, ≤170 U/l; normal value for women, ≤145 U/l; CK-MB activity (immunologic ultraviolet assay)—sensitivity, 5 U/l; intra- and interassay coefficients of variance, <2%; normal value, ≤24 U/l; cTnT (electrochemiluminescence sandwich immunoassay)—sensitivity, >0.01 μg/l; intra- and interassay coefficients of variance, <5%; normal value, <0.01 μg/l; creatinine (Jaffé reaction)—sensitivity, 8.8 μM; intra- and interassay coefficients of variance, <2.5%; normal range for men, 62–106 μM; normal range for women, 44–80 μM; and high-sensitivity C-reactive protein (immunoturbidimetric assay)—sensitivity, 0.3 mg/dl; intra- and interassay coefficients of variance, <6%; normal value, <1 mg/dl. CysC assays were purchased from Dako A/S, Glostrup, Denmark (particle-enhanced turbidimetric assay: sensitivity, 0.2 mg/l; intra- and interassay coefficients of variance, <3.5%; normal range, 0.74–1.50 mg/l).

Holter Electrocardiography

Three-channel digital Holter electrocardiography monitoring was begun at least 12 h before surgery and continued for 72 h postoperatively (8500 series; GE Marquette Medical Systems, WI). Seven bipolar leads were simultaneously recorded using silver–silver chloride electrodes. Lead resistance was tested every 6 h, and faulty leads were replaced. The effect of patient positional variation was measured in supine and upright positions before the study. Holter electrocardiography data were analyzed for ST-segment changes (Marquette Holter analysis system software version 8500; GE Marquette Medical Systems, WI) indicative of ischemia after exclusion of abnormal QRS complexes, such as ventricular ectopic beats or beats with conduction abnormalities. ST-segment changes were trended in three leads for the duration of the recording. Baseline ST-segment levels were defined as the average ST-segment during stable periods (at least 10 min) preceding each ischemic period. Two independent assessors blinded to group assignment, clinical course, and the various plasma parameters reviewed all ischemic periods. Disagreement was resolved by consensus. Electrocardiographic ischemic periods were defined as reversible ST-segment changes lasting at least 1 min and involving either a shift from baseline of ≥0.1 mV of ST depression or a shift from baseline of ≥0.2 mV of ST–T elevation at the J point. ST-segment depression was measured 60 ms after the J point, unless that point fell within the T wave, in which case it was shortened to the J point plus 40 ms. Holter analysis was corrected for positional variation by taking the maximum shift noted by positional changes. The following parameters were measured as indicators of the severity of each ischemic episode: total episodes per patient with ischemia, maximal ST depression, duration of longest ischemic episode, total area under the curve (defined as the integral of ST depression in mV × duration), and maximal area under the curve. Perioperative time was divided into three separate episodes (T1 = preoperative period; T2 = first 24 h postoperatively; T3 = 24–72 h postoperatively). Intraoperative Holter electrocardiography recordings were not used for comparison of the two anesthetic groups due to the many confounding variables such as movement, artifacts due to chest opening, electrocautery interference, ventricular pacing, and electrolyte artifacts. Holter electrocardiography recordings were further analyzed for the occurrence of dysrhythmias using the above-mentioned software. Ventricular and supraventricular ectopic beats were identified and counted as isolated, bigeminal cycles, couplets, or runs. The number of runs and the

percent of recorded time with atrial fibrillation were calculated separately for each period.

Immunohistochemical Analysis of Human Atrial Samples

Translocation of PKC isoforms in response to preconditioning was assessed by immunofluorescence in all samples.[27,28] Briefly, right atrial tissue samples were placed in Hanks solution, immediately frozen in liquid nitrogen, and stored at −70°C. Cryosections (5 μm) were prepared with a cryostat (Cryo-star HM 560 M; Microtom, Kalamazoo, MI) and collected on slides precoated with gelatin. All sections were fixed for 10 min in 100% acetone at −20°C, rinsed with phosphate-buffered saline, and incubated in 10% normal goat serum for 30 min to block nonspecific binding. Sections were incubated for 1 h at room temperature with primary antibodies. Rabbit polyclonal antibodies to PKC δ and PKC ϵ (Santa Cruz Biotechnology, Santa Cruz, CA) diluted in phosphate-buffered saline (1:400–1:1,200) containing 2% normal goat serum were added. Antibodies to PKC were combined with the following: mouse monoclonal α-prohibitin-1 antibody (Research Diagnostics, Flanders, NJ) (1:25) as a mitochondrial marker; guinea pig polyclonal m-dystrophin antibodies (gift from Richard A. Zuellig, Ph.D., Institute of Pharmacology, University Zurich, Switzerland) (1:2,000) as a sarcolemmal marker; mouse monoclonal α-*N*-cadherin antibody (Sigma, St. Louis, MO) as a marker for the intercalated disks (1:500); and rabbit polyclonal α-myomesin antibodies as a marker for cardiomyocytes (gift from Hans M. Eppenberger, Ph.D., Professor of Cell Biology, Department of Cell Biology, Swiss Federal Institute of Technology Zurich, Zurich, Switzerland) (1:200). All sections were then washed with phosphate-buffered saline twice for 5 min each and incubated for 1 h with a mixture of secondary antibodies conjugated to Alexa Fluor 555 goat α-rabbit, Alexa Fluor 488 goat α-mouse, or Alexa Fluor 488 goat α-guinea pig antibody (Molecular Probes, Eugene, OR) (1:500) and 4′,6-diamidino-2-phenylindole-2 hydrochloride (DAPI) (10 ng/ml) (Sigma, St. Louis, MO) in phosphate-buffered saline at room temperature. After washing with phosphate-buffered saline, sections were protected with coverslips using DAKO mounting medium (DAKO, Carpinteria, CA). Sections were analyzed by epifluorescence microscopy using an upright microscope (Axioplan 2; Zeiss, Jena, Germany) with appropriate filter blocks for the detection of fluorescein isothiocyanate, tetramethyl rhodamine isothiocyanate, CY5, and ultraviolet fluorescence. In addition, confocal images were obtained with an LSM Pascal confocal microscope (Zeiss, Jena, Germany) using the appropriate laser lines and filter blocks (488, 540, and 640 nm). Since aged human tissue has a significant amount of lipofuscin-induced autofluorescence and the CY5 filter shows only the lipofuscin autofluorescence, the relative signal intensity in the three channels (fluorescein isothiocyanate, tetramethyl rhodamine isothiocyanate, and CY5) was used to separate on the composite image PKC labeling from unspecific lipofuscin signals.[29] Randomly chosen fields of sections from all samples were examined for translocation of PKC δ and PKC ϵ to sarcolemma (colocalization with dystrophin), mitochondria (colocalization with prohibitin-1), intercalated disks (colocalization with *N*-cadherin), or nuclei (colocalization with DAPI) without prior knowledge of the treatment. Confocal imaging was used to quantify PKC ϵ translocation to nuclei of cardiomyocytes. Five randomly chosen fields at a ×400 magnification from sections of samples from each treatment group (placebo and sevoflurane) collected before and after preconditioning were analyzed for colocalization of PKC δ with nuclei (DAPI staining). Double staining with myomesin was used to assure that only PKC ϵ translocation to cardiomyocytes was counted. The number of PKC ϵ–positive nuclei was expressed as the percentage of total nuclei of myocytes per field.

Clinical Outcome Analysis

Medical charts were reviewed, and the caregivers were interviewed daily for the occurrence of postoperative cardiovascular and renal adverse events. Adverse events (as opposed to myocardial and renal injury markers determined at the end of the study) were diagnosed by the independently managing clinicians. The diagnosis of a new postoperative myocardial infarction required a new Q wave, persistent ST–T-segment changes as defined by Minnesota Codes,[30] and/or association with elevated CK-MB isoenzyme activity (concentration, >100 U/l). The diagnosis of a cerebrovascular insult required the presence of clinical symptoms and/or a positive computer-assisted tomography scan. The diagnosis of significant postoperative renal dysfunction required newly established postoperative hemodialysis or hemofiltration.

Statistical Analysis

The sample size was calculated based on previously reported data for BNP concentrations.[12–16] With an expected difference of 30% between group means, 40% SD of the means, $\alpha = 0.05$, and $\beta = 0.8$, a sample size of 24 patients per group was necessary. A logarithmic transformation was applied to all data to ensure a normal distribution before statistical analysis. Two-factor repeated-measures analysis of variance was used to evaluate differences over time between groups for all parameters determined in plasma samples. The Greenhouse–Geisser correction was used to address the deviation from sphericity. Multiple paired *t* tests were used to compare the parameters at each time point with the respective preoperative baseline measurements within groups, and un-

Table 1. Patient Characteristics

	Placebo Group (n = 35)	Sevoflurane Group (n = 37)
Mean age (yr) ± SD (range)	65 ± 10 (44–79)	62 ± 10 (44–80)
Sex (male/female)	28/7	31/6
Mean preoperative hs-CRP level (mg/l)	2.2 (1.3, 4.1)*	2.8 (1.4, 7.7)*
Mean preoperative hemoglobin (g/dl) ± SD (range)	12.1 ± 2.1 (10.7–16.9)	13.1 ± 2.3 (11.1–16.8)
Mean preoperative ejection fraction (%) ± SD (range)	57.1 ± 11.4 (40–76)	54.4 ± 12.2 (30–78)
Mean no. of diseased vessels ± SD (range)	2.6 ± 0.5 (1–3)	2.6 ± 0.5 (1–3)
No. (%) of patients		
2 vessels with >70% stenosis	33 (94)	34 (91)
Left main stem stenosis	25 (71)	24 (64)
Previous myocardial infarction	13 (37)	10 (27)
Diabetes mellitus	9 (25)	9 (24)
Hypertension	22 (62)	26 (70)
Smoking	19 (54)	24 (64)
Hypercholesterolemia	28 (80)	34 (91)
Preoperative dialysis	0 (0)	1 (2)
Current medication		
β-Blocker	28 (80)	34 (91)
Ca^{2+}-blocker	6 (17)	6 (16)
Nitrates	14 (40)	20 (54)
ACEI	10 (28)	19 (51)
Statins	28 (80)	31 (83)
Diuretics	9 (25)	11 (29)

The two groups were similar in all patient characteristics.

* 25th, 75th percentiles.

ACEI = angiotensin-converting enzyme inhibitor; hs-CRP = high-sensitivity C-reactive protein.

paired *t* tests were used to compare these parameters at each time point between groups. All other data were analyzed using unpaired *t* tests for parametric data or Mann–Whitney tests for nonparametric data. Categorical data were analyzed using the two-tailed Fisher exact test or chi-square test, as appropriate. If not otherwise indicated, values are mean ± SD. *P* values were multiplied by the number of comparisons that were made (Bonferroni correction), and corrected $P < 0.05$ was considered statistically significant. Analyses were performed using SigmaStat and SPSS (SPSS, Chicago, IL).

Results

Demographics, Intraoperative Data, and Clinical Outcome

Patient characteristics are listed in table 1. The placebo and sevoflurane groups were similar with respect to all clinical data. In addition, there was no difference between groups regarding the operative data (table 2), except for phenylephrine administration during the preconditioning process. Sevoflurane-treated patients required significantly more phenylephrine during the pre-

Table 2. Intraoperative Data

	Placebo Group (n = 35)	Sevoflurane Group (n = 37)	*P**
Mean bypass time (min) ± SD (range)	106 ± 31 (54–178)	116 ± 32 (65–194)	0.22
Mean cross-clamp time (min) ± SD (range)	60 ± 24 (15–121)	66 ± 22 (37–131)	0.29
Mean no. of grafts ± SD (range)	3.4 ± 0.9 (2–5)	3.5 ± 1.1 (2–6)	0.87
Mean minimal core temperature during CPB (°C) ± SD (range)	32.1 ± 1.6 (29–35.5)	32.1 ± 1.4 (29–35)	0.92
No. of patients			
Active/passive cooling on CPB	27/8	29/8	1
Cardioplegia			
Anterograde/retrograde/both	23/1/10	22/0/15	1
With/without blood	13/22	17/20	0.48
Median phenylephrine use during preconditioning (μg)	0 (0–100)†	200 (50–525)†	0.0003
Mean total opioid use (μg)‡ ± SD (range)			
Fentanyl only	1,902 ± 613 (850–3850) (n = 22)	2,166 ± 764 (500–3100) (n = 21)	0.32
Fentanyl plus remifentanil	1,943 ± 501 (700–2,500); 6,815 ± 2,573 (3,400–10,500) (n = 13)	1,980 ± 492 (1,400–3,000); 6,680 ± 1,561 (4000–9400) (n = 16)	0.90; 0.75

The two groups were similar except for phenylephrine use during preconditioning on the cardiopulmonary bypass (CPB) circuit.

* Differences were assessed by unpaired *t* test, Mann–Whitney test, Fisher exact test (two-tailed), or chi-square test; $P < 0.05$, significant.

† 25th, 75th percentiles; ‡ One patient each from the placebo group and the sevoflurane group received a small dose (100 μg) of sufentanil.

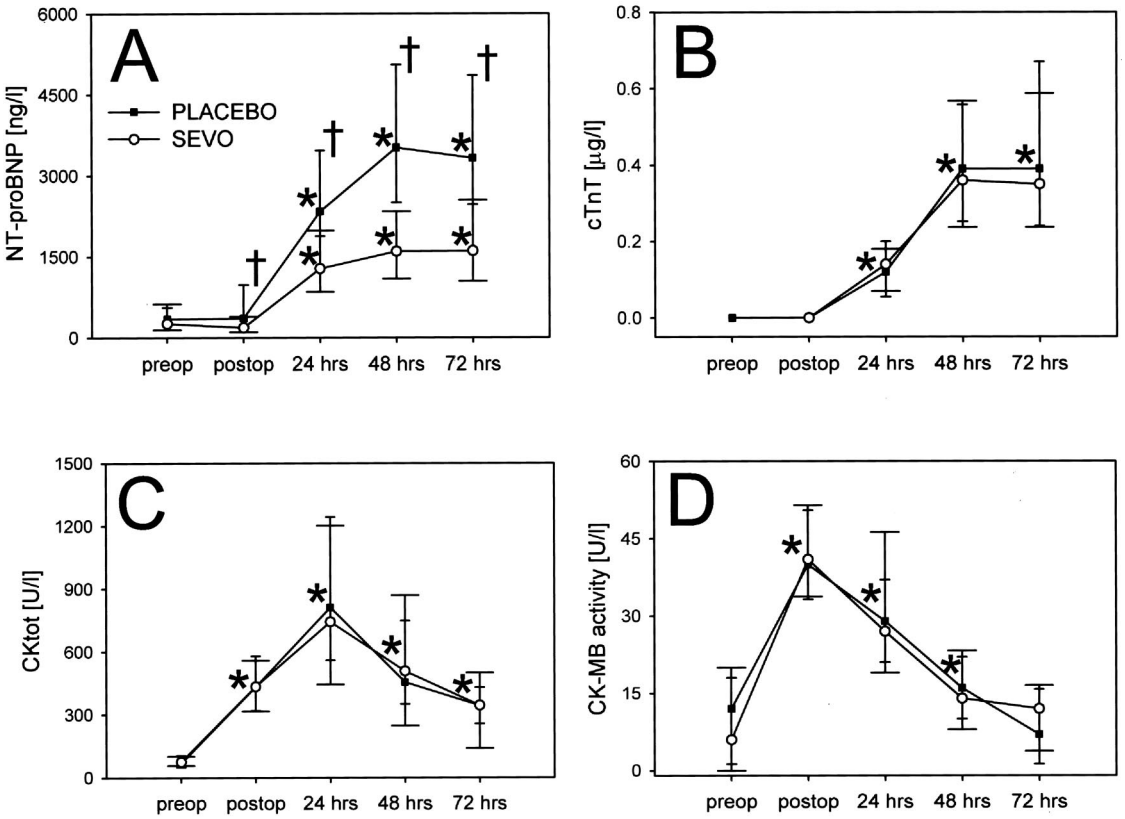

Fig. 2. Biomarkers of myocardial injury at various time points for the placebo (*PLACEBO*)– and sevoflurane (*SEVO*)–treated groups: N-terminal pro brain natriuretic peptide (NT-proBNP) (*A*), cardiac troponin T (cTnT) (*B*), total creatine kinase (CKtot) (*C*), and creatine kinase–MB (CK-MB) isoenzyme activity (*D*). Two-factor repeated-measures analysis of variance indicated that the groups significantly differed in plasma NT-proBNP concentrations (time effect, $P < 0.001$; group effect, $P < 0.001$; group–time interaction, $P = 0.003$). There was no difference for cTnT, CKtot, and CK-MB activity concentrations. Multiple *t* tests with Bonferroni correction for multiple comparisons were used to compare the plasma concentrations of the different biomarkers at each time point with the respective preoperative baseline value within groups and to compare plasma concentrations at each time point between groups. *Significantly increased compared with baseline values ($P < 0.05$). †Significant difference between groups ($P < 0.05$). *PLACEBO* group with narrow cap and *SEVO* group with wide cap. The plot shows medians and lower and upper quartiles.

conditioning process than did placebo-treated patients to maintain blood pressure above 50 mmHg. However, there was no difference in mean arterial blood pressure between the groups during preconditioning, and sevoflurane could be administered safely. Two patients in the placebo group and one patient in the sevoflurane group had a postoperative myocardial infarction as determined by the caring physicians. One placebo-treated patient required transient postoperative inotropic support with an intraaortic balloon pump due to hemodynamic instability. No cerebrovascular insult occurred in any of the patients, and none of the patients required new postoperative hemofiltration or dialysis.

Biochemical Markers for Myocardial Dysfunction

Preoperative NT-proBNP concentrations were similar in placebo- and sevoflurane-treated patients (fig. 2A). In both groups, a marked increase was observed from 24 to 72 h after surgery (time effect, $P < 0.001$). However, postoperative plasma concentrations were significantly lower in sevoflurane-treated patients than in placebo-treated patients (group effect, $P < 0.001$; group–time interaction, $P = 0.003$). In addition, postoperative peak plasma NT-proBNP concentrations were markedly lower in sevoflurane-treated patients than in placebo-treated patients (2,180 ± 1,118 ng/l *vs.* 4,841 ± 2,937 ng/l, respectively; $P < 0.001$).

Biochemical Markers for Myocardial Necrosis

Preoperative plasma concentrations of cTnT, total creatine kinase, and CK-MB activity were within normal limits in all patients and were not different between groups (figs. 2B–D). A significant postoperative increase in concentrations of cTnT, total creatine kinase, and CK-MB activity was observed in the placebo and sevoflurane groups (time effect for all parameters, $P < 0.001$). Although CK-MB activity peaked immediately after surgery, cTnT demonstrated a more protracted course, with the highest plasma concentration 48 h after surgery. There was no significant difference over time between groups (cTnT: group effect, $P = 0.56$; group–time interaction, $P = 0.98$; total creatine kinase: group effect, $P = 0.89$; group–time interaction, $P = 0.92$; CK-MB activity: group effect, $P = 0.79$; group–time interaction, $P =$

Table 3. Incidence of Holter Electrocardiography–Detected ST Segment Depression of ≥1 mm Lasting at Least 1 min

	Placebo Group (n = 35)		Sevoflurane Group (n = 37)		
	n	%	n	%	P*
Preoperative period	7	20	6	16	1.0
First 24 h postoperatively	7	20	4	11	0.33
24–72 h postoperatively	4	11	9	24	0.38

* Differences were assessed by Fisher exact test (two-tailed); $P < 0.05$, significant.

0.88), nor was there a difference with respect to peak concentrations between groups (postoperative peak cTnT concentration: 0.52 ± 0.37 μg/l in the sevoflurane group *vs.* 0.57 ± 0.53 μg/l in the placebo group; $P = 0.65$) (figs. 2B–D). Eighteen patients (25%) had postoperative peak cTnT concentrations of >0.65 μg/l, indicative of major perioperative myocardial damage[31]: 11 (31%) in the placebo group (1.25 ± 0.69 μg/l) and seven (19%) in the sevoflurane group (0.91 ± 0.18 μg/l) ($P = 0.28$). There was no significant increase in plasma NT-proBNP concentrations in patients with postoperative peak cTnT concentrations of >0.65 μg/l, as compared with patients with postoperative peak cTnT concentrations of <0.65 μg/l (4,504 ± 3,025 ng/l *vs.* 3,337 ± 2,686 ng/l, respectively; $P = 0.12$).

Table 5. Incidence of Arrhythmias after Release of Aortic Cross-clamp

	Placebo Group (n = 35)		Sevoflurane Group (n = 37)		
	n	%	n	%	P*
Occurrence of ventricular fibrillation	7	20	4	11	0.52
Use of lidocaine	11	31	7	19	0.42
Occurrence of atrial fibrillation	2	6	1	3	1
Need for electrical defibrillation	7	20	3	8	0.31
Occurrence of atrioventricular block	2	6	2	5	1

* Differences were assessed by Fisher exact test (two-tailed); $P < 0.05$ significant.

Holter Electrocardiography Monitoring: Perioperative ST-segment Changes and Arrhythmias

Holter electrocardiography–detected ST-segment changes were separately analyzed for the preoperative period, the first 24 h immediately after surgery, and from 24 to 72 h after surgery. There was no difference with respect to the incidence (table 3) as well as the severity of ischemic events (table 4) between both groups for any of the analyzed periods. In addition, no difference was detected regarding perioperative ventricular or supraventricular arrhythmias. The incidence of dysrhythmias at the time of aortic cross clamp release was not different between the groups (table 5).

Table 4. Severity of ST Segment Depression of ≥1 mm Lasting at Least 1 min

	Placebo Group (n = 35)	Sevoflurane Group (n = 37)	P*
No. of total episodes per patient with ischemia			
T1	24 (4–32) (7)	22 (4–102) (6)	0.53
T2	5 (2–18) (7)	40 (6–62) (4)	0.81
T3	8 (1–150) (4)	14 (4–50) (9)	0.34
Global median	9 (2–32)	17 (4–62)	0.98
Maximal ST depression (mV)			
T1	1.3 (1.1–1.9)	1.9 (1.7–2.3)	0.20
T2	1.7 (1.1–2.2)	1.5 (1.1–2.1)	0.91
T3	1.6 (1.3–2.3)	1.5 (1.3–2.1)	0.80
Global median	1.4 (1.1–2.1)	1.6 (1.4–2.2)	0.32
Duration of longest episode (min)			
T1	8 (2–28)	24 (3–71)	0.21
T2	2 (1–5)	12 (3–41)	0.86
T3	3 (1–20)	5 (3–21)	0.54
Global median	3 (2–23)	9 (3–27)	0.23
Total area under the curve (mV/min)			
T1	55 (4–142)	111 (7–576)	0.36
T2	8 (7–19)	115 (25–234)	0.98
T3	15 (2–638)	30 (8–135)	0.66
Global median	10 (4–125)	62 (8–165)	0.77
Maximal area under the curve (mV/min)			
T1	8 (2–30)	33 (3–79)	0.16
T2	3 (1–6)	12 (3–48)	0.88
T3	15 (2–638)	30 (8–135)	0.54
Global median	4 (2–27)	10 (4–34)	0.20

Data are median (25th–75th percentile) (no. of patients).

* Differences were assessed by the Mann–Whitney test; $P < 0.05$, significant.

T1 = preoperative period; T2 = first 24 h postoperatively; T3 = 24–72 h postoperatively.

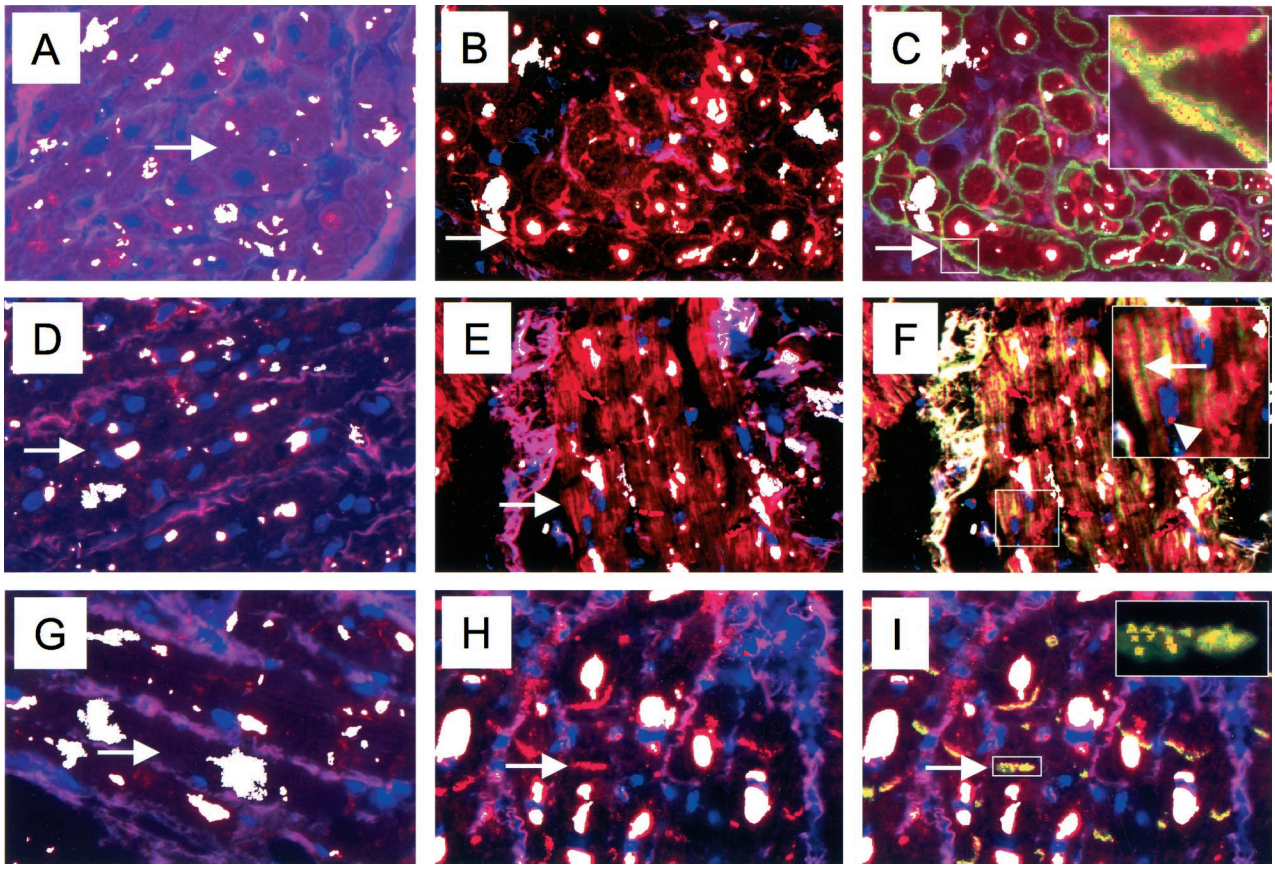

Fig. 3. Immunofluorescence of protein kinase C (PKC) isoforms (see Methods). Translocations of PKC δ (*A–C*) and PKC ϵ (*D–I*) were evaluated in response to sevoflurane preconditioning in human atrial tissue samples from the same patient. (*A, D, G*) Sections from samples before sevoflurane preconditioning indicating diffuse PKC δ and ϵ distribution. (*B, E, H*) Sections from samples after sevoflurane preconditioning indicating translocation to the specific subcellular targets. (*C, F, I*) Colocalization of PKC with the specific markers (same fields as in [*B, E, H*] but double stained for colocalization). (*A*) Diffuse cytoplasmic distribution of PKC δ before preconditioning. (*B*) Increased immunofluorescence of PKC δ is observed in sarcolemma after sevoflurane preconditioning (arrow). (*C*) Yellowish green color represents immunostaining for PKC δ, which is seen over sarcolemma if colocalized (arrow, see small window with ×4 magnification). (*D*) Diffuse PKC ϵ staining before preconditioning. (*E*) PKC ϵ translocation after preconditioning with sevoflurane (arrow). (*F*) PKC ϵ is colocalized between contractile bundles in mitochondria (arrow, small window with ×2.5 magnification). Note PKC ϵ in nuclei (arrowhead). (*G*) Diffuse PKC ϵ staining before preconditioning. (*H* and *I*) PKC ϵ is prominently distributed in the intercalated disks after sevoflurane preconditioning and clearly colocalized with *N*-cadherin (arrow, small window with ×3 magnification). White spots indicate the presence of lipofuscin, a pigment with strong autofluorescence and characteristic for aged human tissue. (*A, D, G*) Epifluorescence micrographs. (*B, C, E, F, H*) Confocal micrographs. All images with ×400 magnification.

Evidence of PKC Translocation in Human Atrial Samples

Translocation of the PKC isoforms δ and ϵ in response to sevoflurane treatment was assessed by colocalization with sarcolemma (dystrophin), mitochondria (prohibitin-1), intercalated disks (*N*-cadherin), and nuclear staining with DAPI. There was clear translocation of PKC δ to sarcolemma in sevoflurane-treated atrial samples as compared with samples without the preconditioning stimulus (figs. 3A–C). In addition, PKC ϵ translocated to mitochondria (figs. 3D–F), intercalated disks (figs. 3G–I), and nuclei after sevoflurane treatment. To quantify the PKC ϵ translocation in response to sevoflurane exposure, the percentage of nuclei with clear PKC ϵ colocalization was determined in cardiomyocytes of atrial samples before and after sevoflurane preconditioning as well as in time-matched placebo-treated samples using confocal microscopy (fig. 4A–D). The results of these experiments demonstrated that the percentage of PKC ϵ–positive nuclei was markedly increased after the application of sevoflurane (before sevoflurane application, 14 ± 11%; after sevoflurane application, 63 ± 14%; $P < 0.001$).

Postoperative Renal Dysfunction

There was no difference in baseline plasma CysC concentrations between groups. CysC concentrations significantly increased immediately postoperatively and peaked at 48 h after surgery for both groups (time effect, $P < 0.001$). CysC concentrations were markedly higher for placebo-treated patients than for sevoflurane-treated pa-

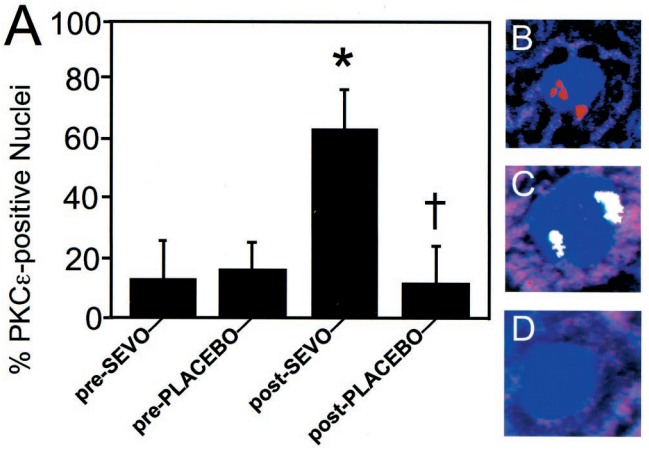

Fig. 4. Translocation of PKC ϵ to cardiomyocytes. (*A*) Percentages of protein kinase C (PKC) ϵ–positive myocytes. Data are mean ± SD. (*B*) Representative nucleus of a PKC ϵ–positive cardiomyocyte. (*C*) Cardiomyocyte with no PKC ϵ but lipofuscin pigment in the nucleus. (*D*) Cardiomyocyte with neither PKC ϵ nor pigment in the nucleus. *Significantly increased compared with *pre-SEVO* (before sevoflurane preconditioning) ($P < 0.001$). †Not significantly different from *pre-SEVO* or *pre-PLACEBO* (before placebo preconditioning). Post-PLACEBO = time-matched control after placebo preconditioning; post-SEVO = after sevoflurane preconditioning.

tients (group effect, $P < 0.009$; group–time interaction, $P < 0.001$), including postoperative peak plasma CysC concentrations (1.58 ± 0.67 mg/l *vs.* 1.21 ± 0.28 mg/l, respectively; $P = 0.0039$) (fig. 5A). Using the prospectively defined cut-off concentration of 1.5 mg/l for CysC, 10 patients in the placebo group but only two patients in the sevoflurane group had new postoperative renal dysfunction ($P < 0.015$). Plasma creatinine concentrations were slightly, but not significantly, increased in placebo-treated patients (time effect, $P < 0.001$; group effect, $P = 0.28$; group–time interaction, $P = 0.98$) (fig. 5B). However, postoperative peak plasma creatinine concentrations were significantly higher in the 10 placebo-treated patients with newly detected postoperative renal dysfunction (148 ± 33 μM) than in patients without postoperative renal dysfunction (96 ± 21 μM) ($P < 0.0001$). As a result of dilution, plasma creatinine concentrations decreased in both groups during the first hours after surgery.

Discussion

To our knowledge, this study is the first double-blinded, placebo-controlled, randomized clinical trial evaluating pharmacological preconditioning elicited by volatile anesthetics. The principal new findings of the study are as follows. First, sevoflurane preconditioning significantly decreased postoperative release of NT-proBNP, a highly sensitive biochemical marker of the myocardial contractile state, in patients undergoing CABG surgery after cardioplegic arrest. Second, the concept of PKC translocation as a pivotal signaling step in the initiation of preconditioning elicited by volatile anesthetics could be directly visualized and confirmed for the first time in human myocardium. Finally, although not a primary outcome measure, the postoperative renal glomerular filtration rate was markedly higher among sevoflurane-preconditioned patients, indicating a preconditioning-like preservation of renal function by systemically administered sevoflurane.

BNP: A Biochemical Marker of Myocardial Dysfunction

The B-type natriuretic peptide (BNP) together with the A-type natriuretic peptide regulate blood pressure by modulating water and salt homeostasis.[32] Although BNP was first isolated from porcine brain in 1988,[33] studies

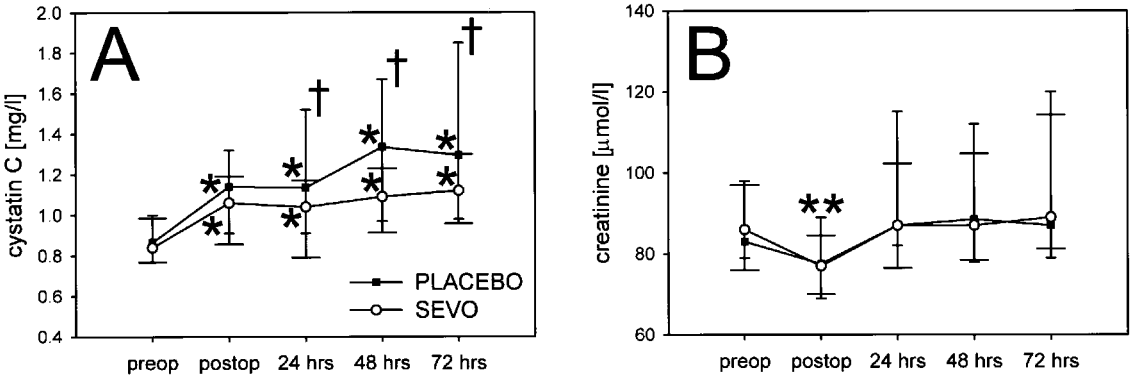

Fig. 5. Biomarkers for perioperative renal function at various time points for the placebo (*PLACEBO*)– and sevoflurane (*SEVO*)–treated groups: CysC (*A*) and creatinine (*B*). Two-factor repeated-measures analysis of variance indicated that the groups significantly differed in plasma CysC concentrations (time effect, $P < 0.001$; group effect, $P < 0.009$; group–time interaction, $P < 0.001$). Multiple *t* tests with Bonferroni correction for multiple comparisons were used to compare the plasma concentrations of CysC and creatinine at each time point with the respective preoperative baseline value within groups and to compare plasma concentrations at each time point between groups. *Significantly increased compared with baseline values ($P < 0.05$). **Significantly decreased compared with baseline values ($P < 0.05$). †Significant difference between groups ($P < 0.05$). *PLACEBO* group with narrow cap and *SEVO* group with wide cap. The plot shows medians and lower and upper quartiles.

over the last decade clearly have shown that the heart is the main source of this hormone.[34] The physiologically active form of BNP (C-terminal BNP) derives from the precursor proBNP, is composed of a 17-amino acid residue ring structure, and is continuously released from ventricular myocytes into the circulation in response to intracavitary pressure and diastolic wall stress (Laplace law).[35] Conversely, the biologically inactive N-terminal cleavage product, NT-proBNP, is also circulating at blood concentrations similar to C-terminal BNP in healthy volunteers,[36] but it exhibits greater proportional and absolute increments for a defined degree of cardiac dysfunction than does C-terminal BNP, suggesting its superior role as an indicator of cardiac impairment and cardiovascular prognosis.[13,37,38] BNP mediates its biologic effects including natriuresis, diuresis, vasodilation, and antagonism to renin, aldosterone, and catecholamine action by the guanylate cyclase–linked receptor natriuretic receptor A, which forms cGMP as a second messenger and is found in a variety of tissues including the endothelium.[39] Results from numerous studies over the last decade strongly confirm the close correlation of blood BNP concentrations increased in proportion to the severity of cardiac dysfunction. Accordingly, increasing BNP concentrations correspond to New York Heart Association (New York, NY) functional classes, closely reflect simultaneously measured hemodynamic indices including pulmonary capillary wedge pressure, and correlate inversely with cardiac output and ejection fraction as determined by angiography, echocardiography, and radionuclide ventriculography.[19–23] After myocardial infarction, C-terminal BNP and particularly NT-proBNP show an inverse relation with ventricular function including left ventricular ejection fraction and ventricular systolic and diastolic volumes, which are indicators of the postinfarct ventricular remodeling process.[13] Moreover, increased BNP concentrations are independent predictors for future cardiovascular adverse events, including heart failure and death, in a variety of clinical settings without the use of other invasive or expensive diagnostic tests.[12,14,17,18] Notably, elevated blood NT-proBNP concentrations were even successfully used as targets for the titration of effective angiotensin-converting enzyme inhibitor therapy for patients with congestive heart failure.[38] Recently, BNP also received attention as a marker of functional recovery of the myocardium following CPB. Morimoto *et al.*[16] found in a heterogeneous population undergoing cardiac surgery that blood BNP concentrations became markedly and acutely elevated starting 12 h after CPB, returning to baseline not until 3 weeks postoperatively. In this study, peak postoperative BNP concentrations were inversely correlated with postoperative left ventricular ejection fraction and cardiac output. In another study of patients undergoing CABG surgery with CPB, peak and cumulative BNP release, as determined from coronary sinus blood during myocardial reperfusion, closely correlated with myocardial lactate production.[40] Taken together, BNP has a pivotal role in the counterregulatory response to cardiac failure, thereby acting as a highly sensitive index of the extent and severity of cardiac dysfunction.

On the basis of this pathophysiologic background, we chose NT-proBNP, beside other cardiac injury markers, to assess the myocardial preconditioning effects of sevoflurane in patients undergoing elective CABG surgery with total CPB. The results of our study demonstrated that a single brief exposure of sevoflurane immediately prior to cardioplegic arrest dramatically reduced the postoperative NT-proBNP concentration, a biochemical marker of myocardial dysfunction. This is the first time the beneficial cardiac effects of preconditioning could be directly linked to and determined by the neurohormonal indicator BNP. Surprisingly, we did not find a significant difference between groups with respect to markers of myocardial necrosis such as CK-MB activity or cTnT concentration. However, since increased concentrations of myocardial enzymes after CABG surgery derive from cannulation of the right atrium, cardioplegia itself, inadequate delivery of cardioplegia in the presence of stenosis or hypertrophy, vigorous manipulations of the heart, prolonged surgery, and differences in surgical techniques, they may not properly reflect the protection afforded by preconditioning. Moreover, properly administered cardioplegia may prevent major structural damage and yet leave postischemic myocardium amenable to functional improvement by preconditioning. The observation that cTnT and NT-proBNP release did not parallel each other in fact supports the recently observed concept that NT-proBNP reflects the contractile state of the myocardium *per se* rather than structural damage or ischemia.[14,17] No difference was observed between groups with respect to perioperative ST-segment changes. However, electrocardiography is less sensitive for the diffuse forms of postcardiac surgery–related myocardial ischemia and may underestimate myocardial injury.[41] We did not observe an antiarrhythmic effect of preconditioning elicited by volatile anesthetics. Preischemic administration of volatile anesthetics can decrease the incidence of postischemic ventricular arrhythmias, particularly in small animals. However, in larger animals and humans, this effect is more controversial.[9,42] Together, each parameter (cTnT, Holter electrocardiography, and BNP) reflects a unique independent axis in the evolution of cardiac outcome and adds complementary incremental information about the cardioprotective mechanisms underlying anesthetic preconditioning in human myocardium.

Previous Studies on Volatile Anesthetics–induced Preconditioning

So far, preconditioning by volatile anesthetics has been evaluated in four unblinded studies with patients under-

going on-pump CABG surgery.[8–11] An additional small study compared sevoflurane with propofol anesthesia in patients who underwent CABG surgery and found improved myocardial function and decreased release of cardiac troponin I with the sevoflurane-based anesthetic.[43] These studies, although partially contradictory in their results, provide evidence that volatile anesthetics may protect human myocardium and support our observation of a cardiac benefit from sevoflurane preconditioning. Penta de Peppo *et al.*[11] applied enflurane, 1.3 vol%, *via* the respirator over 5 min immediately before CPB. Consistent with our results, preconditioning afforded increased postoperative left ventricular contractility, but no decrease in CK-MB or troponin T release was found. In another study,[8] administration of isoflurane, 2.7 vol%, for 5 min on the fully established CPB followed by a 10-min washout period before aortic cross clamping only demonstrated a tendency to lower CK-MB and troponin I release (not statistically significant). Moreover, Haroun-Bizri *et al.*[9] demonstrated increased postoperative cardiac index after administering isoflurane, 0.5–2 vol%, shortly before CPB *via* the respirator. This study further showed decreased intraoperative ST-segment changes, but no reduction was found in reperfusion dysrhythmias after the release of the aortic cross clamp in preconditioned patients. No ST-segment changes or arrhythmias were assessed postoperatively in this study. Tomai *et al.*[10] applied isoflurane, 1.5 vol%, for 15 min through the respirator followed by a washout period of 10 min before starting CPB. In contrast to the aforementioned studies, no difference in postoperative cardiac index and left ventricular ejection fraction was noticed between the control group and the preconditioned group. However, a decrease in postoperative CK-MB and troponin I release could be detected in a subgroup of patients with poor preoperative left ventricular ejection fraction (<50%).

Translocation of PKC Isoforms

Although a series of experimental studies implicated PKC activation as a pivotal step in anesthetic preconditioning,[5] to date it is not clear whether preconditioning-specific translocation of PKC to subcellular targets also occurs in response to volatile anesthetics, particularly in human myocardium. Volatile anesthetics prime the activation of sarcolemmal and mitochondrial K_{ATP} channels,[5,6] the putative end-effectors of preconditioning *via* stimulation of adenosine receptors and subsequent activation of PKC as well as *via* increased formation of NO and free oxygen radicals. Activated PKC acts as a pivotal amplifier of the preconditioning stimulus and stabilizes, by phosphorylation, the open states of the mitochondrial and sarcolemmal K_{ATP} channels. The opening of K_{ATP} channels ultimately elicits cytoprotection by decreasing cytosolic and mitochondrial Ca^{2+} overload. PKC with its isoform-specific translocation is one of the most prominent kinases associated with the preconditioned state.[44] The results of this study clearly showed that PKC δ was translocated to the sarcolemma and PKC ϵ was translocated to mitochondria, intercalated disks, and nuclei in response to sevoflurane preconditioning. Translocation of PKC from the cytosolic to the particulate compartments is commonly used as an index of PKC activation. We used immunohistochemical analysis to determine subcellular translocation of PKC isoforms.[27,28] Since this technique primarily provides qualitative data, we also determined the number of nuclei with clear PKC ϵ colocalization to quantify the preconditioning effect. The results of these studies strongly support the concept that volatile anesthetics induce PKC translocation *in vivo*. These findings are also in clear accordance with a previous observation that ecto-5'-nucleotidase, a marker of PKC activity, is increased in response to isoflurane administration in human myocardium.[8] Interestingly, because PKC δ and PKC ϵ are Ca^{2+}-insensitive isoforms, it appears that volatile anesthetics mainly affect Ca^{2+}-independent PKC signaling pathways. We recently demonstrated that cardioprotection afforded by volatile anesthetics was sensitive to modulations of the NO signaling pathway.[5] Further, NO is known to induce activation and translocation of PKC ϵ in cardiomyocytes.[45] Thus, it may well be possible that volatile anesthetics elicit the subcellular redistribution of PKC in human myocardium at least, in part, by the NO signaling pathway.

Renal Dysfunction after CABG Surgery

It is well documented that CABG surgery with the use of CPB causes ischemia, inflammation, and a significant embolic load to the whole body, leading to global organ dysfunction. We thought that systemically administered sevoflurane might exert beneficial effects on other vital organs over the effects on myocardial tissue. Since renal failure is an important complication in patients undergoing CABG surgery with CPB with an incidence of up to 16% and an associated mortality rate of 13%,[46] we decided to determine the protective effect of sevoflurane preconditioning on postoperative kidney function. Mangano *et al.*[47] recently showed that after cardiac surgery, a reduction of creatinine clearance by only 10% was significantly and independently associated with intensive care unit stay, prolonged hospitalization, and resource utilization. In addition, a mild elevation in the preoperative serum creatinine concentration (130–150 μM) significantly increases the need for postoperative mechanical renal support and in-hospital mortality.[48] Although there was no clinically overt new renal dysfunction in both of the groups in our study, sevoflurane preconditioning markedly improved the postoperative glomerular filtration rate, as determined by serial CysC measurements. CysC is a basic low-molecular-weight cysteine proteinase inhibitor (13,359 Da), which is the product of a "house-

keeping" gene expressed in all nucleated cells at a constant rate.[25] It is freely filtered by the glomerulus but not secreted by the tubulus. Thus, its serum concentration is entirely determined by the glomerular filtration rate. Although plasma creatinine concentrations and even creatinine clearance may significantly overestimate the glomerular filtration rate among patients with impaired renal function, plasma CysC concentrations are independent of age, sex, muscle mass, or dietary factors and more readily and accurately reflect even mild renal dysfunction.[24] The observation that sevoflurane preconditioning may prevent the CPB-associated subtle decrements in the glomerular filtration rate provides evidence that pharmacological preconditioning may protect the kidney and even other vital organs subjected to CPB-associated damage.[49] Whether the observed renoprotective effect is the direct result of a preconditioning effect by sevoflurane operative on renal tissue or the renal vasculature or whether this functional improvement merely reflects the preserved cardiac function, as determined by NT-proBNP, cannot be decided from the present study. However, preconditioning in our study prevented transient renal dysfunction. At present, it is unclear to what extent this treatment may prevent manifestation of clinical disease.

Limitations of the Study

This study has some important limitations. The study was designed with sufficient power to detect differences in postoperative NT-proBNP concentrations. It was not designed to detect differences in in-hospital clinical outcome. In addition, several variables associated with the protective effects of preconditioning were evaluated as secondary outcome measures to support the results of our primary outcome variable NT-proBNP. For these variables, no individual sample size was calculated *a priori*. However, this study had a power of 80% to detect a reduction of 20% in plasma cTnT[43] or CysC concentrations[47,48] and the incidence of arrhythmias but a reduction of 70% in the incidence of ischemic ST-segment changes.[50] Although this study is the largest evaluating anesthetic preconditioning in humans to date, we cannot exclude that for the variables where no difference was found a larger sample size might have resulted in significant differences. The present study did not simultaneously measure hemodynamic parameters to evaluate the cardiac contractile state. However, the diagnostic and prognostic value of BNP as a correlate of myocardial function was previously carefully evaluated and well established by angiography, echocardiography, concomitant measurements of hemodynamic parameters, and radionuclide ventriculography in a variety of clinical settings including the specific situation of cardiac recovery following CABG surgery.[15,16] Specifically, since the BNP concentration is known to remain elevated 1 month after the end of surgical stress,[16] it is highly unlikely that increased postoperative blood NT-proBNP concentrations merely reflect transient volume overload due to perioperative fluid management. Since more phenylephrine was administered during the process of preconditioning in the sevoflurane group to maintain blood pressure above 50 mmHg, we cannot entirely exclude that the stimulation of α-adrenergic receptors may have contributed to the observed cardiac and renal effects. However, in a separate multivariate analysis, the amount of administered phenylephrine did not prove to be a significant predictor of postoperative peak NT-proBNP or CysC concentrations, making a significant contribution of phenylephrine to the observed effects unlikely. Since all patients received aprotinin in their priming volume, it is unlikely that this treatment accounted for the observed differences between the groups. Because this study only evaluated immediate perioperative effects of sevoflurane preconditioning, we can only speculate on potential beneficial long-term effects of this treatment. Future studies should address this important issue.

In summary, pharmacological preconditioning with sevoflurane was found to reduce postoperative myocardial dysfunction in patients undergoing CABG surgery as assessed by the biochemical marker NT-proBNP. This study also directly visualized for the first time the translocation of PKC δ and ϵ to subcellular targets in human myocardium in response to sevoflurane. Finally, we also observed a decrease in CPB-induced renal dysfunction in the preconditioned patients.

The authors thank the cardiac surgeons, intensive care physicians and nurses, and local research associates from the three Swiss medical centers who facilitated the completion of these studies. They also thank Sandra Bühlmann, M.D. (Resident in Anesthesiology, City Hospital Triemli, Zurich, Switzerland), and Christoph Hofer, M.D. (Attending Physician in Anesthesiology, City Hospital Triemli), for the collection of clinical data, and Eliana Lucchinetti, Ph.D. (Laboratory for Biomechanics, Swiss Federal Institute of Technology, Zurich, Switzerland), for helpful discussions and for proofreading the manuscript.

References

1. Warltier DC, al-Wathiqui MH, Kampine JP, Schmeling WT: Recovery of contractile function of stunned myocardium in chronically instrumented dogs is enhanced by halothane and isoflurane. ANESTHESIOLOGY 1988; 69:552–65

2. Zaugg M, Jamali NZ, Lucchinetti E, Shafiq SA, Siddiqui MA: Norepinephrine-induced apoptosis is inhibited in adult rat ventricular myocytes exposed to volatile anesthetics. ANESTHESIOLOGY 2000; 93:209–18

3. Cason BA, Gamperl AK, Slocum RE, Hickey RF: Anesthetic-induced preconditioning: Previous administration of isoflurane decreases myocardial infarct size in rabbits. ANESTHESIOLOGY 1997; 87:1182–90

4. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC: Isoflurane mimics ischemic preconditioning via activation of K(ATP) channels: Reduction of myocardial infarct size with an acute memory phase. ANESTHESIOLOGY 1997; 87:361–70

5. Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Schaub MC: Volatile anesthetics mimic cardiac preconditioning by priming the activation of mitoKATP channels via multiple signaling pathways. ANESTHESIOLOGY 2002; 97:4–14

6. Fujimoto K, Bosnjak ZJ, Kwok WM: Isoflurane-induced facilitation of the cardiac sarcolemmal KATP channel. ANESTHESIOLOGY 2002; 97:57–65

7. de Klaver MJ, Manning L, Palmer LA, Rich GF: Isoflurane pretreatment inhibits cytokine-induced cell death in cultured rat smooth muscle cells and human endothelial cells. ANESTHESIOLOGY 2002; 97:24–32

8. Belhomme D, Peynet J, Louzy M, Launay JM, Kitakaze M, Menasche P: Evidence for preconditioning by isoflurane in coronary artery bypass graft surgery. Circulation 1999; 100:II340–4

9. Haroun-Bizri S, Khoury SS, Chehab IR, Kassas CM, Baraka A: Does isoflurane

optimize myocardial protection during cardiopulmonary bypass? J Cardiothorac Vasc Anesth 2001; 15:418–21

10. Tomai F, De Paulis R, Penta de Peppo A, Colagrande L, Caprara E, Polisca P, De Matteis G, Forlani S, Chiariello L: Beneficial impact of isoflurane during coronary bypass surgery on troponin I release. G Ital Cardiol 1999; 29:1007–14

11. Penta de Peppo A, Polisca P, Tomai F, De Paulis R, Turani F, Zupancich E, Sommariva L, Pasqualetti P, Chiariello L: Recovery of LV contractility in man is enhanced by preischemic administration of enflurane. Ann Thorac Surg 1999; 68:112–28

12. Berger R, Huelsman M, Stecker K, Bojic A, Moser P, Stanek B, Pacher R: B-Type natriuretic peptide predicts sudden death in patients with chronic heart failure. Circulation 2002; 105:2392–7

13. Richards AM, Nicholls MG, Yandle TG, Frampton C, Espiner EA, Turner JG, Buttimore RC, Lainchbury JG, Elliott JM, Ikram H, Crozier IG, Smyth DW: Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: New neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. Circulation 1998; 97:1921–9

14. de Lemos JA, Morrow DA, Bentley JH, Omland T, Sabatine MS, McCabe CH, Hall C, Cannon CP, Braunwald E: The prognostic value of B-type natriuretic peptide in patients with acute coronary syndrome. N Engl J Med 2001; 345: 1014–21

15. Chello M, Mastroroberto P, Perdicone F, Cirillo F, Bevacqua E, Olivito S, Covino E: Plasma levels of atrial and brain natriuretic peptides as indicators of recovery of left ventricular systolic function after coronary artery bypass. Eur J Cardiothorac Surg 2001; 20:140–6

16. Morimoto K, Mori T, Ishiguro S, Matsuda N, Hara Y, Kuroda H: Perioperative changes in plasma brain natriuretic peptide concentrations in patients undergoing cardiac surgery. Surg Today 1998; 28:23–9

17. Sabatine MS, Morrow DA, de Lemos JA, Gibson CM, Murphy SA, Rifai N, McCabe C, Antman EM, Cannon CP, Braunwald E: Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes. Simultaneous assessment of troponin I, C-reactive protein, and B-type natriuretic peptide. Circulation 2002; 105:1760–3

18. Richards AM, Doughty R, Nicholls MG, Macmahon S, Ikram H, Sharpe N, Espiner EA, Frampton C, Yandle TG: Neurohumoral prediction of benefit from carvedilol in ischemic left ventricular dysfunction. Circulation 1999; 99:786–91

19. Burnett JC, Kao PC, Hu DC, Heser DW, Heublein D, Granger JP, Opgenorth TJ, Reeder GS: Atrial natriuretic peptide elevation in congestive heart failure in the human. Science 1986; 231:1145–7

20. Richards AM, Cleland JG, Tonolo G, McIntyre GD, Leckie BJ, Dargie HJ, Ball SG, Robertson JI: Plasma alpha natriuretic peptide in cardiac impairment. BMJ 1986; 293:409–12

21. Raine AE, Erne P, Burgisser E, Muller FB, Bolli P, Burkart F, Buhler FR: Atrial natriuretic peptide and atrial pressure in patients with congestive heart failure. N Engl J Med 1986; 315:533–7

22. Richards AM, Crozier IG, Yandle TG, Espiner EA, Ikram H, Nicholls MG: Brain natriuretic factor: regional plasma concentrations and correlations with haemodynamic state in cardiac disease. Br Heart J 1993; 69:414–7

23. Hirayama A, Yamamoto H, Sakata Y, Asakura M, Sakata Y, Fuji H, Ishikura F, Higuchi Y, Mizuno H, Kashiwase K, Kusuoka H, Hori M, Kuzuya T, Kodama K: Usefulness of plasma brain natriuretic peptide after acute myocardial infarction in predicting left ventricular dilatation six months later. Am J Cardiol 2001; 88: 890–2

24. Laterza OF, Price CP, Scott MG: Cystatin C: An improved estimator of glomerular filtration rate? Clin Chem 2002; 48:699–707

25. Dworkin LD: Serum cystatin C as a marker of glomerular filtration rate. Curr Opin Nephrol Hypertens 2001; 10:551–3

26. Mussap M, Dalla Vestra M, Fioretto P, Saller A, Varagnolo M, Nosadini R, Plebani M: Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients. Kidney Int 2002; 61:1453–61

27. Wang Y, Ashraf M: Role of protein kinase C in mitochondrial KATP channel-mediated protection against $Ca2^{+}$ overload injury in rat myocardium. Circ Res 1999; 84:1156–65

28. Wang Y, Hirai K, Ashraf M: Activation of mitochondrial ATP-sensitive K(+) channel for cardiac protection against ischemic injury is dependent on protein kinase C activity. Circ Res 1999; 85:731–41

29. Kanagratnam P, Rothery S, Patel P, Severs NJ, Peters NS: Relative expression of immunolocalized connexins 40 and 43 correlates with human atrial conduction properties. J Am Coll Cardiol 2002; 39:116–23

30. Blackburn H, Keys A, Simonson E, Rautaharju P, Punsar S: The electrocardiogram in population studies: A classification system. Circulation 1960; 21: 1160–75

31. Carrier M, Pellerin M, Perrault LP, Solymoss BC, Pelletier LC: Troponin levels in patients with myocardial infarction after coronary artery bypass grafting. Ann Thorac Surg 2000; 69:435–40

32. Bonow RO: New insight into the cardiac natriuretic peptides. Circulation 1990; 93:1946–50

33. Sudoh T, Kangawa K, Minamino N, Matsuo H: A new natriuretic peptide in porcine brain. Nature 1988; 332:78–81

34. McDowell G, Shaw C, Buchanan KD, Nicholls DR: The natriuretic peptide family. Eur J Clin Invest 1995; 25:291–8

35. Yandle TG: Biochemistry of natriuretic peptides. J Intern Med 1994; 235:561–76

36. Hunt PJ, Yandle TG, Nicholls MG, Richards AM, Espiner EA: The amino-terminal portion of pro-brain natriuretic peptide (pro-BNP) circulates in human plasma. Biochem Biophys Res Commun 1995; 214:1175–83

37. Hunt PJ, Richards AM, Nicholls MG, Yandle TG, Doughty RN, Espiner EA: Immunoreactive amino-terminal pro-brain natriuretic peptide (NT-PROBNP): a new marker of cardiac impairment. Clin Endocrinol 1997; 47:287–96

38. Troughton RW, Frampton CM, Yandle TG, Espiner EA, Richards AM: Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. Lancet 2000; 355:1126–30

39. Stein B, Levin R: Natriuretic peptides: Physiology, therapeutic potential, and risk stratification in ischemic heart disease. Am Heart J 1998; 135:914–23

40. Mair P, Mair J, Bleier J, Hoermann C, Balogh D, Puschendrof B: Augmented release of brain natriuretic peptide during reperfusion of the human heart after cardioplegic cardiac arrest. Clin Chim Acta 1997; 261:57–68

41. Svedjeholm R, Dahlin LG, Lundberg C, Szabo Z, Kagedal B, Nylander E, Olin C, Rutberg H: Are electrocardiographic Q-wave criteria reliable for diagnosis of perioperative myocardial infarction after coronary surgery. Eur J Cardiothorac Surg 1998; 13:655–61

42. Haessler R, Kuzume K, Chien GL, Wolff RA, Davis RF, Van Winkle DM: Anaesthetics alter the magnitude of infarct limitation by ischaemic preconditioning. Cardiovasc Res 1994; 28:1574–80

43. De Hert SG, ten Broecke PW, Mertens E, Van Sommeren EW, De Blier IG, Stockman BA, Rodrigus IE: Sevoflurane but not propofol preserves myocardial function in coronary surgery patients. ANESTHESIOLOGY 2002; 97:42–9

44. Light PE, Bladen C, Winkfein RJ, Walsh MP, French RJ: Molecular basis of protein kinase C-induced activation of ATP-sensitive potassium channels. Proc Natl Acad Sci USA 2000; 97:9058–63

45. Balafanova Z, Bolli R, Zhang J, Zheng Y, Pass JM, Bhatnagar A, Tang X-L, Cardwell E, Ping P: Nitric oxide (NO) induces nitration of protein kinase C epsilon (PKCε), facilitating PKCε translocation via enhanced PKCε-RACK2 interactions. J Biol Chem 2002; 277:15021–7

46. Mangos GJ, Brown MA, Chan WY, Horton D, Trew P, Whitworth JA: Acute renal failure following cardiac surgery: Incidence, outcomes and risk factors. Aust N Z J Med 1995; 25:284–9

47. Mangano CM, Boisvert D, Zhou S: Small reductions in renal function following CABG independently predict prolonged hospitalisation. Anesth Analg 2000; 90:SCA35

48. Mangano CM, Diamondstone LS, Ramsay JG, Aggarwal A, Herskowitz A, Mangano DT: Renal dysfunction after myocardial revascularisation: Risk factors, adverse outcomes, and hospital resource utilization. Ann Intern Med 1998; 128:194–203

49. Lynch III C: Anesthetic preconditioning. Not just for the heart. ANESTHESIOLOGY 1999; 91:606–8

50. Smith RC, Leung JM, Mangano DT: Postoperative myocardial ischemia in patients undergoing coronary artery bypass graft surgery. ANESTHESIOLOGY 1991; 74:464–73