Prostaglandins in the Pericardial Fluid Modulate Neural Regulation of Cardiac Electrophysiological Properties

Toshihisa Miyazaki, Harald P. Pride, and Douglas P. Zipes

In response to various stimuli, the pericardium produces prostaglandins that might play a role in neural regulation of cardiac electrophysiological properties by modulating epicardial nerve effects. We determined the effects of various epicardial superfusates on efferent cardiac responses induced by bilateral efferent ansae subclaviae (SS) and cervical vagal (VS) stimulation, and afferent cardiac reflexes elicited by intracoronary injections of bradykinin (25 μg) and nicotine (50 μg). Pericardial instillation of arachidonic acid in normal Tyrode’s solution (3 μg/ml) increased the concentration of pericardial prostacyclin (PGI₂), measured by radioimmunoassay as the stable metabolite 6-keto-PGF₁α, and of prostaglandin E₂ (PGE₂). Arachidonic acid superfusion reduced SS-induced shortening of sinus cycle length (SCL), atrio-His interval (AH), and effective refractory period (ERP) of the right and left ventricular myocardium and prevented intra-aortic angiotensin II (30 ng/kg/min) from augmenting SS effects on these variables. Pericardial arachidonic acid plus indomethacin (1 μg/ml) eliminated the prostaglandin increase and restored the responses of SCL, AH, and ERP to SS and to angiotensin II infusion. Pericardial PGE₂ (30 or 50 ng/ml) or PGI₂ (50 ng/ml) reversibly suppressed SS-induced shortening of SCL and ERP. Pericardial arachidonic acid or PGI₂, however, did not blunt the shortening of ERP induced by intravenous infusion of norepinephrine. Pericardial arachidonic acid did not affect VS-induced lengthening of ERP or the duration of sinus arrest, or arterial blood pressure and heart rate responses to bradykinin or nicotine. We conclude that an increase in the concentration of prostaglandins in the pericardial fluid inhibits efferent sympathetic nerve effects on cardiac electrophysiological variables and antagonizes the facilitatory action of angiotensin II on efferent sympathetic stimulation by acting at presynaptic sites. Increased concentration of pericardial prostaglandins in response to various stimuli may constitute a physiological negative-feedback control mechanism that regulates efferent cardiac sympathetic stimulation. (Circulation Research 1990;66:163–175)

We have shown that epicardial superfusion with Tyrode’s solution containing tetrodotoxin instilled into the pericardial cavity in anesthetized dogs attenuates refractory period responses of the deep ventricular myocardium to efferent sympathetic and vagal nerve stimulation without affecting refractory period responses elicited by intravenous infusion of norepinephrine or methacholine.1 These results indicate that vagal and sympathetic axons at some point travel superficially during their course in the heart and were blocked by contact with tetrodotoxin contained in the pericardial fluid. This observation raises the possibility that substances in the pericardial fluid, which are produced by the pericardium and/or epicardium normally or during disease, might also modulate cardiac autonomic nerve effects, making the pericardium an essential link in a cardiac autonomic feedback control system.

Dusting et al2 have shown that prostacyclin (PGI₂) and prostaglandin E₂ (PGE₂) are released in large amounts from the parietal pericardium and the epicardial surface during epicardial irrigation in situ with Krebs’ solution in anesthetized dogs and suggested an important effect of pericardial prostaglandins on the large coronary vessels in the epicardial
surface. The output of PGI₂ was increased further by adding arachidonic acid to the irrigating fluid or by increasing afterload and heart rate, and by angioten-
sin II infusion.² In fact, the pericardium in vitro produces prostaglandins in greater concentration than does the myocardium and is considered a major source of PGI₂.³⁴

In addition to the direct effects on coronary vascular smooth muscle, prostaglandins exert modulating influences on efferent autonomic stimulation in the heart⁵⁻⁷ and on afferent cardiac reflexes.⁸ For example, PGE₂ is known to inhibit sympathetic neurotrans-
mission in the rabbit heart.⁹ Thus, prostaglandins in the pericardial fluid might have a potential role, hitherto unrecognized, in modulating neural regulation of cardiac properties. Therefore, the present study was conducted to test this hypothesis. We did this in anesthetized, open-chest dogs by determining cardiac responses induced by efferent ansae subclaviae stimulation and cervical vagal stimulation and by determining afferent cardiac reflexes elicited by intracoronary injections of bradykinin and nicotine during epicardial superfusion with various Tyrode’s solutions instilled into the pericardial cavity.

Materials and Methods

Surgical Preparation

Studies were carried out in 60 mongrel dogs of either sex weighing 14–26 kg. Forty-nine dogs in which efferent cardiac responses were examined were anesthetized with secobarbital or pentobarbital (30 mg/kg i.v.). Another 11 dogs used for cardiac reflex studies were anesthetized with α-chloralose (100 mg/kg i.v.). Additional doses were injected as needed to maintain anesthesia. Dogs were intubated and ventilated with room air by a constant volume-cycled respirator (model 607, Harvard Apparatus, South Natick, Massachusetts). A fluid-filled cannula was placed in the right femoral artery and was connected to a transducer (Statham P-23Db, Gould Instruments, Cleveland, Ohio) to monitor arterial pressure. A femoral venous cannula was used to infuse normal saline at 100–200 ml/hr to replace spontaneous fluid losses. Lead II electrocardiogram was monitored throughout the study. The chest was opened through a median sternotomy. A small incision was made in the anterior surface of the pericardium. The edges of the incision were tied with sutures at four points so that tension applied to the sutures produced a square opening approximately 2.5×2.5 cm. This provided a pericardial cavity sufficient to contain 40–80 ml of the solution to bathe the epicardial surface of the heart (Figure 1). A thermistor (model 400, Yellow Springs Instruments, Yellow Springs, Ohio) was used to monitor epicardial temperature, which was main-
tained between 36° and 38° C by covering the thoracotomy with a plastic sheet and by adjusting the proximity of an operating table lamp.

Figure 1. A diagram showing pericardial cradling tech-
nique for epicardial superfusion. Through the pericardial opening, six unipolar electrodes were inserted into the right and left ventricles to determine the effective refractory period. Tyrode’s solution was instilled into the pericardial cavity to bathe the epicardial surface of the heart. See text for details.

Electrode Placement

Through the pericardial opening, four hook elec-
trodes made from Teflon-coated wires, insulated except for their tips, were inserted in the anterior and posterior myocardium of the basal and apical left ventricle to a depth of 4–6 mm, and two additional electrodes were placed in the subendocardium of the right ventricular outflow and apex. These electrodes served as the cathode for unipolar stimulation to determine the effective refractory period (ERP). An anodal electrode was placed in the abdominal wall. A bipolar plunge electrode was placed in the left ventricle to record activation. A quadripolar catheter electrode (6F, USCI, Billerica, Massachusetts) was advanced from the right carotid artery to the noncor-
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at a constant cycle length of 250 msec were averaged to obtain the AH and HV interval, respectively.

**Measurement of Ventricular Effective Refractory Period**

The ERP was determined at six test sites by the extrastimulus technique employing a programmable stimulator (Krannert Medical Engineering, Indianapolis, Indiana) and a constant current isolator. Each ventricular test site was driven with a 2-msec rectangular cathodal stimulus twice the diastolic threshold, which was measured during each intervention. A train of eight stimuli (S₈) was followed by a late premature stimulus (S₉) that produced a propagated response. The S₈-S₉ interval was 250 msec in dogs tested for sympathetic response and 280 msec in dogs tested for vagal response and was kept constant throughout the experiment. The ventricular response to S₉ was recorded in lead II electrocardiogram and from the bipolar left ventricular electrode and was displayed on a storage oscilloscope. The S₈-S₉ interval was shortened in steps of 2 msec until S₉ failed to produce a propagated response. The S₈-S₉ interval was then increased by 5 msec and was shortened by 1 msec decrements until S₉ failed to produce a propagated response. The ERP was determined twice at each test site, and the values were within 1 msec of each other or the data were discarded and the determination was repeated.

**Neural Stimulation**

The ansae subclaviae were isolated as they exited from the stellate ganglia, doubly ligated, and cut. Shielded bipolar electrodes were placed on the right and left anterior and posterior ansae subclaviae to stimulate the efferent cardiac sympathetic nerves. Bilateral ansae subclaviae were stimulated with separate constant current isolators driven by a programmable stimulator (Pulsar 4, Frederick Haer and Co, Brunswick, Maine). Stimuli were 4-msec pulses at a frequency of 2–4 Hz and at 1.5–3.5 mA.

The cervical vagi were isolated, doubly ligated, and transected. In dogs tested for vagal response, both cervical vagi were stimulated through two Teflon-coated wire electrodes embedded in the cardiac end of each vagal nerve. Rectangular pulses of 4-msec duration were delivered at a frequency of 20 Hz using separate constant isolators. The current strength was 0.05 mA greater than that required to produce asystole for the right vagus and asystole or complete atrioventricular block for the left vagus. The effects of efferent vagal stimulation were determined during intravenous infusion of norepinephrine at a constant rate of 0.125 μg/kg/min to achieve a constant background of sympathetic effect. The ERP determined during norepinephrine infusion served as control for the determination of the efferent vagal effects on ventricular refractoriness.

The conditions of neural stimulation were kept constant in each experiment.

**Intracoronary Injections of Bradykinin and Nicotine**

In 11 dogs with both cardiac autonomic limbs intact, the left anterior descending coronary artery was carefully dissected at its midportion, sparing any pericoronary nerves, and was cannulated nonocclusively with a PE-10 catheter through a pericardial opening. We have shown that a careful dissection does not interrupt neural innervation. Bradykinin triacetate (Sigma Chemical, St. Louis, Missouri) and nicotine (Sigma) were dissolved in normal saline solution, which was kept at 37°C–38°C. Twenty-five micrograms of bradykinin in 0.25 ml normal saline or 50 μg nicotine in 0.25 ml normal saline was injected via this catheter over 5 seconds while the electrocardiogram, right atrial electrogram, and mean arterial blood pressure were being recorded. Bradykinin was used to stimulate sympathetic afferent nerve endings of the left ventricle. Nicotine was used to stimulate vagal afferent nerve endings.

**Solutions Used for Epicardial Superfusion**

Normal Tyrode’s solution was used for control epicardial superfusion. The millimolar composition of the solution was MgCl₂ 0.5, NaH₂PO₄ 0.9, CaCl₂ 2.0, NaCl 137.0, NaHCO₃ 12.0, KCl 4.0, and glucose 5.0. This resulted in a pH of 7.35. The test solution was prepared by adding substance(s) to normal Tyrode’s solution. The solution was prewarmed on a hot plate stirrer to 36°C–38°C and gassed with 95% O₂-5% CO₂, resulting in an oxygen tension of about 400 mm Hg, before it was instilled into the pericardial cavity. Arachidonic acid (sodium salt form; Sigma), indomethacin (Sigma), PGI₂ (Sigma), and PGE₂ (Sigma) were dissolved in normal Tyrode’s solution immediately before it was instilled into the pericardial cavity.

**Radioimmunoassay of Prostaglandins**

Radioimmunoassay was used to measure the concentration of PGE₂ and 6-keto-PGF₁α, the stable metabolite of PGI₂, in the pericardial superfusate during epicardial superfusion (in groups 2 and 4, see below). At the end of each superfusion, 10 ml of the fluid sample was taken from the pericardial cavity and placed in tubes that contained indomethacin (10 μg/ml), kept at room temperature for 60 minutes, and then stored frozen until assay using commercially available assay kits (Amersham, Arlington Heights, Illinois). The “normal” pericardial fluid sample was also obtained for the assay (in group 8, see below).

The assay was performed in polypropylene tubes containing [³H]bicyclic PGE₂ or [¹⁴C]6-keto-PGF₁α, each antibody, and either standards or samples. After vortexing, the tubes were incubated at 4°C overnight. The unbound bicyclic PGE₂ was precipitated with dextran-coated charcoal, and after centrifugation (1,000g for 10 minutes), the supernatant was decanted into the vials containing scintillation fluid. The antibody-bound 6-keto-PGF₁α was reacted
with the Amerlex-M second antibody (Amersham) that is bound to magnetizable polymer particles, then separated from free fraction by magnetic separation. The amounts of bound \[^{3}H\]bicyclic PGE\(_{2}\) and \[^{[28]}I\]6-keto-PGF\(_{1\alpha}\) were determined by counting in a beta scintillation counter (model 2000, Packard Instrument, Meridian, Connecticut) and in a gamma counter (model 5530, Packard Instrument), respectively. The concentration of unlabeled bicyclic PGE\(_{2}\) or 6-keto-PGF\(_{1\alpha}\) in the sample was then determined from each standard curve. The bicyclic PGE\(_{2}\) antibody had less than 0.001% cross-reactivity with PGF\(_{2\alpha}\), 6-keto-PGF\(_{1\alpha}\), PGD\(_{2}\), PGA\(_{1}\), and thromboxane B\(_{2}\). The 6-keto-PGF\(_{1\alpha}\) antibody had less than 0.8% cross-reactivity with PGF\(_{2\alpha}\), PGE\(_{2}\), PGE\(_{1}\), and thromboxane B\(_{2}\).

**Experimental Protocol**

After removal of all pericardial fluid by suction, each dog received instillations of various Tyrode’s solutions into the pericardial cavity. Each solution was removed by suction after each set of measurements was completed. The amount of Tyrode’s solution used for each superfusion was kept constant in each dog.

**Group 1: Time course of the effects of epicardial superfusion with arachidonic acid on ERP shortening elicited by bilateral ansae subclaviae stimulation.** Studies were done in six dogs that received control superfusion with normal Tyrode’s solution followed by superfusion with Tyrode’s solution containing arachidonic acid (3 \(\mu\)g/ml). ERP response to bilateral ansae subclaviae stimulation was determined at two or three ventricular test sites during control superfusion and 5, 10, 20, 30, and 40 minutes after the installation of the test solution.

**Group 2: Effects of epicardial superfusion with arachidonic acid on changes in cardiac electrophysiological variables elicited by bilateral ansae subclaviae stimulation and angiotensin II infusion.** Shortening of SCL, AH interval, HV interval, and ERP elicited by bilateral ansae subclaviae stimulation was determined during control superfusion with normal Tyrode’s solution and during subsequent superfusion with arachidonic acid (3 \(\mu\)g/ml) in seven dogs. During the latter superfusion, shortening of SCL, AH, HV, and ERP was also determined during intra-aortic infusion of angiotensin II (30 ng/kg/min). Data collection was started 30 minutes after the initiation of each superfusion. Another seven dogs received control superfusion with normal Tyrode’s solution followed by suction and instillation of normal Tyrode’s solution containing arachidonic acid (3 \(\mu\)g/ml) plus indomethacin (1 \(\mu\)g/ml), a cyclooxygenase inhibitor. Data collection was done in a same way as in the first seven dogs.

**Group 3: Effects of epicardial superfusion with arachidonic acid on changes in ventricular ERP elicited by bilateral ansae subclaviae stimulation and by norepinephrine infusion.** To test whether arachidonic acid superfusion exerts its effect presynaptically or postsynaptically, stimulation frequency–ERP response curves and norepinephrine dose–ERP response curves were obtained. Shortening of ERP elicited by bilateral ansae subclaviae stimulation was determined at different frequencies, ranging from 0.5 to 8 Hz during control superfusion with normal Tyrode’s solution and during subsequent superfusion with arachidonic acid in four dogs. Shortening of ERP induced by intravenous infusion of norepinephrine was determined at different doses, ranging from 0.1 to 1.5 \(\mu\)g/kg/min during control normal Tyrode’s superfusion and during subsequent arachidonic acid superfusion. Data collection was started 10 minutes after the initiation of each superfusion. Stimulation at different frequencies and norepinephrine at different doses were given at 10-minute intervals.

**Group 4: Effects of epicardial superfusion with arachidonic acid on changes in ventricular ERP elicited by bilateral cervical vagal stimulation.** In a separate group of four dogs, the change in ERP elicited by bilateral vagal stimulation was determined during control epicardial superfusion with normal Tyrode’s solution and during subsequent superfusion with Tyrode’s solution containing arachidonic acid (3 \(\mu\)g/ml).

In groups 2 and 4, at the end of the data collection period during each superfusion, a fluid sample of the superfusate was taken from the pericardial cavity for radioimmunoassay determination of prostaglandin concentrations.

**Group 5: Effects of epicardial superfusion with extrinsic PGI\(_{2}\) and PGE\(_{2}\) on shortening of SCL and ERP elicited by bilateral ansae subclaviae stimulation.** The effects on ansae subclaviae stimulation of adding extrinsic amounts of PGI\(_{2}\) and PGE\(_{2}\) to the pericardial fluid were examined. Five control dogs received three instillations of normal Tyrode’s solution, separated by suction. Eight dogs received normal Tyrode’s solution, normal Tyrode’s solution containing PGI\(_{2}\) (50 ng/ml), and then normal Tyrode’s solution. Five dogs received normal Tyrode’s solution, normal Tyrode’s solution containing PGE\(_{2}\) (30 or 50 ng/ml), and then normal Tyrode’s solution. Thirty minutes after the instillation of each solution, shortening of SCL and ERP during bilateral ansae subclaviae stimulation was determined.

**Group 6: Effects of epicardial superfusion with extrinsic PGI\(_{2}\) on ventricular ERP responses to ansae subclaviae stimulation and intravenous infusion of norepinephrine.** In three of eight dogs in which the effects of extrinsic PGI\(_{2}\) on shortening of SCL and ERP during ansae subclaviae stimulation were examined, shortening of ERP induced by intravenous infusion of norepinephrine (0.25 \(\mu\)g/kg/min) was also determined.

**Group 7: Effects of epicardial superfusion with arachidonic acid on afferent cardiac reflexes elicited by intracoronary injections of bradykinin and nicotine.** Bradykinin (25 \(\mu\)g) and nicotine (50 \(\mu\)g) in 0.25 ml normal saline were injected in a random order at a 10-minute interval in the absence of epicardial superfusion. Then, injections were repeated during superfusion with normal Tyrode’s solution containing arachidonic acid (3 \(\mu\)g/ml) and during subsequent superfusion with normal Tyrode’s solution containing indomethacin (1 \(\mu\)g/ml), starting 15 minutes after the
initiation of each superfusion. Changes in heart rate and mean arterial blood pressure from the baseline values were determined 10, 20, 30, 40, 50, 60, 80, 100, and 120 seconds after each injection.

**Group 8: Radioimmunoassay of prostaglandins in the “normal” pericardial fluid.** After anesthesia and thoracotomy, pericardial fluid was collected from seven dogs by suction with a syringe to determine the concentrations of 6-keto-PGF$_{1\alpha}$ and PGE$_2$.

**Analysis of Data**

Data from ventricular test sites with <9 msec shortening of ERP elicited by bilateral ansae subclaviae stimulation or <3 msec lengthening of ERP induced by bilateral vagal stimulation during the first control superfusion with normal Tyrode’s solution were discarded because of possibly insufficient neural effect at that particular site.\textsuperscript{16} This resulted in exclusion of data from 13 of 233 ventricular sites. In group 7, data were excluded from dogs in which intracoronary injection of bradykinin or nicotine elicited no or only a modest change in heart rate (<10 beats/min) during control determination period in the absence of epicardial superfusion. This resulted in exclusion of data from five out of 11 dogs treated with bradykinin and five out of 11 dogs treated with nicotine.

Data were expressed as mean±SEM. The difference among mean values was determined using an analysis of variance for repeated measurements. When multiple comparisons were made, the $t$ test was modified using the Bonferroni method.\textsuperscript{17} Paired $t$ test was used when two measurements were compared. A statistical significance was set at a value of $p<0.05$.

**Results**

**Group 1: Time Course of the Effects of Epicardial Superfusion With Arachidonic Acid on ERP Shortening Elicited by Bilateral Ansae Subclaviae Stimulation**

Shortening of ERP elicited by bilateral ansae subclaviae stimulation during control superfusion with normal Tyrode’s solution became attenuated 5 minutes after the instillation of arachidonic acid (Figure 2). This suppressive effect lasted a minimum of 40 minutes.

**Group 2: Effects of Epicardial Superfusion With Arachidonic Acid on Changes in Cardiac Electrophysiological Variables Elicited by Bilateral Ansae Subclaviae Stimulation and Angiotensin II Infusion**

Shortening of SCL, AH, and ERP elicited by bilateral ansae subclaviae stimulation during control epicardial superfusion with normal Tyrode’s solution was reduced 30 minutes after the onset of superfusion with arachidonic acid (Figure 3). HV interval was not affected by bilateral ansae subclaviae stimulation. During superfusion with arachidonic acid, intra-aortic infusion of angiotensin II did not modify the suppressive effects on bilateral ansae subclaviae stimulation of arachidonic acid superfusion alone. Superfusion with arachidonic acid plus indomethacin did not blunt the shortening of SCL, AH, and ERP induced by bilateral ansae subclaviae stimulation during normal Tyrode’s superfusion. Angiotensin II infusion during superfusion with arachidonic acid plus indomethacin augmented the shortening in SCL, AH, and ERP elicited by bilateral ansae subclaviae stimulation compared with that during arachidonic acid plus indomethacin alone or during normal Tyrode’s solution.

In both groups of dogs, baseline SCL, AH interval, and ERP were unaffected by superfusion with arachidonic acid or by angiotensin II infusion without ansae subclaviae stimulation (Table 1). Radioimmunoassay demonstrated a significant increase in the concentration of 6-keto-PGF$_{1\alpha}$ and PGE$_2$ in the superfusate during superfusion with arachidonic acid compared with control normal Tyrode’s superfusion. During superfusion with arachidonic acid plus indomethacin, this increase was eliminated (Table 2).

**Group 3: Effects of Epicardial Superfusion With Arachidonic Acid on Changes in Ventricular ERP Elicited by Bilateral Ansae Subclaviae Stimulation and by Norepinephrine Infusion**

During arachidonic acid superfusion, shortening of ventricular ERP induced by bilateral ansae subclaviae stimulation was reduced at all frequencies ranging from 0.5 to 8 Hz, compared with normal Tyrode’s superfusion, and the stimulation frequency–ERP response curve shifted downward and rightward ($p<0.001$). These data indicated an effect of arachidonic acid superfusion independent of stimulation frequency (Figure 4). In contrast, norepinephrine dose–ERP response curve was similar for both conditions ($p=0.403$).
FIGURE 3. Effects of stimulation of prostaglandin release into the pericardial fluid on changes in cardiac electrophysiological variables elicited by bilateral ansae subclaviae stimulation. Left panels indicate efferent sympathetic-induced shortening of spontaneous sinus cycle length (ΔSCL), AH interval (ΔAH), and ventricular effective refractory period (ΔERP) determined during control superfusion with normal Tyrode’s solution (NT) and during subsequent superfusion with arachidonic acid (3 μg/ml, AA) with and without intra-aortic infusion of angiotensin II (30 ng/kg/min, AII). Right panels indicate the data obtained from another seven dogs that received AA plus indomethacin (1 μg/ml, IND). N, number of test dogs; n, number of ventricular test sites.

Also, heart rate increase in response to norepinephrine infusion remained unaffected (3±3 and 57±5 beats/min, respectively, at norepinephrine 0.1 and 1.5 μg/kg/min during normal Tyrode’s superfusion; 6±2 and 55±3 beats/min, respectively, during arachidonic acid superfusion).

**Group 4: Effects of Epicardial Superfusion With Arachidonic Acid on Changes in Ventricular ERP Elicited by Bilateral Cervical Vagal Stimulation**

ERP increase elicited by bilateral vagal stimulation during control superfusion with normal Tyrode’s solution remained unchanged during superfusion with arachidonic acid (Figure 5). Baseline ERP was constant (165±2 to 166±2 msec, n=21). Also, the duration of sinus arrest induced by bilateral efferent vagal stimulation was unaffected (2.3±0.3 to 2.1±0.4 seconds, N=4). In these dogs, 6-keto-PGF₁α and PGE₂ were increased by epicardial superfusion with arachidonic acid (Table 2).

**Group 5: Effects of Epicardial Superfusion With Extrinsic PGI₂ and PGE₂ on Shortening of SCL and ERP Elicited by Bilateral Ansae Subclaviae Stimulation**

In control dogs that received three pericardial instillations of normal Tyrode’s solution, shortening of SCL and ERP elicited by bilateral ansae subclaviae stimulation were unchanged throughout the determination period. Superfusion with PGI₂ attenuated shortening of SCL and ERP induced by bilateral ansae subclaviae stimulation. Superfusion with PGE₂ also inhibited shortening of SCL and ERP produced by bilateral ansae subclaviae stimulation. The shortening of SCL and ERP induced by bilateral ansae subclaviae stimulation returned toward the control values after removal of PGI₂ and PGE₂ solution from the pericardial cavity and subsequent superfusion with normal Tyrode’s solution (Figure 6).

Baseline SCL and ERP were constant in each group throughout three instillation periods (Table 3).

| TABLE 1. Baseline Electrophysiological Values During Epicardial Superfusion With Normal Tyrode’s Solution, Arachidonic Acid, and Indomethacin |
|----------------|---------------|---------------|
| I. Seven dogs that received epicardial superfusion with arachidonic acid following superfusion with normal Tyrode’s solution | AII infusion | NT (-) | AA (-) | AA (+) |
| Sinus cycle length (msec) | 495±29 | 495±33 | 482±31 |
| AH interval (msec) | 110±10 | 112±10 | 117±12 |
| Ventricular effective refractory period (msec) | 158±1 | 158±1 | 157±1 |
| II. Seven dogs that received epicardial superfusion with arachidonic acid plus indomethacin | AII infusion | NT (-) | AA+IND (-) | AA+IND (+) |
| Sinus cycle length (msec) | 487±19 | 493±24 | 495±22 |
| AH interval (msec) | 114±6 | 114±7 | 120±7 |
| Ventricular effective refractory period (msec) | 162±1 | 164±1 | 165±1 |

Mean±SEM. AII infusion, intra-aortic infusion of angiotensin II (30 ng/kg/min); NT, normal Tyrode’s solution; AA, arachidonic acid; IND, indomethacin.
TABLE 2. Radioimmunoassay of Prostaglandins in the Superfusate

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<th>NT</th>
<th>AA</th>
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<tr>
<td>6-keto-PGF1α</td>
<td>18.0±4.0</td>
<td>30.3±4.6</td>
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<td>PGE2</td>
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<td>9.2±0.9</td>
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<td>6-keto-PGF1α+PGE2</td>
<td>21.4±4.1</td>
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II. Four dogs in which bilateral cervical vagal stimulation was performed

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<td>6-keto-PGF1α</td>
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<td>PGE2</td>
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<td>6-keto-PGF1α+PGE2</td>
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III. Seven dogs that received epicardial superfusion with arachidonic acid plus indomethacin in which bilateral ansae subclaviae stimulation was performed

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<th>NT</th>
<th>AA+IND</th>
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<td>6-keto-PGF1α</td>
<td>18.4±2.5</td>
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<tr>
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<td>4.1±0.8</td>
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<tr>
<td>6-keto-PGF1α+PGE2</td>
<td>22.5±2.8</td>
<td>13.3±3.5</td>
<td>&lt;0.05</td>
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Mean±SEM in nanograms per milliliter. NT, normal Tyrode's solution; AA, arachidonic acid; PG, prostaglandin; IND, indomethacin.

Group 6: Effects of Epicardial Superfusion With Extrinsic PGI2 on Ventricular ERP Responses to Ansae Subclaviae Stimulation and Intravenous Infusion of Norepinephrine

In three dogs, shortening of ERP elicited by bilateral ansae subclaviae stimulation during control epicardial superfusion with normal Tyrode's solution became attenuated during subsequent superfusion with PGI2, while shortening of ERP induced by intravenous infusion of norepinephrine was not affected (Figure 7).

Group 7: Effects of Epicardial Superfusion With Arachidonic Acid on Afferent Cardiac Reflexes Elicited by Intracoronary Injections of Bradykinin and Nicotine

In six of 11 dogs, intracoronary injection of bradykinin caused an increase in heart rate ≥10 beats/min with a peak effect at 20 seconds after the injection (167±12 baseline to 196±8 beats/min, N=6; p<0.05). Bradykinin at the same time reduced mean arterial blood pressure (106±3 baseline to 79±7 mm Hg at 20 seconds after the injection, N=6; p<0.01) in the absence of epicardial superfusion (Figure 8, top). In these six dogs, similar responses were elicited by bradykinin during epicardial superfusion with arachidonic acid and during subsequent superfusion with indomethacin (Figure 8, bottom). There was no significant difference in the magnitude of heart rate and blood pressure changes induced by bradykinin among three conditions, that is, in the absence of epicardial superfusion, during arachidonic acid superfusion, and during indomethacin superfusion.

Intracoronary injection of nicotine caused a decrease in heart rate ≥10 beats/min in six of 11 dogs with a peak effect at 10 seconds after the injection.
FIGURE 5. Effects of epicardial superfusion with arachidonic acid (3 μg/ml, AA) on lengthening of ventricular effective refractory period (ERP) elicited by bilateral cervical vagal stimulation. Control superfusion was done with normal Tyrode’s solution (NT). n, number of ventricular test sites.

(168±12 baseline to 148±16 beats/min, N=6; p<0.05) and a decrease in mean blood pressure (96±6 baseline to 86±7 mm Hg at 10 seconds after the injection, N=6; p<0.05) in the absence of epicardial superfusion (Figure 9, top). In these six dogs, similar responses were elicited by nicotine during epicardial superfusion with arachidonic acid and during subsequent superfusion with indomethacin (Figure 9, bottom). There was no significant difference in the magnitude of heart rate and blood pressure changes elicited by nicotine among three conditions.

Group 8: Radioimmunoassay of Prostaglandins in the “Normal” Pericardial Fluid

The concentrations of 6-keto-PGF$_{1α}$ and PGE$_2$ in the “normal” pericardial fluid samples (0.9~2.0 ml in volume) taken from seven dogs are indicated in Table 4. The concentration of 6-keto-PGF$_{1α}$ was at a great variance from dog to dog ranging from 10.6 to 152.0 ng/ml (mean value, 60.5 ng/ml). The mean concentration of PGE$_2$ was 5.3 n/ml with a small variance.

Discussion

Major Findings

The present data indicate that epicardial superfusion in situ with arachidonic acid instilled into the pericardial cavity resulted in increased concentration in the pericardial superfusate of PGE$_2$ and PGI$_2$, measured as the stable metabolite 6-keto-PGF$_{1α}$. The increase in these prostaglandins in the pericardial fluid reduced the amount of shortening of SCL, AH interval, and ventricular ERP elicited by bilateral ansae subclaviae stimulation and prevented intra-aortic infusion of angiotensin II from augmenting the effects of bilateral ansae subclaviae stimulation on these variables. A similar increase of prostaglandins in the pericardial fluid did not affect efferent vagal-induced prolongation of ERP or the duration of sinus arrest. Afferent cardiac reflexes elicited by intracoronary injections of bradykinin and nicotine were also not affected. Epicardial superfusion with arachidonic acid plus indomethacin prevented the increase in 6-keto-PGF$_{1α}$ and PGE$_2$ and therefore did not reduce

FIGURE 6. Effects of epicardial superfusion with extrinsic PGI$_2$ and PGE$_2$ on shortening of spontaneous sinus cycle length (∆SCL) and ventricular effective refractory period (∆ERP) elicited by bilateral ansae subclaviae stimulation. NT, normal Tyrode’s solution; N, number of test dogs; n, number of ventricular test sites.
the amount of shortening of SCL, AH interval, and ventricular ERP elicited by bilateral ansae subclaviae stimulation. In these dogs, angiotensin II infusion augmented the effects of bilateral ansae subclaviae stimulation on SCL, AH interval, and ventricular ERP. Epicardial superfusion with either extrinsic PGI₂ or PGE₂ reversibly suppressed shortening of SCL and ventricular ERP elicited by bilateral ansae subclaviae stimulation. However, epicardial superfusion with arachidonic acid or PGI₂ did not blunt the shortening of ventricular ERP induced by intravenous infusion of norepinephrine.

**Consideration of Experimental Model**

In the present model, 6-keto-PGF₁α and PGE₂ were detected in normal Tyrode’s solution instilled in the pericardial cavity. Adding arachidonic acid increased the concentration of these prostaglandins approximately twofold. If other prostaglandins synthesized from arachidonic acid, such as PGF₂α and PGD₂, were also measured, the amount of increase in the total concentration of the prostaglandins in the pericardial fluid would have been greater. Our observations are consistent with the previous reports that the pericardium and epicardium are active producers of prostaglandins.²⁻⁴ Using a method of biological assay, Dusting et al.⁵ showed that epicardial and pericardial irrigation of the dog heart in situ with Kreb’s solution resulted in release of PGI₂, equivalent to 2–10 ng/ml, into the solution continuously withdrawn from the pericardial cavity. Our direct assay of prostaglandins in the “normal” pericardial fluid demonstrated the presence of 6-keto-PGF₁α and PGE₂ in concentrations of approximately 10–150 ng/ml and 4–7 ng/ml, respectively. Although it is possible that prostaglandins detected in the pericardial fluid were derived also from the endothelial cells of the coronary vasculature, which are considered to be one of the major sources of cardiac prostaglandins,¹⁰ this seems unlikely. In an epicardial irrigation model in situ, intravenous infusion of PGI₂ at rates achieving supraphysiological concentrations in the blood stream was not accompanied by a detectable increase of PGI₂ in the irrigating fluid.²

We did not measure leukotrienes and thromboxanes in the superfusate. In addition to cyclooxygenase activity, the pericardium exhibits lipoxygenase activity.³ Thus, it might have produced leukotrienes in response to arachidonic acid added to the superfusate. However, the leukotrienes do not seem to contribute to a modulation of efferent sympathetic nerve effects, since superfusion with arachidonic acid plus indomethacin did not affect efferent sympathetic responses compared with control superfusion with normal Tyrode’s solution. Also, amounts of thromboxanes produced by the pericardium in vitro in

**TABLE 3. Baseline Electrophysiological Values During Epicardial Superfusion in Dogs Receiving Normal Tyrode’s Solution, PGI₂, or PGE₂**

<table>
<thead>
<tr>
<th></th>
<th>NT</th>
<th>NT</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus cycle length (msec)</td>
<td>519±14</td>
<td>518±10</td>
<td>521±4</td>
</tr>
<tr>
<td>Ventricular effective refractory period (msec)</td>
<td>160±1</td>
<td>160±1</td>
<td>163±3</td>
</tr>
</tbody>
</table>

**II. Eight dogs that received epicardial superfusion with PGI₂**

<table>
<thead>
<tr>
<th></th>
<th>NT</th>
<th>PGI₂</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus cycle length (msec)</td>
<td>536±36</td>
<td>532±36</td>
<td>518±34</td>
</tr>
<tr>
<td>Ventricular effective refractory period (msec)</td>
<td>159±1</td>
<td>159±2</td>
<td>162±2</td>
</tr>
</tbody>
</table>

**III. Five dogs that received epicardial superfusion with PGE₂**

<table>
<thead>
<tr>
<th></th>
<th>NT</th>
<th>PGE₂</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus cycle length (msec)</td>
<td>541±15</td>
<td>519±16</td>
<td>521±26</td>
</tr>
<tr>
<td>Ventricular effective refractory period (msec)</td>
<td>164±3</td>
<td>164±3</td>
<td>163±3</td>
</tr>
</tbody>
</table>

Mean±SEM. NT, normal Tyrode’s solution; PG, prostaglandin.

**FIGURE 7. Effects of epicardial superfusion with extrinsic PGI₂ on shortening of ventricular effective refractory period (ΔERP) induced by bilateral ansae subclaviae stimulation and by intravenous infusion of norepinephrine (0.25 μg/kg/min). Control superfusion was done with normal Tyrode’s solution (NT). n, number of ventricular test sites.
response to arachidonic acid in the superfusate are negligible compared with those of PGI$_2$ and PGE$_2$. Therefore, modulation of efferent sympathetic nerve effects during superfusion with arachidonic acid could be attributed largely to an increase in the pericardial fluid prostanoids.

In the present study, an increase in the total concentration of 6-keto-PGF$_{1\alpha}$ and PGE$_2$ in the pericardial fluid from 21 to 40 ng/ml was associated with a reduction in the effects of efferent sympathetic stimulation on several cardiac electrophysiological properties. Therefore, the presence of these prostanoids in the pericardial fluid in a concentration of approximately 40 ng/ml seems sufficient to modulate efferent sympathetic input to the heart. The concentration of PGE$_2$ detected in the pericardial superfusate or in the “normal” pericardial fluid was considerably lower than that of 6-keto-PGF$_{1\alpha}$ or the concentration of extrinsic PGE$_2$ that suppressed the efferent sympathetic responses. Therefore, PGE$_2$ alone may not contribute much to the modulation of cardiac efferent sympathetic nerve effects. PGI$_2$ or possibly a synergistic action of several prostanoids may be more important.

Although we did not monitor contractile changes, it is likely that efferent sympathetic nerve effects on these variables were also affected.

The absence of an effect of pericardial fluid prostanoids on baseline electrophysiological parameters in neurally decentralized hearts (Tables 1 and 3)
Mechanism of Action of Pericardial Fluid
Prostaglandins on Efferent Sympathetic Responses of the Heart

In the present model, epicardial superfusion with arachidonic acid or extrinsic PGI$_2$, as well as tetrodotoxin, did not affect the refractory period shortening of the deep intramyocardial test sites in response to infused norepinephrine. Therefore, prostaglandin-induced reduction of efferent sympathetic nerve effects is more likely due to a modulation of cardiac sympathetic neurotransmission than to an alteration in postjunctional responsiveness; that is, the reactivity of the ventricular effector sites to infused norepinephrine remained unchanged by the increase in pericardial prostaglandins. Two possible mechanisms may be responsible. Prostaglandins in the pericardial fluid can affect neurotransmission in the superficial postganglionic axons directly or by a presynaptic receptor mechanism. The fact that an increase in pericardial fluid prostaglandins did not affect efferent vagal actions on the ventricles supports a presynaptic receptor mechanism, since a drug like tetrodotoxin, which blocks axonal neurotransmission directly by suppressing sodium current, when instilled into the pericardial cavity, suppresses both efferent sympathetic and vagal responses in the ventricles.$^1$

Many endogeneous substances such as norepinephrine, dopamine, angiotensin, acetylcholine, prostaglandins, and peptides related to morphine are known to modulate transmitter release in response to impulses from sympathetic neurones, possibly by acting via presynaptic receptors.$^{20}$ Although presynaptic receptor modulation of neurotransmitter release is a popular concept, the mechanisms and whether it functions during normal physiological conditions in vivo are for the most part unknown.$^{21}$ Presynaptic receptors are thought to be located on or in the nerve endings. However, there is little direct evidence that the receptors that are activated or blocked are confined to the vicinity of the prejunctional membrane.$^{21}$ If presynaptic receptors of prostaglandins were present in the superficial cardiac sympathetic nerve fibers, pericardial fluid prostaglandins could have modulated nor-epinephrine release even at the deep intramyocardial test sites via a presynaptic receptor modulation.

Table 4. Radioimmunoassay of Prostaglandins in the “Normal” Pericardial Fluid

<table>
<thead>
<tr>
<th>Dog</th>
<th>Collected volume (ml)</th>
<th>Concentration of 6-keto-PGI$_{1}$ (ng/ml)</th>
<th>Concentration of PGE$_2$ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>10.6</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>1.8</td>
<td>45.0</td>
<td>3.6</td>
</tr>
<tr>
<td>3</td>
<td>2.1</td>
<td>100.0</td>
<td>7.3</td>
</tr>
<tr>
<td>4</td>
<td>1.1</td>
<td>152.0</td>
<td>5.1</td>
</tr>
<tr>
<td>5</td>
<td>0.9</td>
<td>29.0</td>
<td>4.0</td>
</tr>
<tr>
<td>6</td>
<td>1.1</td>
<td>66.0</td>
<td>5.3</td>
</tr>
<tr>
<td>7</td>
<td>1.4</td>
<td>21.0</td>
<td>5.6</td>
</tr>
</tbody>
</table>

PG, prostaglandin.

An antagonism by Pericardial Fluid Prostaglandins of the Effects of Angiotensin II

A facilitatory effect of angiotensin II on norepinephrine release evoked by efferent sympathetic nerve stimulation has been demonstrated in isolated rabbit$^{22}$ and rat$^{23}$ hearts and in canine heart without intact pericardium.$^{24}$ This effect of angiotensin II was suppressed by infusion of PGE$_2$ and PGI$_2$ into the heart and was enhanced during inhibition of prostaglandin synthesis with indomethacin infusion.$^{23}$ In the present study, we clearly demonstrated that when prostaglandin release into the pericardial fluid was stimulated, the facilitatory effects of angiotensin II on changes in electrophysiological variables elicited by efferent sympathetic stimulation were eliminated. Therefore, the effects of angiotensin II may be antagonized by pericardial prostaglandins in the intact heart. Angiotensin II itself may increase the release of PGI$_2$ from the pericardium and epicardium.$^2$ Thus, pericardial release of prostaglandins may operate as a negative feedback control mechanism antagonizing angiotensin II effects on the heart.

Angiotensin II is thought to be important in arrhythmogenesis, particularly in the ischemic heart,$^{21}$ because increased plasma catecholamines can augment renin release directly, which increases plasma concentration of angiotensin II, thereby resulting in a positive feedback mechanism to augment further the intramyocardial release of catecholamines. However, it is possible that ischemia-induced regional bulging would increase stretch of the epicardium and pericardium, thereby increasing PGI$_2$ release.$^7$ Also, PGE$_2$ and other prostaglandins are known to be released from the ischemic myocardium.$^{25}$ These may antagonize the effects of angiotensin II.

A Possible Negative-Feedback Mechanism Constituted by Pericardial Fluid Prostaglandins in the Regulation of Efferent Sympathetic Input to the Heart

In the present study, prostaglandin synthesis was stimulated by adding arachidonic acid to the pericardial fluid. As shown in Figure 2, the onset of the effect on efferent sympathetic-induced changes in ventricular refractoriness was very quick. Therefore,
any stimulus for prostaglandin synthesis by the pericardium and/or epicardium may result in a rapid inhibition of efferent sympathetic responses in the heart. In an epicardial irrigation model in situ, PGI₂ release from the epicardial surface was also increased when heart rate and force of contraction were stimulated by isoprenaline, and afterload was increased by aortic constriction, and when angiotensin II was infused.² All of these stimuli might be expected to result in increased cardiac work and oxygen consumption and might be associated with increased stretching of the epicardium and pericardium in the intact heart.² Thus, when efferent sympathetic input to the heart is heightened or plasma catecholamines are increased, it seems likely that the pericardium and epicardium would produce PGI₂ and other prostaglandins in response to the stimuli, thereby limiting efferent sympathetic input to the heart and further release of catecholamines.

**Pericardial Fluid Prostaglandins and Reflex Cardiac Responses**

In the present model, stimulation of pericardial prostaglandin synthesis had little or no effect on the magnitude of heart rate and arterial blood pressure changes elicited by intracoronary injections of bradykinin and nicotine. Since not only afferent sympathetic neural component but also efferent sympathetic component are involved in the reflex cardiac responses evoked by bradykinin, a lack of significant influence of pericardial prostaglandins on the responses seems somewhat contradictory in the light of their suppressive effects on efferent cardiac sympathetic neurotransmission.

Staszewska-Barczak et al.²⁸ have shown the effects of topical application of prostaglandins to the surface of the ventricle on reflex cardiovascular responses evoked by epicardial applications of bradykinin and nicotine. After intravenous administration of indomethacin (5 mg/kg), bradykinin-induced vasopressor responses are reduced,²⁶ and epicardial application of PGI₂, PGE₂ (0.01 to 0.1 µg/min), or PGI₁ (0.1 to 0.3 µg/min) potentiates the responses.²⁶,²⁷ They attributed this potentiation to the ability of epicardial prostaglandins to sensitize (reduce the excitation threshold of) afferent nerve endings in the epicardium to chemical stimuli that activate cardiac reflex. These results appear to be concordant with a study²⁸ using action potential recordings, which demonstrated that stimulation of A and C sympathetic afferent fibers evoked by epicardial application of bradykinin was reduced after administration of aspirin.

Our data show quite clearly that prostaglandins reduce the cardiac responses to efferent sympathetic stimulation. It is possible, therefore, that prostaglandins applied to the epicardium or present in the pericardial fluid exert opposite actions that sensitize afferent sympathetic nerve endings but reduce efferent sympathetic neurotransmission. The "net" response would depend on which one of these actions predominates, and the overall lack of the effects of pericardial prostaglandin synthesis on bradykinin-evoked reflex cardiac responses in the present model may be explained by such differential effects. In contrast to the topical application of prostaglandins to the ventricular epicardium used in the previous studies,²⁸,²⁶,²⁷ in our study, the entire surface of the heart should have come in contact with prostaglandins in the pericardial superfusate. Therefore, efferent cardiac sympathetic neurotransmission through epicardial nerves should have been affected to a greater extent in the present model than in the model used in the previous studies.²⁸,²⁶,²⁷ This may account, at least in part, for the difference in the influence of epicardial prostaglandins on bradykinin-evoked reflex cardiac responses between the previous studies²⁸,²⁶,²⁷ and the present one.

The difference of the route of bradykinin administration, that is, topical epicardial application versus intracoronary injection, may also account for the results. Unlike epicardial application, intracoronary injection of bradykinin produced predominantly vasodepressor responses in the present model, which may be explained by the direct bradykinin action on the heart and vascular bed,²⁹ or by the bradykinin stimulation of chemosensitive afferent vagal fibers.³⁰ In this respect, topical application of bradykinin to the epicardium has the advantage of eliciting predominately sympathetically mediated cardiac responses. However, because of global superfusion of the heart with Tyrode's solution instilled into the pericardial cavity, we had to give bradykinin by intracoronary injection. Therefore, no data are available from the present study on the influence of stimulation of pericardial prostaglandin synthesis on reflex cardiac responses elicited by the stimuli applied to the epicardium.

Epicardial application of prostaglandins did not affect nicotine-induced reflex cardiac responses.²⁶,²⁷ These results are concordant with our present results.

**Implication of the Study**

In the present study, we have shown that an increase in prostaglandins in the pericardial fluid inhibited efferent sympathetic nerve effects on several cardiac electrophysiological variables, while it did not affect efferent vagal nerve actions. These differential effects of prostaglandins on each cardiac autonomic limb may act to suppress arrhythmia development in various situations, especially when efferent sympathetic stimulation to the heart is maximally increased, for example, during acute myocardial ischemia/infarction.³¹ As predicted by Herman et al.,³ it is likely that prostaglandins produced by the pericardium and epicardium play a physiological and/or defensive role in the control of the external environment of the heart. If this is true, then physicians in general, but particularly cardiovascular surgeons, will need to reassess the importance of the pericardium and how it is handled, especially during and after cardiac surgery.
Acknowledgments

The authors thank John K. Lourie, MS, for help with some of the experiments, Naomi S. Fineberg, PhD, for statistical analysis of the data, and Peter B. Corr, PhD, for helpful discussions.

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Key Words: prostaglandins • epicardial superfusion • efferent cardiac response • sinus cycle length • atrioventricular conduction • ventricular refractoriness • cardiac reflexes
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T Miyazaki, H P Pride and D P Zipes

doi: 10.1161/01.RES.66.1.163

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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