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# Myocardial Drug Distribution Generated from Local Epicardial Application: Potential Impact of Cardiac Capillary Perfusion in a Swine Model Using Epinephrine

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## Abstract

Prior studies in small mammals have shown that local epicardial application of inotropic compounds drives myocardial contractility without systemic side effects. Myocardial capillary blood flow, however, may be more significant in larger species than in small animals. We hypothesized that bulk perfusion in capillary beds of the large mammalian heart enhances drug distribution after local release, but also clears more drug from the tissue target than in small animals. Epicardial (EC) drug releasing systems were used to apply epinephrine to the anterior surface of the left heart of swine in either point-sourced or distributed configurations. Following local application or intravenous (IV) infusion at the same dose rates, hemodynamic responses, epinephrine levels in the coronary sinus and systemic circulation, and drug deposition across the ventricular wall, around the circumference and down the axis, were measured. EC delivery via point-source release generated transmural epinephrine gradients directly beneath the site of application extending into the middle third of the myocardial thickness. Gradients in drug deposition were also observed down the length of the heart and around the circumference toward the lateral wall, but not the interventricular septum. These gradients extended further than might



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be predicted from simple diffusion. The circumferential distribution following local epinephrine delivery from a distributed source to the entire anterior wall drove drug toward the inferior wall, further than with point-source release, but again, not to the septum. This augmented drug distribution away from the release source, down the axis of the left ventricle, and selectively towards the left heart follows the direction of capillary perfusion away from the anterior descending and circumflex arteries, suggesting a role for the coronary circulation in determining local drug deposition and clearance. The dominant role of the coronary vasculature is further suggested by the elevated drug levels in the coronary sinus effluent. Indeed, plasma levels, hemodynamic responses, and myocardial deposition remote from the point of release were similar following local EC or IV delivery. Therefore, the coronary vasculature shapes the pharmacokinetics of local myocardial delivery of small catecholamine drugs in large animal models. Optimal design of epicardial drug delivery systems must consider the underlying bulk capillary perfusion currents within the tissue to deliver drug to tissue targets and may favor therapeutic molecules with better potential retention in myocardial tissue.

**Keywords**

epicardial drug delivery; myocardial drug distribution; inotrope; contractility; systemic vascular resistance

## 1. Introduction

Local controlled drug delivery provides pharmacologic therapy with elevated target tissue levels and minimal peripheral side effects [1–6]. Prior studies have demonstrated pharmacologic response following epicardial (EC) application of antiarrhythmic drugs [7–14], proarrhythmic agents [15, 16], vasodilators [17–21], and antiproliferative chemotherapeutics [22]. Other studies have demonstrated favorable pharmacokinetics, with elevated myocardial drug levels and minimal peripheral concentration, when drug is given to the pericardial sac [23–28]. We have shown in small animals that local EC application of inotropic compounds leads to elevated myocardial drug and intracellular second messenger concentrations in target ventricular tissue, enhanced contractile response with lower systemic levels, and less atrial and peripheral vascular responses than IV infusion [29, 30]. EC epinephrine delivery in rats required only 1/3$^{rd}$ of the IV dose rate to provide an equal contractile effect without raising plasma drug levels or deleterious systemic side effects such as tachycardia or peripheral vasodilation [30].

While these phenomena from small animals are promising and intriguing, they may not necessarily translate to larger species. Indeed, the mechanisms of drug transport within and clearance from the myocardium may be more complex in larger species. Local delivery of soluble drug relies on diffusion through interstitial spaces and thus distribution may be impacted by physical dimensions [4, 31–34]. Furthermore, intramyocardial capillaries may clear drug from the heart [34]. Given these concerns, we sought to extend our findings from small rodents to larger animal models, where the thickness of the heart wall and vascular networks are similar to human cardiac anatomy, and we have the means to sample venous effluent from the heart to quantify the myocardial capillary contribution to systemic loading. We used an anesthetized, adolescent swine model to study the distribution of the drug in the

myocardial tissue, its route of clearance from the myocardium, and subsequent systemic levels when delivered EC or by intravenous (IV) infusion. We hypothesized that the surface area of release might impact myocardial distribution and biological response. Therefore, epinephrine was delivered to the anterior wall of the heart of swine using either a point-source release, in the form of a small 1.5 $cm^2$ diameter polymeric disc, or a dispersed-source release, in the form of an adherent hydrogel covering the entire anterior wall of the left ventricle. Cardiovascular function was continuously monitored, epinephrine levels in cardiac venous effluent and arterial blood were measured, and after sacrifice and tissue harvest, drug distribution in the left ventricle was quantified.

## 2. Methods

### 2.1. Fabrication and Characterization of Epicardial Point-Source Alginate Drug Delivery Platform

An alginate polymeric local drug releasing system, designed to precisely administer animal-weight based amounts of epinephrine to the epicardium, was fabricated from calcium-cross-linked alginate hydrogels [29, 30]. Briefly, 0.65 ml of 2% alginate (#71238; Sigma-Aldrich) slurry in double distilled water (ddH20) was pipetted onto the upper side of the permeable membrane of a transwell support (#3472, 24 mm, polyester, 3 μm pore size; Corning). Immersion of the transwell support in 1.5 ml of 3% $CaCl_2$ in $ddH_20$ using a leveled culture plate (#353047, 15.75 mm; Corning) for 30 min at room temperature cross-linked the alginate to form a flexible solid concave disk (diameter 24 mm, minimal thickness [center] 2.0 mm, maximal thickness [perimeter] 3.0 mm, lower surface area 452 $mm^2$, volume 700 $mm^3$). Free $Ca^{2+}$ in the alginate was removed by placing the disks in $ddH_2O$ for 30 min.

A series of in vitro experiments quantified the epinephrine released over time as a function of the applied concentration. The alginate disk was placed in a new transwell support and immersed in a leveled 24 well culture plate filled with 1.5 ml of normal saline representing the released drug receiving chamber. The culture plate was placed on an orbital shaker at 20 RPM (#3520; Labline). Epinephrine (10 μl of 1, 2, 5, 10 or 20 mg/ml in $ddH_2O$, # 0409-4921-34; Hospira) was added every 10 minutes to the upper free concave surface to smooth the release at the lower surface to a steady rate. These experiments were repeated in triplicate. At regular intervals, a 60-μl sample from the receiving chamber was removed to evaluate the amount of released drug and 60 μl of normal saline was added immediately to the wells to restore the receiving chamber volume. The concentration of epinephrine in each sample was determined by spectrophotometric methods (Victor$^3$ Multilabel Counter, Perkin Elmer) [35]. Metaperiodate (6 μl of 2% $NaIO_4$ in $ddH_2O$, #S1878, Sigma-Aldrich) and ethanol (9 μl, 100%) were added to the samples and the absorbance at 490 nm was measured to calculate the amount of released epinephrine at each time point using a standard curve. For each concentration of applied drug solution to the alginate disk, the release rate was determined using a linear least-squares correlation. Each release rate was then linearly correlated to the applied concentration. This relationship, specific to these disks at the fixed volume and interval of applied drug solution, allows the release rate to be prescribed solely through adjustments in applied concentration [29, 30]. This novel method of controlling

epicardial drug release allows for precise animal weight-based dosing without any chemical modifications to the polymer platform.

### 2.2. Fabrication and Characterization of Epicardial Dispersed-Source Poloxamer Based Drug Delivery Platform

As an alternative to the point-source alginate disk delivery platform, a poloxamer hydrogel was used to distribute the source of drug release over the entire anterior wall of the LV. Epinephrine (1 mg/ml, # 0409-4921-34; Hospira) was mixed into a 30 w/v% solution of Poloxamer 407 (#16758; Sigma) in ddH20 at 5°C. The resulting solution (10, 50, 100, or 200 μg/ml) was poured into a precooled beaker and mixed overnight at 5°C with a magnetic stir plate. Poloxamer 407 solutions remain free-flowing liquids at temperatures below 15°C; above this temperature, viscosity increases forming a hydrogel that allows for controlled drug release.

In vitro release was characterized using precooled (5 C°) 12-well culture plates (#3513; Corning) coated with 760 μl of epinephrine-poloxamer solution (10, 50, 100, or 200 μg/ml) with an average thickness of 2 mm. The poloxamer was allowed to gel at 37°C for 5 minutes before 300 μl of normal saline was added to each well and the plates placed on an orbital shaker set at 80 RPM. Samples (60 μl) were drawn only once from each well at either 5, 10, 15 and 30 minute time points and were pipetted into a 96-well plate (#3799; Corning) for quantification by spectrophotometric methods as above. Thus, 12 poloxamer hydrogels were needed at each starting epinephrine concentration for the release experiment to be repeated in triplicate.

### 2.2. Surgical Procedures

All studies were approved by the Institutional Animal Care and Use Committee at Steward St Elizabeth's Medical Center, Boston, Massachusetts. Twelve adolescent Yorkshire swine (36 – 41 kg) were fasted for 12 hours preceding the experiment, but had access to water.

The animals were sedated with an intramuscular (IM) injection of telazol 250 mg, ketamine 125 mg, and xylazine 125 mg and restrained supine on heating pads. The airway was secured with a 6.0 mm cuffed endotracheal tube and ventilated (10 breaths per minute, initial tidal volume 500 ml, Excel 110 anesthesia machine with 7000 series ventilator, Ohmeda) to maintain end-tidal carbon dioxide between 35 and 40 mmHg (Poet-IQ gas monitor; Criticare Systems). Anesthesia was maintained with isoflurane 1–2% during catheterization. A femoral artery catheter (#CS-04300, Arrow) was placed by the Seldinger technique for continuous blood pressure monitoring and plasma sampling. A Pressure–Volume conductance catheter (Ventricath 507, Millar instruments) was passed retrograde into the left ventricle under fluoroscopic guidance from the contralateral femoral artery. A pulmonary artery catheter (#746HF8, Edwards Life Sciences) was placed from the right external jugular vein to measure core temperature and continuous thermodilution cardiac output (CO). Other acquired hemodynamic parameters were mean arterial pressure (MAP), central venous pressure (CVP), heart rate, and an index of contractility (max dP/dt). Systemic vascular resistance (SVR) was calculated as (SVR = (MAP – CVP) / CO). Following catheterization, the isoflurane based anesthetic was transitioned to total

intravenous anesthesia with midazolam (0.25 mg/kg/hr), fentanyl (12–25 μg/kg/hr), and ketamine (5 mg/kg/hr) and stabilized for 30 minutes prior to experimentation. After opening the pericardial sac and draining pericardial fluid, a 14 Fr retrograde cardioplegia catheter (#RC014IT, Edwards Life Sciences) was placed into the coronary sinus from a right atriotomy. At the end of the study, which was 15 and 60 min for dispersed- and point-source release systems, respectively, the heart was excised and dissected for further assessment of myocardial epinephrine levels.

### 2.3. Drug Delivery

Epinephrine was delivered by EC administration using a point-source alginate disk (0.1 μg/kg/min, N=3) and a dispersed-source poloxamer hydrogel (0.03 μg/kg/min, N=3). Epinephrine was infused in two additional groups of animals at these doses as IV controls (N=3). Preliminary data suggested that these EC doses increased max dP/dt by 40–60%. Prior to drug administration, endomyocardial tissue from the anterior wall was sampled under fluoroscopic guidance using biopsy forceps (#190080, Argon Jawz™ Endomyocardial Biopsy Forceps, Argon Medical Devices) to measure pretreatment epinephrine levels.

The point-source release alginate platform was placed on the anterior wall of the beating heart between the circumflex and left anterior descending artery (Fig. 1A). Epinephrine was pre-delivered to the alginate devices for 30 minutes before placement on the heart to eliminate the time delay needed for the drug to diffuse through the thickness of the platform. After the placement of the point-source alginate disk on the heart, appropriate dilutions of epinephrine were applied (10 μl) every 10 min to the free upper surface of the alginate disk. The absence of liquid-drug overflow was visually confirmed throughout the experiment. EC epinephrine administration continued for 60 min before sacrifice and harvesting myocardial tissues.

When using the poloxamer dispersed-source platform, epinephrine was embedded within the hydrogel prior to the experiment in concentrations that provided the desired weight based dose determined by the in vitro release data. Poloxamer solution (3 ml) was applied to the anterior wall of the heart in 0.05 ml increments, which gelled on contact. Application of the poloxamer hydrogel took less than 2 minutes. The area of contact between poloxamer hydrogel and the heart was 15 $cm^2$ (Fig. 1B) and the mean thickness was 2 mm. Preliminary experiments showed that the stable attachment of the hydrogel to the heart surface is limited to 15–20 min after which it tended to slide off the surface. Therefore, drug application from the poloxamer dispersed-source platform was limited to 15 minutes before sacrifice and harvesting of myocardial tissues.

All IV infusions of epinephrine at doses corresponding to the EC release rates were at a rate of 10 ml/hr via syringe pump (#74900-00; Cole Parmer), and corresponding sham EC devices with no drug were placed on the heart. Saline (10 ml/hr IV) was infused during all EC epinephrine delivery experiments.

### 2.4. Tissue and blood harvesting and processing

Coronary sinus and arterial blood was sampled for plasma epinephrine concentration at 0, 5, 10, 15, 30, 45 and 60 min when using alginate and at 0, 5, 10 and 15 min when using

poloxamer platforms. At the end of the study, the heart was quickly excised and rinsed with saline at 4°C. Transmural cores (10 mm) of myocardium were rapidly harvested from the anterior wall just under the release device and from the lateral, inferior, septal and apical walls using a biopsy punch (#P1025, Acu-punch$^R$, AcuDerm$^R$ Inc). An adjacent core was taken from the anterior wall under the release device for high-resolution immunofluorescent quantification of the transmural drug gradient. Harvested tissue samples were immediately rinsed in saline, blotted, and snap frozen with pre-cooled tongs in liquid nitrogen and stored at −80°C for future analysis.

Frozen myocardial tissue cores from the anterior, lateral, inferior, and septal walls were trisected into adjacent 3 mm thick slices using a tissue matrix cutting guide (#TM2000, ASI instruments) to separate epicardial, mid-myocardial and endocardial layers for epinephrine quantification. Apical cores were dissected in half. Frozen myocardial tissue slices were crushed in liquid $N_2$ using a ceramic mortar and pestle, mixed with lysis buffer (#250006; Cell Biolabs Inc) containing phosphatase and protease inhibitor cocktails (#P2850, P5726 and P8340; Sigma) [36, 37] and mechanically homogenized (VirTishear 1700, Lab/Pilot). The lysate was incubated at 4°C for 60 min, centrifuged at 1100 g for 15 minutes, and the supernatant stored at −80°C. Blood samples were centrifuged at 1100 g for 15 minutes and the supernatant stored at −20°C. Epinephrine content in plasma and cardiac tissue extracts were measured using a colorimetric enzyme-linked immunosorbent assay (ELISA) (#IB89551; Immuno-Biological Laboratories Inc) [38]. Cardiac epinephrine levels were normalized to protein content, which was measured by Bradford's method with bovine serum albumin as the standard [39] (#500–0006; Bio-Rad).

The depth of receptor-bound epinephrine deposition was evaluated in anterior wall transmural cores harvested beneath the point- or dispersed-source release device. Frozen transmural myocardial sections (15 μm thick) were fixed with 4% paraformaldehyde, triple washed with PBS solution, and sequentially incubated with 10% goat serum for 60 min (to prevent non-specific antibody binding) with rabbit anti-adrenaline primary antibody for 16 hours (#A0906, 1:2000 dilution, USBiological) and with fluorescein isothiocyanate (FITC)-conjugated secondary antibody for 30 min (#AP182F, 1:80 dilution, Millipore) [40, 41]. Immunofluorescent imaging was carried out using confocal microscopy (Zeiss LCM 150, USA). Transmural profiles of the intensity of the fluorescence were computed by averaging values laterally over 500 μm at each transmural location (Image J 1.45S, NIH). Values of autofluorescense intensity from myocardial tissue from untreated animals (Data not shown) were subtracted from those in EC and IV groups. All data were scaled between control tissues and maximal values observed with EC treatment.

### 2.5 Data analysis and statistics

Myocardial drug concentration data is presented as mass normalized to protein content in the tissue extract. A one-way ANOVA analysis with Bonferroni's correction was used to compare epinephrine concentrations from different myocardial locations in the same treatment group (GraphPad Prism, 5.0). A one-way ANOVA analysis was also used to compare plasma epinephrine levels at different time points in the same treatment group. Plateau steady-state hemodynamic responses to epinephrine infusion or local release are

presented as percentage change from the baseline. An unpaired, two-tailed t-test was used to compare hemodynamic responses to equal doses during EC and IV delivery. The unpaired, two-tailed t-test was also used to compare EC and IV delivery at the same dose in terms of plasma epinephrine levels at corresponding time points, and tissue epinephrine concentrations from corresponding tissue sites. Data were considered statistically distinct if the p-value was less than 0.05.

## 3. Results

### 3.1 In Vitro Epinephrine Release Kinetics

The release kinetics of epinephrine through a point-source alginate disk in vitro was characterized for applied concentrations of 1, 2, 5, 10 and 20 mg/ml (Fig. 2A). The release was linear for over 120 minutes suggesting zero$^{th}$ order kinetics, with the release rate solely dependent on the concentration of the applied drug. These release rates were linearly correlated with the applied epinephrine concentration (Fig. 2B). This relationship was used to calculate the concentration of applied epinephrine to provide the desired EC delivery rate in vivo. These data indicate that the alginate drug release platform allows delivery of the precise release rate for any animal weight without having to redesign the system to change the dose.

As an alternative to the point-source delivery using an alginate disk, a poloxamer hydrogel was used to disperse the point of release over a wide surface area of the anterior wall of the heart. The kinetics of epinephrine release from the poloxamer hydrogel was linear with time for epinephrine loading of 10 μg/ml. In vitro data for higher loading concentrations (50–200 μg/ml) showed an initial burst release of 2.2% of the loaded drug during first 5 minutes and then zero$^{th}$ order kinetics thereafter (Fig. 2C). By 15 minutes 3.6% and at 30 minutes 6% of the loaded drug had been released. The total drug released between by 15 minutes, the length of the dispersed-source in vivo experiment, was correlated to the initial concentration of drug in the poloxamer hydrogel (Fig. 2D). This relationship was used to calculate the epinephrine concentration in the poloxamer hydrogel to provide the desired EC delivery rate.

### 3.2. Tissue epinephrine distribution in EC delivery

EC epinephrine delivery from a point-source at 0.1 μg/kg/min over 60 minutes (Fig. 1A) led to significant drug deposition near the point of release with a transmural gradient that tapered across the wall of the myocardium (Fig. 3A). Drug deposition around the LV circumference of the heart shows asymmetric distribution with elevated concentrations in the epicardial layer of the lateral wall and apex, but not in the inferior wall or septum. Drug deposition in the inferior and septal walls, as well as all endocardial layers, following EC application did not differ from all of the sites following IV treatment (Fig. 3B) and was elevated 2.2 to 2.6 fold above levels detected in pretreatment biopsy samples.

Immunostaining of the transmural sections of the anterior wall showed the transmural receptor-bound drug gradient, providing greater spatial resolution than the tissue ELISA measurements. Confocal microscopy of the immunostained transmural sections showed a visually detectable accumulation of the drug up to 400 μm deep from the epicardial surface

where the EC alginate device was applied (Fig. 4A, C). Of note, the outermost parietal epicardial membrane that remains attached to the myocardium contains few adrenergic receptors and therefore low fluorescent signal. Myocardial epinephrine deposition during IV treatment was below the detection threshold for this technique (Data not shown).

EC epinephrine delivery to the entire anterior wall (15 $cm^2$) using dispersed-source release over 15 minutes (Fig. 1B) provided significant deposition in the epicardial layer of the anterior, lateral, inferior and apical walls but not in the septum (Figs. 3C). LV epinephrine levels in the mid-myocardium and endocardium was similar to that observed during IV treatment (Figs. 3D). Immunostaining techniques did not show any receptor-bound epinephrine in myocardium sampled from under the polymer hydrogel due to the limited sensitivity of the method (Data not shown).

### 3.3 Systemic epinephrine distribution in EC delivery

Epicardial epinephrine delivery led to elevated drug concentrations in the coronary sinus blood (Fig. 5). Coronary sinus plasma epinephrine levels rapidly reached steady state 5 minutes into the treatment and were 11-fold and 10-fold higher than pretreatment levels with point-source (Fig. 5A) and dispersed-source (Fig. 5B) release, respectively. The coronary sinus plasma epinephrine levels remained elevated well beyond the time of the burst from dispersed-source release predicted by in vitro data (Fig. 2C). Arterial blood epinephrine levels were not different between EC and IV administrations for either point- or dispersed-source release (Figs. 5A, B).

### 3.4 Contractile, chronotropic and vascular effects of epinephrine EC delivery

The inotropic effect of epinephrine delivered locally using either point- or dispersed-source release was similar to the corresponding IV infusions (Figs. 6A, D). With EC application using point-source release and equal dose IV infusion, max dP/dt increased by 76 ± 7% and 86 ± 27% at 60 minutes, respectively (Fig. 6A). The increase in max dP/dt following EC epinephrine administration using dispersed-source release and equal dose IV infusion was 34 ± 15% and 44 ± 19% at 15 minutes, respectively (Fig. 6D). Non-ventricular hemodynamic effects, such as an increase in heart rate and decrease in systemic vascular resistance (SVR), were also present to similar extents as with IV infusions (Figs. 6B, C, E and F). EC application using point-source release and corresponding IV infusion increased HR by 39 ± 23% and 33 ± 9% at 60 minutes, respectively (Fig. 6B). Similarly, dispersed-source release and corresponding IV infusion increased HR by 36 ± 24% and 32 ± 16% at 15 minutes, respectively (Fig. 6E). EC point-source application and equal dose IV infusion decreased SVR by 29 ± 10% and 35 ± 14% at 60 minutes, respectively (Fig. 6C), and with dispersed-source release and corresponding IV infusion, by 24 ± 9% and 23 ± 15% at 15 minutes, respectively (Fig. 6F). MAP and stroke volume (SV) responses to EC and systemic epinephrine are shown in the Supplementary Material (S1). Neither MAP nor SV changed significantly by the end of the study in both, point- and dispersed-source EC delivery or IV infusion (Figs S1A–D, Supplementary Material). Of note, epinephrine can be arrythmogenic in high concentrations. In this model, we did not observe any ventricular ectopy or arrhythmia during point- or dispersed-sourced EC epinephrine delivery.

## 4. Discussion

In contrast to systemic treatment, where drug evenly distributes throughout the body and sought primary biological response is inevitably accompanied by proportional side effects, local controlled delivery elevates target tissue drug levels, initiating local biological responses and minimizing peripheral effects [1–6]. In particular, local epicardial drug delivery of catecholamine inotropic drugs leads to augmented myocardial contractility with less peripheral vasodilation and tachycardia than systemic delivery in small rodent models [29, 30]. These data demonstrate the potential and precise targeting of local drug delivery techniques to one tissue within the heart as local beta agonist therapies increased ventricular myocardial contractility but not HR, which is driven by the SA node high in the right atrium. While these small rodent studies clearly demonstrate the focused therapy of local EC treatments, the deposited drug may be less confineable in animal models with larger hearts such as swine due to clearance by myocardial capillaries.

Myocardial drug distribution after local application is the net result of the complex interplay between diffusive spread driven by concentration gradients, transmural convection driven by the pressure gradient from endocardial to epicardial surface, bulk transport by capillary perfusion, and clearance by the same capillaries [29, 30, 31, 34]. Diffusive forces, though likely augmented by LV contraction and relaxation, decline with distance from the source. Therefore, the thickness of the tissue and large axial and circumferential dimensions of the heart may become limiting factors in achieving extensive drug distribution. The dimensions of the swine heart may also limit transmural convection as the hydraulic resistance of any tissue increases with the thickness [42]. In EC drug delivery, convective forces oppose the direction of drug diffusion and, if present, may limit drug penetration through the tissue. Estimates of the velocity of transmural hydraulic flow and diffusivity of epinephrine in tissues suggest that convective forces are overwhelmed by diffusion (Supplementary Material S2). This is supported by the fact that drug penetrates several millimeters into the tissue in less than 1 hour (Figure 3A). The remaining mechanism of drug distribution in this experimental model is transport within capillaries. By contrast to diffusion, capillary transport may be more uniform throughout the tissue and may dominate the local pharmacokinetics at greater depths and distances from the point of local administration.

Given that swine hearts are 6-fold thicker and 200-fold more massive than in small rodents, it is expected that the movement of drug within the heart following EC administration might differ between these two species. We hypothesized that in large animal hearts, gradients of deposited drug might exist from the point of local release toward more distant sites [30], that these gradients may be shaped by the flow of blood in capillary networks, and that these capillaries may remove drug from the tissue and elevate systemic drug levels. We used epinephrine as a model compound as it exerts quantifiable biologic responses and is readily measured in plasma and tissues [30]. We delivered epinephrine to the epicardial surface of adolescent swine to closely characterize the extent of transmural, circumferential, and longitudinal drug distribution, and clearance in coronary sinus effluent following local EC delivery using both point-source and dispersed-source polymeric drug releasing systems.

### 4.1 Transmural Longitudinal and Asymmetric Circumferential Epinephrine Distribution in the Myocardium in EC Delivery

The swine model revealed unexpected drug deposition with local EC epinephrine delivery that could not be resolved in small animals. Tissue cores from under the release matrix showed that drug levels were elevated above distant sites in the epicardial and mid-myocardial, but not in the endocardial layers (Figs. 3A). Thus, drug diffused up to 6 mm transmurally during the experiment (Fig. 3A). In contrast, elevated drug levels were observed up to 4 cm further toward the apex of the heart and 3 cm circumferentially past the edge of the release device, but only in the leftward direction. The drug did not travel from the anterior wall to the septum at all. Such asymmetric, circumferential drug distribution is unlikely to arise from passive diffusion alone. In addition, drug is seen much further from the release device, both circumferentially and longitudinally, than one might expect from the extent of transmural diffusion. Therefore, there must be some force augmenting the distribution of drug down the length of the heart and towards the lateral and inferior walls.

Our data show that the drug is rapidly absorbed by capillaries, as significant amounts are detected in the coronary sinus blood after only 5 minutes (Figs. 5A). In both point- and dispersed-source release, the polymer was applied near the bifurcation between the left anterior descending and the circumflex arteries (Fig. 1) and it appears that the vector of the tissue drug distribution follows the net direction of blood flow from branches and capillaries arising from these major vessels (Fig. 7A). Capillaries drive bulk flow through the tissue that is away from these arteries towards the venous drainage, which feed the coronary sinus along the atrial-ventricular groove on the inferior wall of the heart. Thus, bulk perfusion may transport the drug towards distal myocardial segments and around the circumference of the heart, toward the apex and lateral and inferior walls, and also remove drug from the tissue. The drug did not move from the anterior wall to the septum, perhaps because the bulk myocardial capillary perfusion under the release device is in the opposite direction. These data suggest that septal wall myocytes may be very difficult to treat from epicardial approaches alone.

### 4.2. Biologic Effect in EC Delivery is Driven by Systemic Distribution

Systemic levels of epinephrine were similar whether given by IV infusion or local controlled release (Fig. 5). Cardiac epinephrine levels in remote regions from the site of EC delivery were also similar following local or systemic delivery and higher than pretreatment levels (Fig. 3), suggesting systemic, rather than local, origin of much of the drug in the heart. As a result, inotropic, chronotropic, and vascular effects were similar in both EC and IV administration (Figs. 6A, D). Even though EC epinephrine delivery using both point- and dispersed-source release increased drug levels in a limited part of the myocardium, these treated myocytes did not raise global cardiac contractility (max dP/dt) above the level seen with an equal dose IV infusion. These disappointing results stand in marked contrast to promising prior rodent data, where elevated myocardial drug by local delivery led to augmented global indices of contractility for the whole ventricle in excess of equivalently dosed IV treatments [29, 30].

One possible reason for why local application of inotropic drugs led to augmentation of contractility in small rodents to a greater extent than an equivalent IV dose [30] is that the devices were large relative to the anterior wall and that they treated the whole of the anterior myocardium rather than just a small part of the anterior wall. Therefore, in this swine study we used a dispersed hydrogel delivery system. Even with this dispersed delivery system, enhanced contractile effect from local delivery could not be demonstrated to be more effective than with IV infusion (Fig. 6D), nor could we show lower plasma levels with local application (Fig. 5). The coronary sinus drug concentration was elevated for both the point-source alginate (Fig. 5A) and for the dispersed poloxamer hydrogel EC delivery systems (Fig. 5B). Therefore, the clearance of drug from the myocardium may have been much more significant in swine than in rats.

Another reason why local epinephrine in swine did not augment contractility above the systemic contribution is the limited penetration depth from the surface relative to the thickness of the target tissue (Figs. 3A, 3C, 4C). To a first order approximation, the diffusivity of epinephrine in rat and swine myocardium should be similar, and therefore the diffusive length, the depth to which drug can diffuse into a medium, should also be similar over the same time interval (Figs. 7B and 7C). However, the greater thickness of swine myocardium implies that a far lower fraction of the myocytes are exposed to a therapeutic level and that the endocardium may see very little drug. Therefore, even though epicardial myocytes may have profound augmentation of their contractile state, inotropic effect measured on the whole ventricle, such as with max dP/dt calculations, may not substantially increase. These relationships are valid, even if one neglects the contributions of capillary clearance.

The direction and depth of epinephrine deposition in myocardial tissue is determined by the interplay of the EC drug diffusion and capillary clearance (Figs. 7B and 7C). Hence, at some depth, drug gradients diminish and diffusion is overridden by the capillary clearance, preventing further forward motion [35] (Fig. 7B). Histological evidence also suggests that capillary density increases with both thickness [43], and distance from the surface of vascularized tissues [44]. In addition, left ventricular perfusion in swine also increases from epicardium to endocardium by 16% [45] and may contribute to a higher rate of drug removal deeper into the heart muscle. Moreover, the high concentrations of epinephrine in the myocardium following EC application (Figs. 3A, 3C, 4A) may increase local myocardial perfusion via beta-2 adrenergic arterial dilation, and thus facilitate its own clearance from the tissue. All these potential mechanisms suggest that the deposition in large animal species following local drug delivery to the epicardial surface is complex and determined by much more than passive diffusion, including flow in myocardial capillaries.

### 4.3 Implications of Intramyocardial Pharmacokinetics and Potential Clinical Applications

We envision several potential applications of EC delivery for both open chest heart surgery patients and minimally invasive therapies. Open chest heart procedures, including heart valve, coronary artery bypass grafting and heart transplant surgery, may benefit from local drug applications aimed to support cardiac function, improve postsurgical tissue regeneration, reduce the risk of rejection of the implanted tissues or reduce inflammatory

complications. Basic lessons learned about rapid circumferential and axial transport should be valid in a closed chest application, since drug diffusion and myocardial capillary perfusion should not be significantly altered. Minimally invasive therapies may include perioperative cardiac support with inotropes or chronic therapy of infarcted or failing myocardium with small molecules or growth factors to decrease infarction-related inflammatory reactions and enhance regeneration of cardiac tissue.

There are several means for deploying drug eluting systems on the EC surface for both open and closed applications. These technologies include drug eluting polymeric patches or hydrogels placed directly on the epicardial surface, such as during cardiac surgery. Closed chest approaches include injection of viscous or self polymerizing drug eluting systems into the pericardial sac using percutaneous, minimally invasive techniques, either through direct subxiphoid pericardial puncture or through catheters advanced from the femoral vein across the right atrial appendage and into the pericardium [46–48]. The pericardial membrane will be intact in closed chest applications, unlike open cardiac surgical applications. Residual pericardial fluid may potentially dilute locally delivered drug, or may provide an extramyocardial pathway of circumferential and axial distribution. Further study is required to decipher the impact of pericardial fluid on drug transport, and for the development of polymeric systems that mitigate such potential effects.

Local drug delivery strategies imply targeted treatment of cells within a confined tissue of interest. Organ blood perfusion, in turn, opposes drug accumulation following local delivery as it clears drug from the tissue target [29, 34, 49, 50]. This phenomenon suggests the need for drug formulations designed specifically for local delivery that impede local clearance and prolong tissue retention. Other local drug delivery strategies have faced similar challenges. Initial attempts at modulating the vascular response to injury from mechanical revascularization in small animal models utilized soluble heparin-like compounds [2, 6]. Detailed pharmacokinetic analyses of heparin in the vascular wall after local delivery suggested that its high solubility, and therefore, easy diffusibility, made it difficult to retain within the target tissue to a therapeutic level [31, 51, 52]. Furthermore, the relative lack of potency of heparin, combined with its solubility and rapid clearance, demanded large reservoirs for use over prolonged periods. These insights were part of the impetus for developing a now widely used technology to locally deliver highly potent and hydrophobic drugs, such as Paclitaxel [53], Rapamycin [54], and Tacrolimus [55] to the vascular intima to prevent arterial restenosis following coronary stenting. Inotropic therapy by EC delivery may need to undergo a similar evolution based on similar detailed intramyocardial pharmacokinetic analyses. The data presented in this study may provide the first of many steps in such an evolution.

## 5. Conclusion

EC epinephrine delivery from both point- and dispersed-source release platforms generates transmural, longitudinal and asymmetric left-sided circumferential tissue gradients. The vector of myocardial drug distribution coincides with the predominant direction of the blood flow away from the nearest large epicardial coronary arteries suggesting the role of bulk tissue perfusion in the movement and clearance of the drug. Epinephrine delivered EC is

rapidly cleared by capillaries, increasing the level in coronary sinus and arterial blood. Thus, coronary blood flow which drives capillary perfusion is a plausible mechanism of both drug distribution and clearance from myocardium. These critical insights suggest that inotropic compounds with physicochemical properties that lend themselves to tissue retention may be better suited for epicardial applications. The demonstrated local myocardial pharmacokinetics may allow for rational designs for epicardial drug therapy systems.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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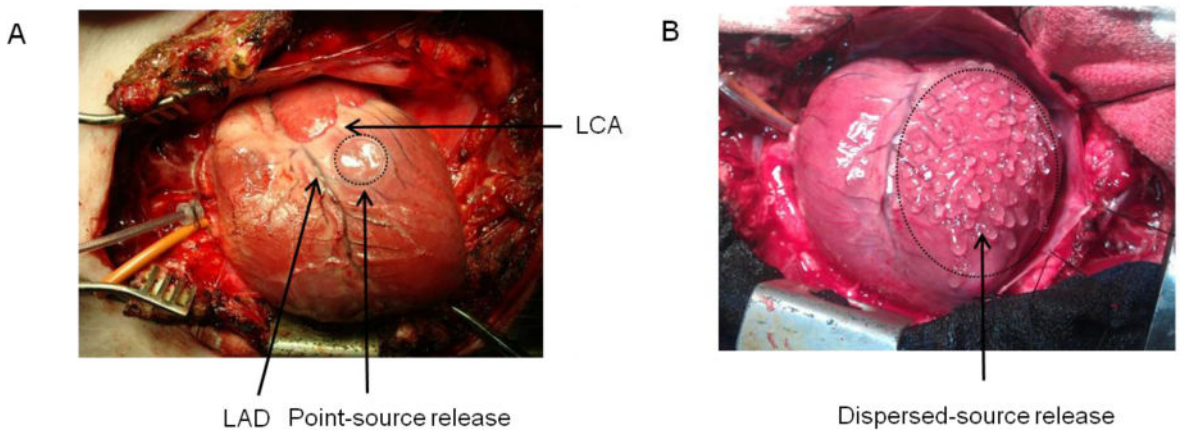


**Figure 1.**

Epinephrine EC delivery to the anterior wall of the left ventricle (LV) using point-source release (A) and dispersed-source release (B). The edge of each drug release platform was approximately 2 mm from the left anterior descending (LAD) and 1.5 cm from the left circumflex arteries (LCA).

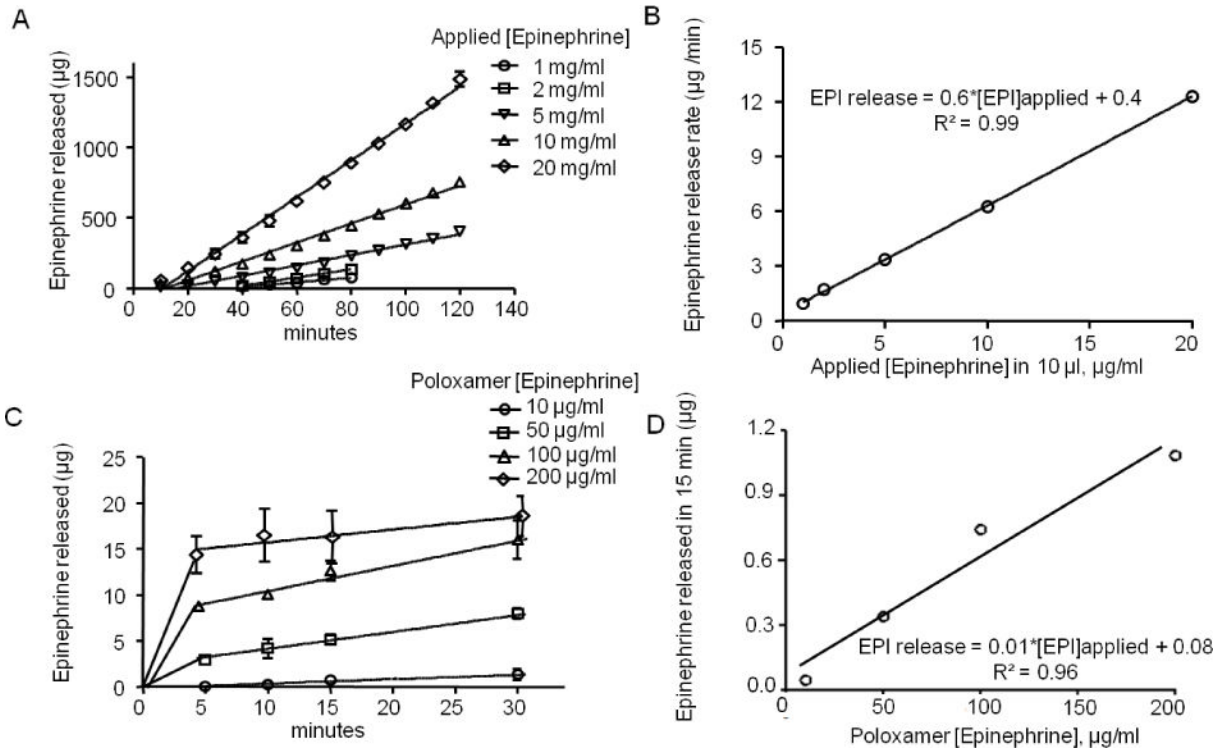


**Figure 2.**

In vitro release of epinephrine from point- and dispersed-source release epicardial platforms. Epinephrine released from the point-source is linear with time for each applied concentration studied (A). The slope of each of these in vitro release data is the steady state release rate. A strong correlation between applied concentration and release rate shows that the release rate can be tailored with precision by selecting the appropriate applied drug concentration (B). Epinephrine release rate from the dispersed-source is linear for loading of 10 µg/ml (C). For higher drug loading, there is an initial burst release followed by zero$^{th}$ order kinetics from 5–30 minutes (50–200 µg/ml) (C). There is a strong correlation between applied concentration and the drug released during first 15 min period (D) that allows determination of the concentration of drug to embed within the poloxamer hydrogel to obtain the required animal weight-based delivery rate in vivo. N = 3, Avg ± SD.

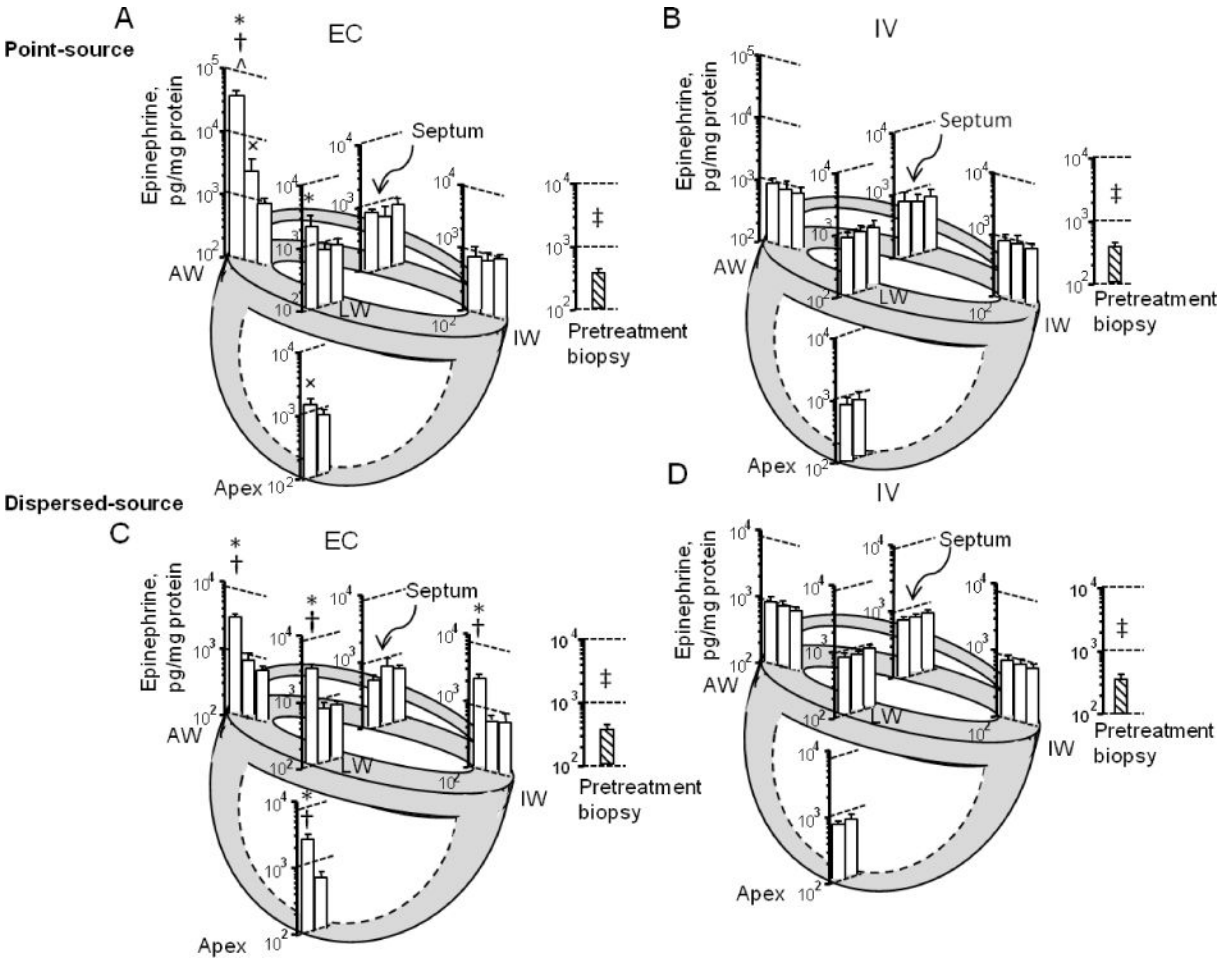


**Figure 3.**

LV tissue epinephrine distribution following epicardial (EC) delivery using point-source (A; 0.1 μg/kg/min) and dispersed-source release (C; 0.03 μg/kg/min). Corresponding equal dose intravenous (IV) infusions are also shown (B and D). EC epinephrine delivery using point-source release leads to prominent drug deposition in the epicardial layer of the anterior wall (AW) and, to a lesser extent, in the mid-myocardium (A). Accumulation of epinephrine was also observed in the epicardial layer of the lateral wall (LW) and apex during. EC delivery using dispersed-source release provides accumulation of epinephrine in the epicardial layer directly beneath the site of application (anterior and lateral walls) and extends laterally to the inferior wall (C). IV infusion leads to homogenous deposition with no appreciable transmural, circumferential or longitudinal gradients (B and D). N = 3, Avg ± SD.

***** – $p<0.05$ compared to mid-myocardial and endocardial layers of the same heart wall,

× – $p<0.05$ compared to endocardial layer of the same heart wall,

ˆ $p< 0.05$ compared to epicardial layers of other walls,

† – $p<0.05$ compared to the corresponding tissue sample in the IV group,

‡ – $p<0.05$ compared with the IV or EC treatment.

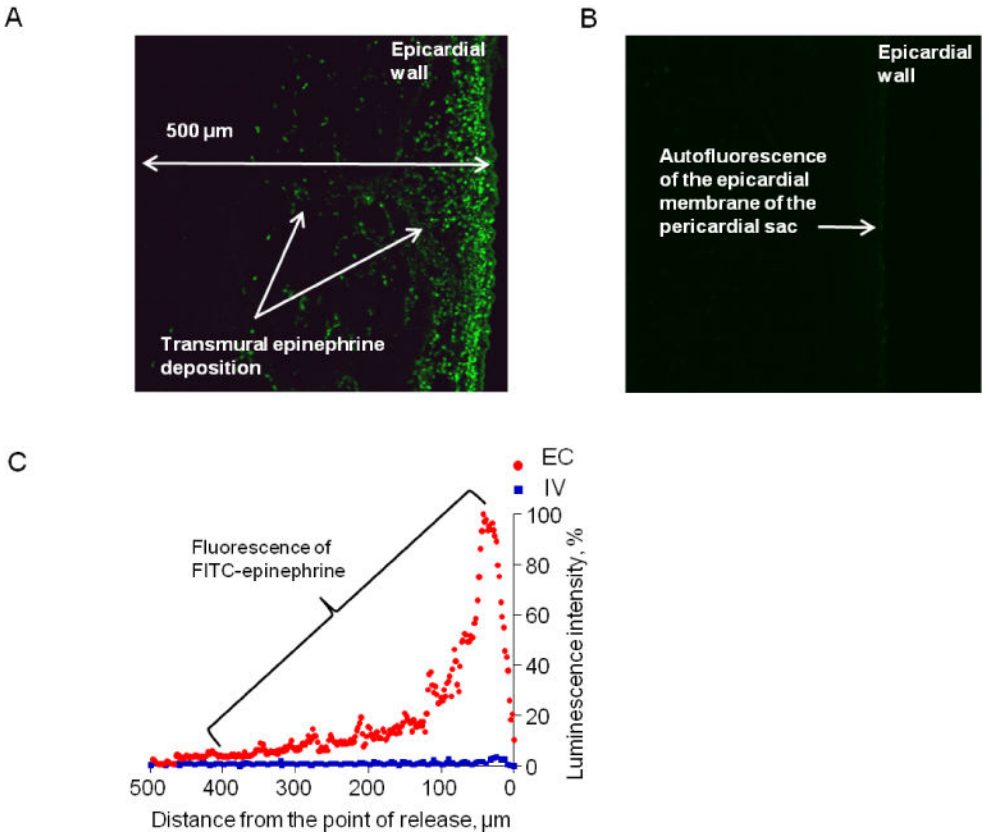


**Figure 4.**

Immunohistochemical microscopy shows the transmural distribution of epinephrine bound to adrenergic receptors sampled from tissue cores just under the point-source release device. EC epinephrine treatment leads to detectable accumulation of receptor-bound epinephrine to a depth of 400 μm from the epicardial surface (A) FITC-epinephrine staining (green). Limited sensitivity of this method cannot distinguish epinephrine accumulation following IV treatment (B) from autofluorscense (Data not shown). Transmural profiles of the intensity of the luminescence were derived using ImageJ 1.45S software, NIH (C).

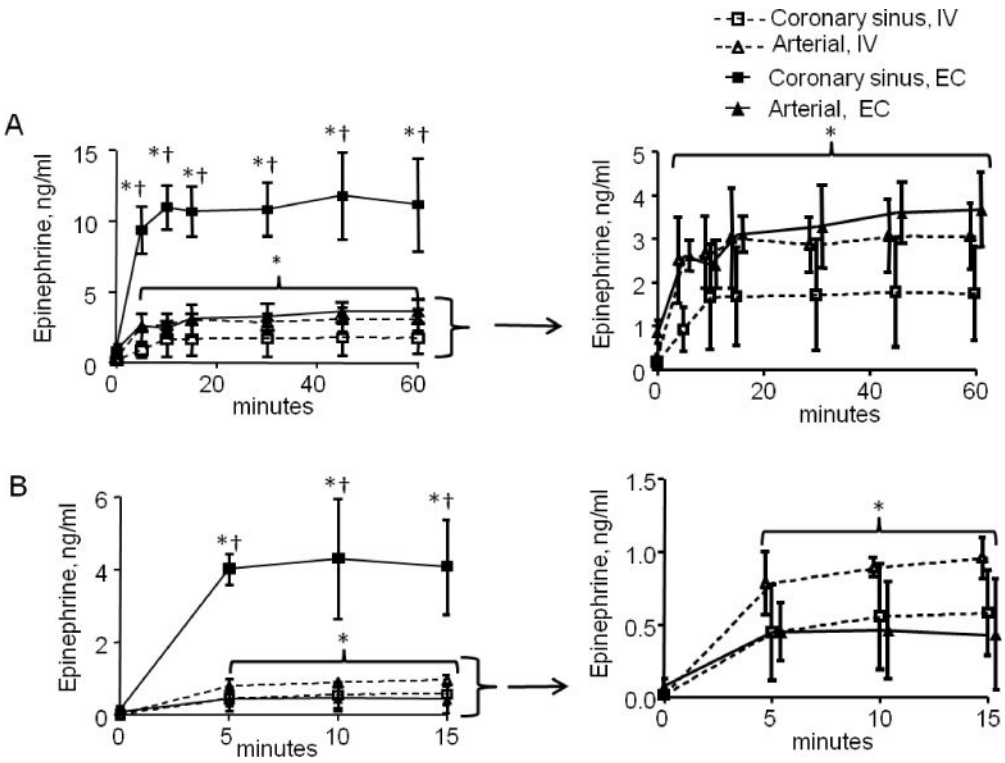


**Figure 5.**

Blood plasma epinephrine levels following epicardial point-source (A) and dispersed-source release (B) and corresponding equal dose intravenous (IV) infusions. Epinephrine levels in plasma sampled from the coronary sinus was elevated within 5 minutes of EC application and remained high for the duration of the experiment. Data points on expanded scale graphs (right) are temporally shifted to help visualize the error bars. Arterial plasma epinephrine levels were not different between EC and IV groups. N = 3, Avg ± SD.

* – <0.05 compared with baseline; † – <0.05 compared with corresponding IV group

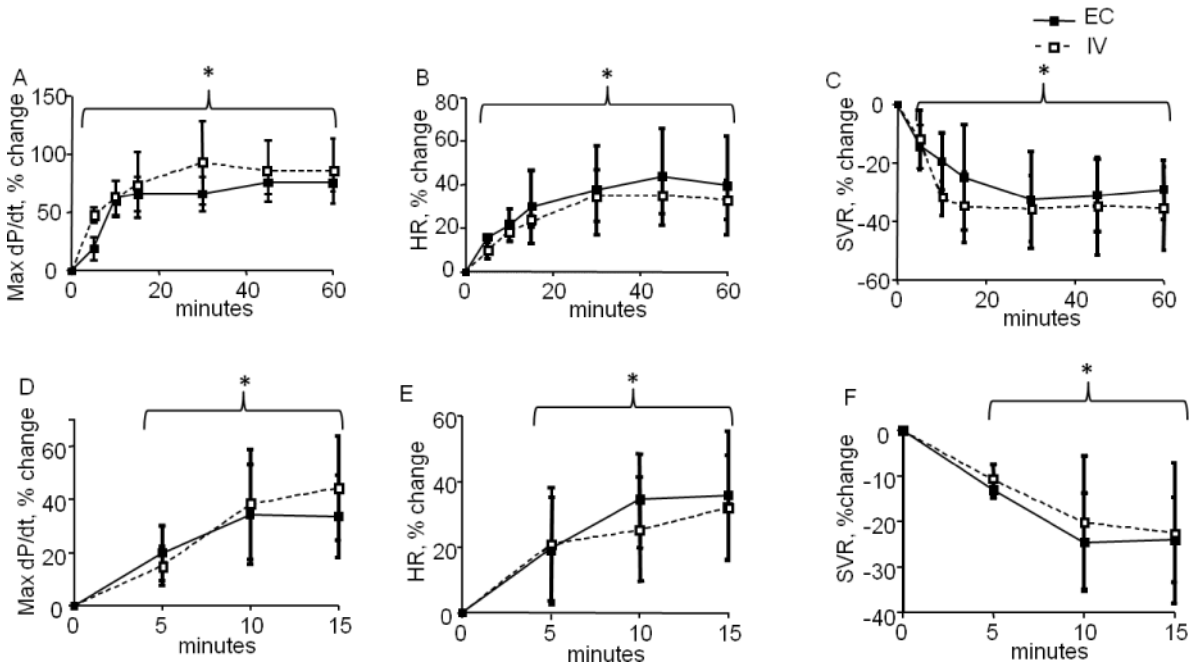


**Figure 6.**

Physiologic effects of EC epinephrine (solid lines) using point-source (0.1 μg/kg/min, A, B and C) and dispersed-source release (0.03 μg/kg/min, D, E and F) and IV infusion (IV, dotted lines) at equivalent doses. Contractility (max dP/dt, A, D), heart rate (HR; B, E) and systemic vascular resistance (SVR; C, F) responses to local and systemic epinephrine are shown. N = 3, Avg ± SD.

***** – <0.05 compared with baseline; † – <0.05 compared with corresponding IV group

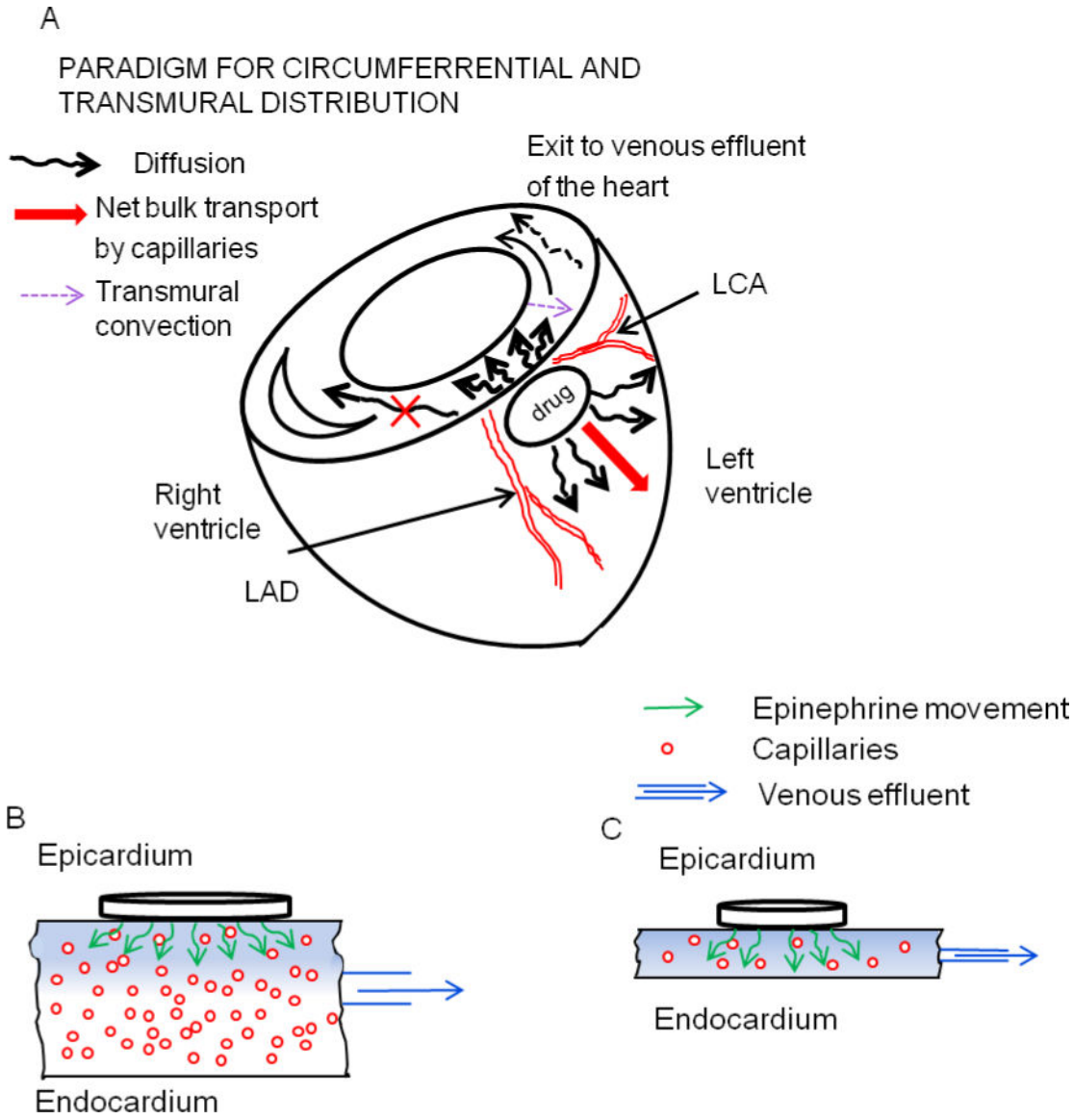


**Figure 7.**

Potential mechanisms of drug transport in myocardium following EC delivery (A). Left anterior descending (LAD) and left circumflex arteries (LCA) may provide bulk perfusion and transport of drug to the apical and lateral walls. Epinephrine distribution in myocardial tissue in EC delivery for thick myocardium such as in swine or humans (B) and thin myocardium such as small rodents is driven by diffusion in interstitial spaces and bulk tissue perfusion (C). The diffusivity of epinephrine in myocardial tissue should be similar for both species and therefore the diffusive length should be the same over the same time interval. Therefore, a larger fraction of myocardium may be treated in the thinner tissue. Thicker tissues have a more extensively developed vasculature and therefore, the clearance of drug by capillaries may be greater in large animals and may limit the penetration depth after EC application. The penetration depth of EC applied drug may therefore cover a greater fraction

of the tissue thickness in small animals (29, 30) than in large ones leading to enhanced pharmacologic response in the former (C).