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High Concentrations of Drug in Target Tissues Following Local Controlled Release Are Utilized for Both Drug Distribution and Biologic Effect: An Example with Epicardial Inotropic Drug Delivery

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Abstract

Local drug delivery preferentially loads target tissues with a concentration gradient from the surface or point of release that tapers down to more distant sites. Drug that diffuses down this gradient must be in unbound form, but such drug can only elicit a biologic effect through receptor interactions. Drug excess loads tissues, increasing gradients and driving penetration, but with limited added biological response. We examined the hypothesis that local application reduces dramatically systemic circulating drug levels but leads to significantly higher tissue drug concentration than might be needed with systemic infusion in a rat model of local epicardial inotropic therapy. Epinephrine was infused systemically or released locally to the anterior wall of the heart using a novel polymeric platform that provides steady, sustained release over a range of precise doses. Epinephrine tissue concentration, upregulation of cAMP, and global left ventricular response were measured at equivalent doses and at doses equally effective in raising indices of contractility. The contractile stimulation by epinephrine was linked to drug tissue levels and commensurate cAMP upregulation for IV systemic infusion, but not with local epicardial delivery. Though cAMP was a powerful predictor of contractility with local application, tissue epinephrine levels were high and variable - only a small fraction of the deposited epinephrine was utilized in second messenger signaling and biologic effect. The remainder of deposited drug was likely used in diffusive transport and distribution. Systemic side effects were far more profound with IV infusion which, though it increased contractility, also induced tachycardia and loss of systemic vascular resistance, which were not seen with local application. Local epicardial inotropic delivery illustrates then a paradigm of how target tissues differentially handle and utilize drug compared to systemic infusion.

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Keywords

epicardial drug delivery; inotrope; cAMP; contractility; systemic vascular resistance; heart rate

1. Introduction

Local controlled drug delivery provides pharmacologic therapy with elevated target tissue levels and minimal peripheral side effects [1, 2, 3]. There is, however, little direct relationship between applied dose and biologic response [4, 5, 6, 7] as seen and expected from systemic infusion. While intravascular injection brings drug into a tissue through dense uniform capillary networks [8, 9] providing uniform distribution [10], local application only applies drug to the target tissue surface. Locally applied drug must enter the tissue and diffuse through the target area down concentration gradients (Fig. 1) [4, 10, 11, 12, 13]. The very high supratherapeutic drug concentration near the point of release may lead to saturation of proximal binding sites or exceed their capacity for binding. The remaining unbound drug in a tissue participates in diffusive spread [14]. Supratherapeutic concentrations of drug near the release device ensure that a large fraction of the drug is unbound, and provides a driving gradient for trans-target distribution. This unbound fraction is not biologically active until drug diffuses to a region where receptors are unsaturated and available. Progression of effect, through longer periods of treatment or higher doses, therefore, depends on the geographic extent of therapeutic concentrations to the target tissue and the extent of saturated binding. While increasing administered systemic dose increases target organ concentrations and biologic effect as long as receptors are available (Fig. 1b), tissue capillaries remove locally released drug from a tissue [11 13] rather than deliver it. Thus, capillary clearance and enzymatic degradation limit the extent of tissue under pharmacologic control even with substantial increments of local drug release and, unlike systemic delivery, extra steps are required for drug to reach receptors suggesting a non-linear nature of the dose-response [4, 5, 6].

We examined the hypothesis that local application dramatically reduces systemic circulating drug levels, but leads to significantly higher tissue drug concentration than might be needed with systemic infusion when dosed to similar therapeutic endpoints. A biologic system including a drug with a cell surface receptor mediated function, detectable intracellular intermediates, and a functional organ-wide response was needed to test this hypothesis and provide underlying mechanisms. For these reasons, epinephrine, a drug with well described systemic pharmacokinetics [15], was administered to a focal region of the epicardial surface of the anterior wall of the rat heart in a preparation that measures indices of global left ventricular (LV) contractility in real time, and tissue levels of drug and cyclic adenosine monophosphate (cAMP) at the time of organ harvest. This intracellular intermediate was measured as a marker of the intracellular biological effect of epinephrine, as cAMP is upregulated by adrenergic receptor activation and increases calcium release into the cytosol of the myocyte leading to more forceful contractions [16, 17]. These experiments required a novel, finely tunable polymeric platform that allows precise, steady animal weight based controlled release. Epicardial administration of epinephrine at a single weight based dose

rate was compared to IV systemic infusion at an equivalent dose rate at which preliminary data suggested would provoke an equivalent global LV contractile response.

2. Methods

2.1. Fabrication and Characterization of the Epicardial Drug Delivery Platform

A novel polymeric local epicardial (EC) drug releasing system designed to permit precise animal-weight based epinephrine release to the anterior wall of the beating rat heart was fabricated from calcium-cross-linked alginate hydrogels [18]. Briefly, 45 mcl 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, 6.5mm, polyester, 3 micrometers pore size; Corning). Immersion of the transwell support in 1 ml of 3% $CaCl_2$ in ddH_20 using a leveled 24 well culture plate (#353047, 15.75mm; Corning) for 25 min at room temperature cross-linked the alginate to form a solid but flexible disk. Free Ca^{2+} in alginate disks was removed by placing the disks in ddH_2O for 60 min. The end product had the shape of a concave disk with diameter of 6.5 mm, with minimal thickness of 0.52 mm at the center and maximal thickness of 1.2 mm along the perimeter, lower surface area of 32 mm^2 and the volume of the concave of 30 mm^3. These devices were formed without embedded drug. When epinephrine is applied in solution to the upper free concave surface at regular intervals they serve to smooth the release at their lower surface to a linear rate and disperse drug over an area approximately the size of the disk.

A series of in vitro experiments quantified the epinephrine released over time as a function of the applied concentration to the upper surface of the concave disk (Fig. 2a). The formed alginate disk was placed in a new transwell support and immersed in a leveled 24 well culture plate filled with 320 microliters (mcl) of ddH_2O representing the released drug receiving chamber. The culture plate was placed on an orbital shaker at 80 RPM (#3520; Labline). Epinephrine (Hospira) solution (10 mcl of 0.05, 0.1, 0.25, 0.5, 0.75 or 1 mcg/mcl in ddH_2O) was added every 10 minutes onto the upper surface of the alginate disks. These experiments were repeated in triplicate. At regular intervals, a 60 mcl sample from the receiving chamber was removed to evaluate the amount of the released drug and 60 mcl of ddH_2O was added immediately to the wells to restore receiving chamber volume. The concentration of epinephrine in each sample was determined by spectrophotometric methods [19]. Metaperiodate (6 mcl of 2% $NaIO_4$ in ddH_2O, #S1878, Sigma-Aldrich) and ethanol (9 mcl, 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 (Fig. 2a). 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 (Fig. 2b). 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 [18]. This novel method of controlling epicardial drug release allows for precise animal-weight based dosing without any chemical modifications of the platform.

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. Twenty four adult male Sprague-Dawley rats (375-475 grams) underwent identical surgical procedures but different routes of epinephrine administration. Four groups of animals (N=6) were studied: 0, 0.1 and 0.3 mcg/kg/min IV and 0.1 mcg/kg/min epicardial (EC). All animals had an identical alginate disk applied to the anterior wall of the heart. Hemodynamic measurements were made prior to treatment and at steady-state. The hearts were harvested during steady state treatments and transmural tissue cores (6mm, Miltex biopsy punch, VWR, #21909-144) from the inferior wall and the anterior wall under the release device were rapidly harvested for measurement of the epinephrine concentration and cAMP.

Animals were anesthetized with ketamine hydrochloride (90 mg/kg ip) and xylazine (10 mg/kg i.p.). Anesthesia was maintained by continuous infusion of ketamine hydrochloride (40 mg/kg/hr, i.p.) and xylazine (2.5 mg/kg/hr, i.p.). A heating lamp and pad were adjusted to maintain rectal temperature at 37°C. Animals were shaved in the neck, thorax and inguinal areas and the skin prepped with alcohol. A 20 gauge endotracheal tube was inserted through a tracheotomy and the animal was placed on a rodent ventilator (#683, Harvard) according to the following formulas [20]: tidal volume (ml) = $6.2\times M^{1.01}$ (M = animal mass, kg), respiration rate = $53.5 \times M^{-0.26}$ (BPM). The right femoral artery and vein, and internal jugular vein were cannulated for arterial blood pressure measurements, resuscitation, and epinephrine or saline infusions, respectively. Bovine albumin (10%) (#A7906, Sigma-Aldrich) in 0.9% saline was continuously infused (0.5 ml/kg/hr IV) throughout the experiment to maintain blood oncotic pressure and electrolyte balance [21]. Bilateral anterolateral thoracotomies were performed to expose the heart. A 16 gauge needle was used to make a ventriculotomy near the apex of the heart for insertion of a Millar™ conductance catheter (#SPR-869, 6mm, Millar catheter, MPVS-300 system) into the left ventricular (LV) chamber. An alginate drug releasing platform was placed on the anterior surface of the LV.

Hemodynamic data were acquired continuously throughout the experiment. Femoral arterial and central venous pressures (CVP) were transduced (#TRN050; Kent Scientific) and amplified (#TRN005; Kent Scientific). Left ventricular pressure and volume signals from the Millar catheter were acquired using a Powerlab A/D converter (AD Instruments, Colorado Springs, CO). All signals were recorded with Chart 5.0 (AD Instruments) for instantaneous display, analysis, and storage. Heart rate (HR) and the first derivative of left ventricular pressure (dP/dt) were recorded from the pressure transducer of the Millar catheter. Mean arterial pressure (MAP) was calculated from femoral arterial pressure data. Real time calculated hemodynamic parameters include relative cardiac output (CO) and systemic vascular resistance (SVR) (SVR = [MAP – CVP] / CO). The central venous pressure (CVP) was periodically monitored by transiently halting the drug infusion with a 3-way stopcock. The optimal position of the catheter inside the LV was continuously ensured by slight manipulation of the catheter until the maximum value of the SV with 4 distinct phases of the cardiac cycle was displayed. Hemodynamic parameters were analyzed at the baseline and at steady state, as determined by max dP/dt, HR, and MAP signals, always between 20 and 25 minutes of continuous epinephrine administration. Hemodynamic

measurements were recorded for 20 minutes during placebo control experiments (0 mcg/kg/min).

2.3. Drug Delivery

Epinephrine was administered either as an intravenous infusion at the rate of 0.1 or 0.3 mcg/kg/min, or by epicardial (EC) controlled release from alginate devices at 0.1 mcg/kg/min. Preliminary data suggested that 0.1 mcg/kg/min EC and 0.3 mcg/kg/min IV raised indices of contractility similarly and are thus equipotent. For all IV infusions, epinephrine solutions were diluted and then infused at a rate of 0.5 ml/hr via a syringe pump (#74900-00; Cole Parmer). For EC application, epinephrine was diluted according to the in vitro release correlations (Fig. 2b) to provide the required animal-weight based release rate. Epinephrine solution (10 mcl in ddH_20) was added every 10 minutes using a micropipette to the concave top of alginate devices placed on the anterior wall of the beating left ventricle. Saline was infused IV (0.5 ml/h) during EC epinephrine delivery. Sham EC devices were placed on the heart with 10 mcl of ddH_2O applied every 10 minutes during IV infusion and placebo control animals. The ONLINE SUPPLEMENT shows still photographs and video of the alginate device on the rat anterior epicardial surface

2.4. Tissue and blood harvesting and processing

At the end of the in vivo experiment, a blood sample was taken from the femoral artery and the heart quickly excised and rinsed with saline at 4°C. Transmural cores of myocardium were rapidly harvested from the anterior wall just under the alginate disk and from the opposing inferior wall (6 mm Miltex biopsy punch, VWR, #21909-144) for epinephrine and cAMP quantification. Harvested tissue samples were rinsed in saline, blotted, and snap frozen with pre-cooled tongs in liquid nitrogen and stored at −80°C for future analysis. Myocardial tissue was crushed in liquid nitrogen 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) [22, 23] 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 isolated and stored at −80°C. Blood samples were centrifuged at 1100 g for 15 minutes and supernatant isolated and stored at −20°C. Epinephrine content in plasma and cardiac tissue extracts were evaluated using a colorimetric enzyme-linked immunosorbent assay (ELISA #IB89551; Immuno-Biological Laboratories, Inc) [24]. cAMP content in cardiac tissue extracts were measured using a chemiluminescent cAMP ELISA (# STA-501; Cell Biolabs Inc.) [17]. Cardiac epinephrine and cAMP levels were normalized to protein content, which was measured by Bradford's method with bovine serum albumin as the standard [25] (#500-0006; Bio-Rad). Standard curves for the ELISA assays are shown in the ONLINE SUPPLEMENT.

2.5 Data analysis and statistics

Plateau steady-state hemodynamic responses to epinephrine infusion or local release are presented as percentage change from the baseline. Biochemical data is presented as molar or weight values normalized to protein content in the tissue extract. A one-way ANOVA analysis with Bonferroni's correction was used to compare hemodynamic responses to different doses and routes of epinephrine delivery (GraphPad Prism, 5.0). A t-test was used

to compare epinephrine and cAMP levels in the anterior and inferior walls of the left ventricle within the groups. Data were considered statistically distinct if the p - value was less than 0.05.

3. Results

3.1. Specifiable In Vitro Epinephrine Release Kinetics

The in vitro release through the alginate disks was measured for a range of applied epinephrine concentrations (Fig. 2a). Release was perfectly linear (Fig. 2b) with applied concentration (0.05 to 1 mcg/mcl) over the duration used in the in vivo experimentation, up to 25 minutes, suggesting that the rate of release depended only on the amount of applied drug. This relationship allowed us to select the concentration of the drug to apply to the EC alginate disk to give a desired linear rate of release in subsequent in vivo experiments. This flexible system provides animal-weight based linear zero order release without having to modify the polymer matrix or test each needed release rate in vitro.

3.2. Tissue epinephrine, cAMP, and Global Ventricular Contractility

EC delivery led to a pronounced epinephrine and cAMP accumulation in the left ventricular anterior wall (LVAW) that was not observed in the inferior wall (LVIW). Anterior wall epinephrine and cAMP were 14- fold and 2 fold higher than in the inferior wall, respectively (Figs. 3a and 3b). In contrast, the distribution of drug and cAMP in the anterior and inferior walls were homogenous following IV infusion. Epinephrine in the anterior wall of the heart (Fig. 3a) increased most significantly following epicardial delivery and to a far lesser extent with intravenous infusion. IV infusion at 0.1 mcg/kg/min did not raise myocardial epinephrine concentration above baseline. At a three-fold increased IV dose (0.3 mcg/kg/min), myocardial drug was still lower than with EC (0.1 mcg/kg/min). The epinephrine concentration in the anterior wall with local EC administration at 0.1 mcg/kg/min was 19 and 6.7 fold higher than 0.1 and 0.3 mcg/kg/min IV, respectively, and 31 fold higher than placebo treated animals. cAMP expression in the anterior followed suit (Fig. 3b) rising linearly with tissue epinephrine after IV infusion but remaining flat with EC application (Fig. 3c). In a fascinating manner, the global index of contractility (max dP/dt) was proportional to anterior myocardial concentrations of epinephrine (Fig 4a) and cAMP (Fig. 4b) for systemic IV infusion, but increased only with cAMP and not tissue drug for EC administration. Higher tissue levels of drug were observed with local EC application, but these did not result in greater contractility, however, increasing myocardial cAMP levels directly increased the inotropic response.

3.3. Confirmation of Local Epicardial Inotropic Drug Delivery

Plasma epinephrine levels were also revealing. EC delivery which drove up anterior myocardial levels did not raise plasma epinephrine above 125 pg/ml, whereas the same dose IV achieved plasma levels twice as high (Fig. 5). Moreover, the linear systemic dose-response observed with contractility, heart rate and vascular tone with IV infusion was not observed with EC delivery. Plasma levels never exceeded the threshold that elicits these systemic responses. These data imply that drug is restricted to its local environment after EC

release where anterior myocardial epinephrine levels can well exceed that which can be seen with three fold greater infusion rate IV.

4. Discussion

Local drug delivery has appeal for several reasons including reduced systemic side effects, high concentrations of drugs in target tissues, and potentially reduced drug costs. Several studies using local drug delivery have noted a poor correlation between response and released dose [4, 5, 6, 7]. The behavior of the drug once inside the target tissue when given locally may be quite different from when given systemically. With IV infusion, the drug in target tissues is delivered by capillaries and the levels are consistent with the rest of the body. At these concentrations, unbound and bound drug are at steady state and evenly distributed across tissues (Fig. 1b). With local delivery, the distribution is driven by diffusion down concentration gradients away from the point of release (Fig. 1a). This diffusion may be in equilibrium with clearance from capillaries and enzymatic degradation [13, 18] so that as long as the release continues, the gradient will persist. Local delivery may flood the tissue with drug; some small fraction binds to specific receptors and activates biological effect while the majority remains unbound. At proximal sites within the tissue, specific receptors may be saturated while at distant sites, the drug concentration may be subtherapeutic. The magnitude of the biologic response of a tissue to locally released drug dose may therefore depend more on the geographic extent of therapeutic concentration in the target tissue and less on administered dose or subsequent tissue concentrations.

In order to demonstrate this distinction between local and systemic delivery, we needed a model where we could readily find equi-efficacious doses, where both modes of application resulted in similar organ-wide biologic responses, and drug levels in target tissues, as well as intracellular intermediate signaling molecules, could be measured. Inotropic myocardial pharmacotherapy with adrenergic agonists serves as a perfect model for demonstrating the differential response to tissue concentrations of a drug when given locally and systemically. Epinephrine was chosen as its systemic pharmacokinetics are well described [15], it is easily measured in both plasma and tissue, and it upregulates a quanitifiable and biologically significant intracellular intermediate, cAMP. This experimental preparation allowed us to describe the target tissue drug distribution, the intracellular intermediate upregulation, and subsequent mechanisms of biologic response following local application.

4.1. Intracellular Signaling and Biologic Response in Local and Systemic Delivery

Locally applied epinephrine leads to an order of magnitude higher drug concentration in the anterior wall of the heart, even at one third the dose rate of systemic infusion (Fig 3a). In contrast, EC administration increases cAMP, a biological result of adrenergic stimulation, only marginally higher over IV infusion (Fig. 3b). The amount of epinephrine needed in tissue to raise cAMP is far greater for local application than IV infusion (Fig. 3c). Thus, after EC delivery, much of the drug observed in tissue is not biologically active. With local EC delivery, the adrenergic receptors in a zone of tissue near the release device may be saturated and unavailable for binding and the majority of detectable drug in the tissue is in the unbound fraction. In contrast to IV infusion, epinephrine concentration in anterior

myocardium following local application does not drive cAMP upregulation (Fig. 3c). The global LV contractile response data, as quantified through max dP/dt measurements, indicate that far more drug is needed in the anterior myocardium with local EC application than IV infusion to achieve the same biologic effect (Fig. 4a). Whereas max dP/dt follows anterior tissue epinephrine concentration in IV delivery, little correlation can be seen in EC application. While epinephrine concentrations may be lower in the myocardium but evenly distributed with IV infusion, therapeutic concentration of drug with local EC delivery is confined to an area under the zone of release. Indeed, the amount of epinephrine in the anterior wall of the heart following local application is 14 fold greater than in the inferior wall, and thus forms a circumferential gradient (Figs. 3a). Limited territory of the myocardium may be pharmacologically augmented by EC application. Thus, far higher concentrations of epinephrine are needed in the limited treatment zone of the anterior wall to augment myocyte contraction enough to change the contractility of the whole ventricle with EC application than with IV infusion.

These very different patterns of drug deposition in tissue following IV and EC delivery lead to remarkably similar cAMP upregulation in the anterior and inferior walls (Fig. 3b). Indeed, the anterior wall epinephrine concentrations are 6.7 fold higher, but the cAMP concentration is only 1.6 fold higher in local EC application over equipotent IV infusion. More cAMP is needed in the treated anterior wall tissue to increase global contractility when the distribution is limited circumferentially by local application than when upregulated equally around the ventricle by systemic infusion (Fig. 4b). However, the global contractility responses to incremental levels of cAMP are identical whether given locally or systemically. With IV infusion, epinephrine tissue deposition is predictable and homogenous. Following local application, the multiple steps between release, uptake, binding and distribution create variable tissue deposition (Fig. 3c) and yet, the biologic response does not change with deposited drug (Fig. 4a). Therefore, the biologic response with local delivery is solely dependent on second messenger concentrations (Fig. 4b). While tissue epinephrine drives cAMP production and myocardial contractility in IV infusion, (Fig. 4a), ultimate biologic effect for local EC delivery is not driven by the tissue level of drug, but rather the upregulated second messenger, cAMP.

4.2. Novel Polymeric Hydrogel System for Epicardial Applications

An innovation that made these analyses possible was the development of a simple experimental polymeric disk platform that steadily released large amounts of drug to the anterior wall of the heart. In applications that require high rates of release, it may not be possible to load sufficient drug into a small volume polymer. In addition, each new desired dose may require reformulation and in vitro characterization and thus are not amenable to precise animal weight based dosing in vivo. Rather than embedding the drugs into the polymer matrix, our experimental platform uses an alginate hydrogel shaped with a concave upper surface that serves as a well for drugs applied in very small volumes at regular intervals (Fig. 2). This polymer disperses drug to an area of the anterior wall of the heart and buffers the release to be steady. By defining the linear release over a range of applied drug concentrations, and through correlation with the release rates, this very flexible system does not require new in vitro characterization for every needed rate of release. The release rate is

finely selectable by simply changing applied drug concentration tailored to the weight of each animal.

4.3. Validation of local delivery scheme

One of the perceived benefits of local drug delivery systems are reduction in systemic side effects, and epicardial inotrope delivery fits well into this paradigm. Local epicardial epinephrine delivery increased contractility to the same extent as IV infusion at one-third the dose rate (Fig. 5a). Plasma levels were lower in EC application than with IV infusion suggesting that the experimental platform succeeded in confining drug to the target tissue. Systemic consequences in this application are peripheral vasodilatation from beta-2 agonist effects in the viscera and beta-1 agonism driven tachycardia. Tachycardia can be considered a systemic side effect as the sino-atrial (SA) pacemaking cells of the heart are high in the right atrium away from the treated ventricular myocardium. The most likely pathway of drug released onto the ventricular surface to the SA node is absorption from myocardium by capillaries and entry into the systemic circulation [8]. Thus, pharmacologically induced tachycardias should be considered a systemic side effect. Heart rate increased linearly with plasma levels in IV infusion (Fig. 5b). Likewise, systemic vascular resistance dropped in proportion to plasma levels (Fig. 5c). With local EC application, HR increased and SVR decreased far less than with IV infusion. Furthermore, there did not appear to be a relationship between plasma levels and these side effects with local EC delivery. Thus, this method of local inotropic drug delivery appears to confine drug to the heart and function as an ideal local release system that limits peripheral effects.

4.4 Clinical applications

Inotropic compounds increase the force of myocardial contraction, however, adverse effects such as tachycardia and loss of systemic vascular resistance narrow its therapeutic index and may limit its clinical utility [26, 27]. While systemic infusion is currently the only method to deliver inotropic compounds to the failing myocardium, epicardial application of this class of drugs may serve as a better alternative as it allows separating sought contractile and undesirable chronotropic and vascular responses (Figs 5a-c). Envisioned technologies for epicardial application include drug eluting polymeric patches placed directly on the epicardial surface, such as during cardiac surgery, or percutaneously through minimally invasive techniques, such as injection of viscous or self polymerizing drug eluting systems into the pericardial sac. The latter can be accomplished through direct subxiphoid pericardial puncture or through catheters advanced from the femoral vein across the right atrial appendage and into the pericardium [28-32]. Thus, epicardial inotropic drug delivery may find multiple applications in heart failure patients beyond the cardiac surgical environment.

5. Conclusions

A novel paradigm for the disposition of locally delivered drug to target tissues is presented. Following local application, drug concentrations are very high near the point of release and form a gradient across the target tissue. Drug from this high concentration either binds to receptors to exert a biologic effect or remains in the unbound fraction to drive the diffusion of drug across the tissue. Implicit in this construct is that much of the drug measured in the target tissue does not exert a biologic effect and much more drug will be in the tissue with

local delivery than with systemic infusion, even when given in equipotent doses. Our data, generated by a model system wherein we can measure target tissue drug concentration, intracellular intermediate upregulation and organ wide biologic response, confirm that there is greater tissue drug concentration with local delivery than with IV infusion. Given the complexity of the distribution and fate of locally released drug, dose and response are circuitously related and less predictable. Thus, with EC delivery, biologic response correlates well with second messenger levels such as cAMP, but not with tissue drug levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations

EC	Epicardial
IV	Intravenous
Max dP/dt	Maximum rate of change of pressure
SVR	Systemic vascular resistance
HR	Heart rate
LVAW	Left ventricular anterior wall
LVIW	Left ventricular inferior wall

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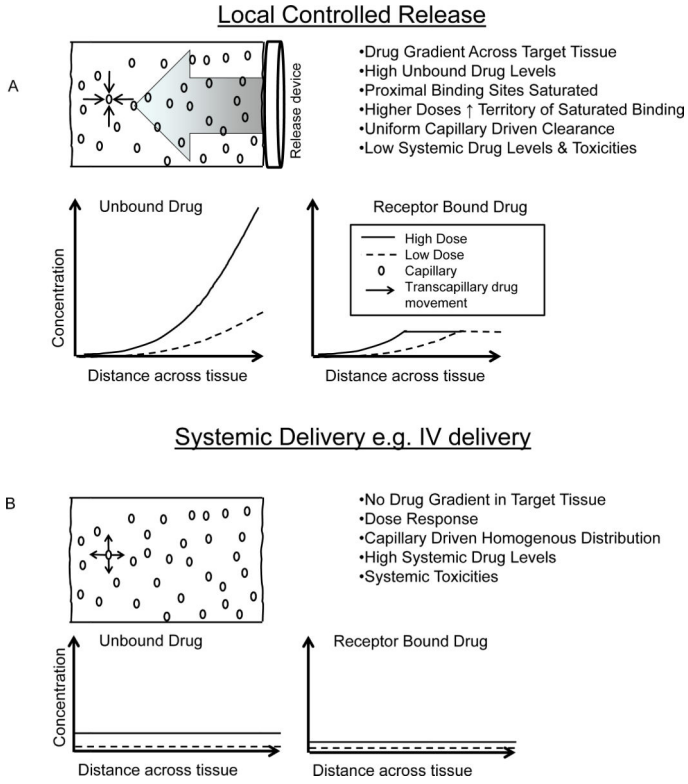

Figure 1.

A paradigm of drug distribution in local and systemic delivery. Panel A: Local drug delivery leads to a gradient in drug distribution across the target tissue. The unbound fraction may exceed by many fold the bound active drug fraction. Close to the release matrix where unbound concentrations are high, receptors are saturated and the bound phase uniform. Increased release rate may increase the extent of saturated binding which might increase biologic effect. Panel B: Systemic drug delivery provides homogenous target tissue drug distribution from capillary networks with low levels of unbound drug that are comparable to bound drug fractions. Higher doses lead to higher concentrations of drug in tissue and increased effect, provided receptors are not saturated.

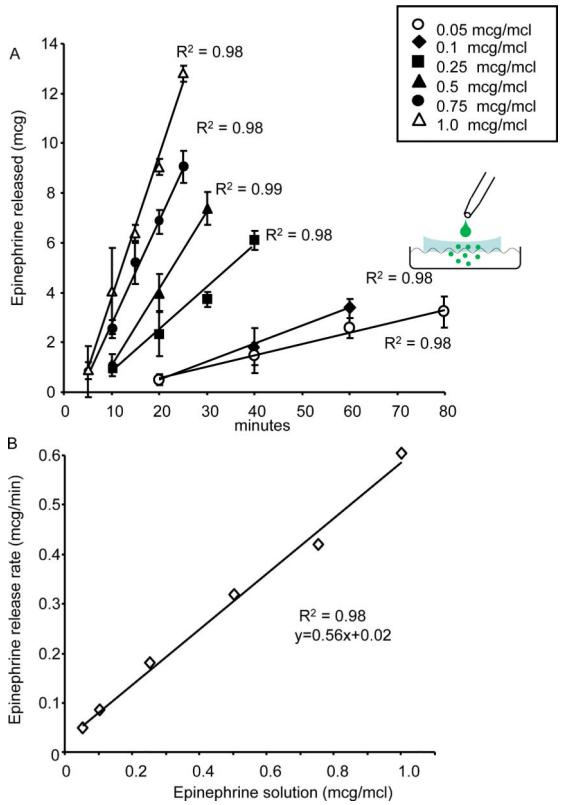

Figure 2.

In Vitro release of epinephrine from local epicardial platforms (diameter=6.2 mm, height = 1.2 mm, surface area of the bottom of the platform = 32 mm^2 and the volume of the concave = 30 mm^3. Epinephrine release rate is linear for each applied concentration studied (Panel A, N=3, Avg ±SD). 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 an appropriate applied concentration of drug (Panel B)

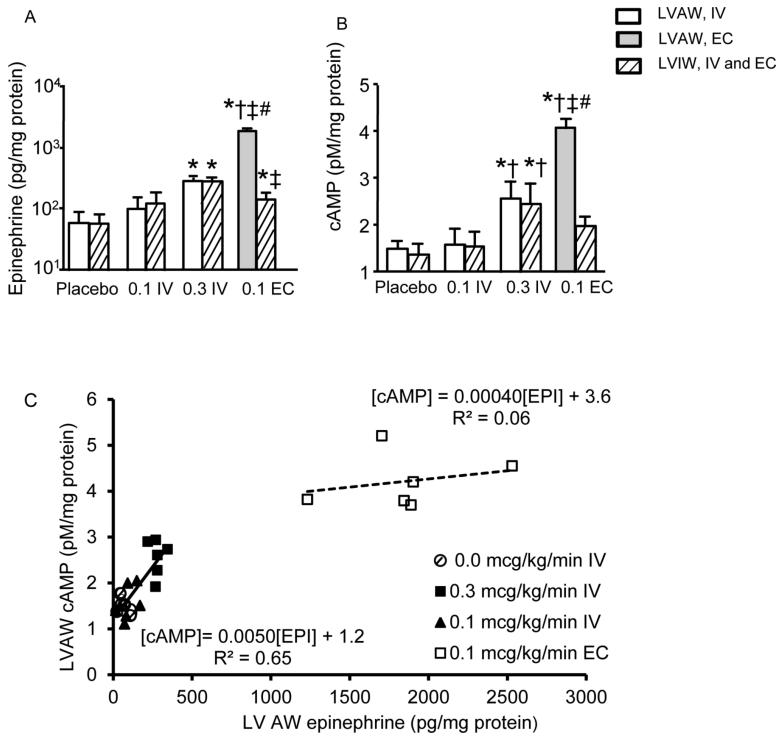

Figure 3.

Concentration of epinephrine and cAMP in the left ventricular anterior (LVAW) and inferior walls (LVIW) following local epicardial (EC) and intravenous (IV) drug deliveries. Local EC application (0.1 mcg/kg/min) leads to 6.7-fold higher epinephrine (Panel A) and 1.6-fold higher cAMP (Panel B) concentration in the LVAW compared to equipotent IV delivery at three fold higher dose (0.3 mcg/kg/min). Epinephrine and cAMP distribution was homogenous in the LV during IV infusion (Panels A and B). EC application provided significantly higher accumulation of epinephrine and cAMP in the LVAW (Panels A and B). Much greater epinephrine concentrations in myocardium are needed with local EC delivery to produce similar amounts of cAMP as systemic infusion (Panel C).

[EPI] – epinephrine

* - <0.05 compared with 0.0 mcg/kg/min, † - <0.05 compared with 0.1 mcg/kg/min IV

‡ - <0.05 compared with 0.3 mcg/kg/min IV, # <0.05 compared with LVIW; N=6 in all groups

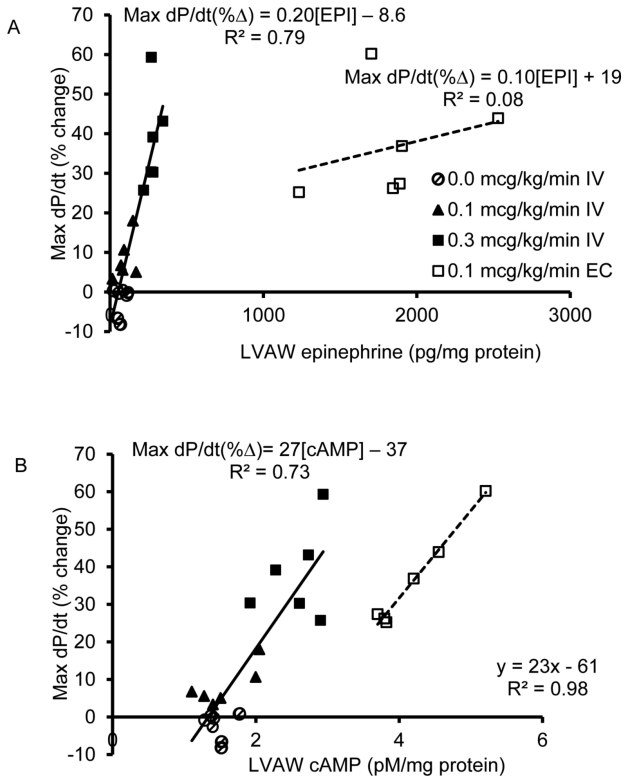

Figure 4.

Relationship between an index of global left ventricular contractility (Max dP/dt) and left ventricular anterior wall (LVAW) epinephrine (Panel A) and cAMP (Panel B) concentrations. Cardiac contractility correlates strongly with epinephrine and cAMP for systemic IV infusion. With local epicardial (EC) application a correlation is only seen with cAMP concentration and not LVAW epinephrine levels. [EPI] – epinephrine, %Δ - %change

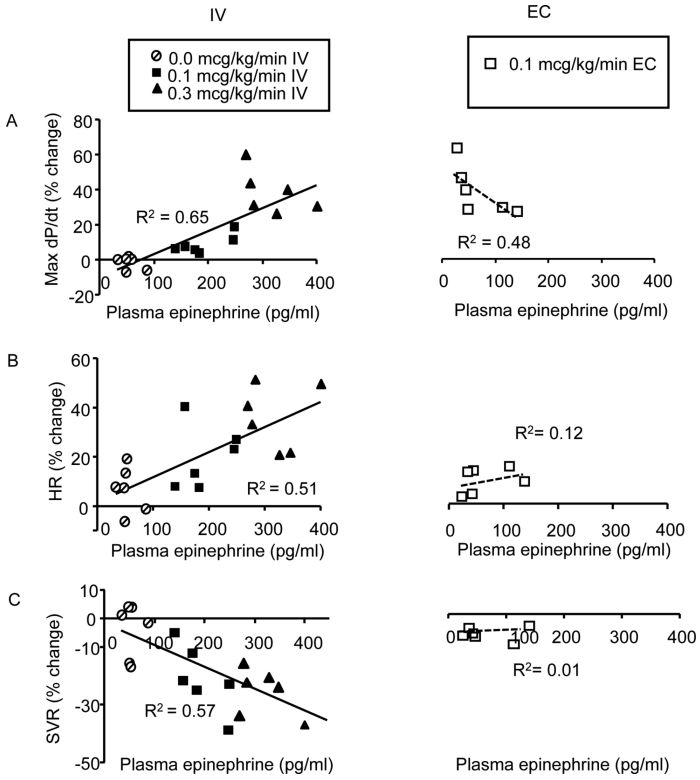

Figure 5.

Relationship between plasma epinephrine and changes in hemodynamic parameters including max dP/dt (Panel A) , heart rate (HR) (Panel B) , and systemic vascular resistance (SVR) (Panel C) with systemic IV and local EC delivery. The change in max dP/dt, HR and SVR significantly correlates with plasma epinephrine following IV drug infusion but not in local EC delivery. Thus, the local delivery systems provide for local contractile effect without the systemic effects of tachycardia and peripheral vascular dilatation seen in systemic IV infusion.