Pharmacokinetic Advantage of Intrapericardially Applied Substances in the Rat

J. J. ROB HERMANS, HELMA VAN ESSEN, HARRY A. J. STRUIJKER-BOUDIER, RANDOLPH M. JOHNSON, FELIX THEEUWES, and JOS F. M. SMITS

Department of Pharmacology and Toxicology, Cardiovascular Research Institute, Universiteit Maastricht, Maastricht, The Netherlands (J.J.R.H., H.v.E., H.A.J.S.-B., J.F.M.S.); and DURECT Corporation, Cupertino, California (R.M.J., F.T.)

ABSTRACT

Intrapericardial application of therapeutic agents may open perspectives for target-directed therapy of the diseased heart. This study was performed to investigate whether intrapericardial drug application is beneficial from a pharmacokinetic point of view. Male Wistar rats were provided with intrapericardial and intravascular catheters for substance administration and sampling. Intrapericardial bolus injections of fluorescent macromolecules [fluorescein isothiocyanate (FITC)-rat IgG, molecular weight about 155 kDa; Texas Red rat serum albumin, mol. wt. 67 kDa; Texas Red fibroblast growth factor (FGF), mol. wt. 18 kDa; and FITC heparin, mean mol. wt. 18 kDa] resulted in substance concentrations in pericardial fluid that exceeded those in plasma, for several hours. Pericardial fluid volumes of catheter-instrumented rats, derived from (initial) central compartment volumes, ranged between 0.5 and 0.9 ml/kg. After chronic (7 days) intrapericardial infusions with osmotic minipumps, pericardial fluid/plasma concentration ratios (local advantages) were 7 to 10 for the fluorescent proteins and >30 for FITC-heparin. This can be explained by the low substance clearances in pericardial fluid compared with plasma. Local advantages of the small substances cortisol (mol. wt. = 362.5) and a carbonic acid derivative thereof (mol. wt. = 348) were 14 and 420. Intrapericardial infusion of ^{125}I-FGF-2 yielded 8 times higher cardiac tissue levels than systemic infusion, whereas ^{125}I-FGF-2 was found in the entire heart. Pharmacokinetic profiles of intrapericardially applied substances are such that desired local drug concentrations can be obtained at lower dosages, whereas systemic concentrations remain low (thus reducing the potential risk of peripheral side effects). Therefore, intrapericardial application of therapeutic agents provides a promising strategy for site-specific treatment of heart or coronary diseases.

In the pharmacological treatment of heart diseases, one of the problems to overcome is to achieve satisfactory concentrations of therapeutic agents at the target site. When agents are applied systemically, the potential risk of side effects exists and therapeutic efficacy may be low, due to metabolism and tissue binding of the agent. In addition, delivery of systemically administered substances to the heart or coronary circulation may be hampered by diminished local blood supply, e.g., in occlusive coronary artery disease. Higher therapeutic efficiencies and smaller risks of peripheral side effects are anticipated if agents are administered locally. This issue has become a matter of intense debate with recent attempts to induce myocardial angiogenesis by targeting genes or gene-derived products to the heart (Kornowski et al., 2000).

Various approaches exist to deliver agents to the heart, such as intracoronary, intramyocardial, and transvascular application or local installation of slow releasing polymers (Sellke and Simons, 1999; Kornowski et al., 2000). An alternative approach is administration of agents into the pericardial space. This approach has been successfully applied for a number of agents (see Spodick, 2000 for a brief overview). For instance, in animal models of cardiac ischemia, intrapericardially applied FGF-2 was shown to induce myocardial angiogenesis (Landau et al., 1995; Uchida et al., 1995), to increase collaterals and to improve coronary blood flow and perfusion of the ischemic heart region (Laham et al., 2000). Intrapericardially applied nitric oxide-donors (Willerson et al., 1996; Waxman et al., 1999) and antiarrhythmic agents (Ayers et al., 1996) have been shown to elicit clear effects on heart function or heart circulation in animals. In humans, pericardial effusions have been successfully treated by intrapericardially applied chemotherapeutics (Kohnoe et al., 1994) and glucocorticoids (Buselmeier et al., 1978). Finally, genes have been transferred to heart tissue with high efficiency, by applying gene-containing vectors into the pericardial space of animals (Lazarous et al., 1999; March et al., 1999; Zhang et al., 1999).

These pharmacodynamic studies suggest that intrapericardial drug application may represent a promising strategy for site-specific drug targeting to the heart. Also, from a pharmacokinetic point of view, there is reason to assume that

---

ABBREVIATIONS: FITC, fluorescein isothiocyanate; FGF-2, fibroblast growth factor 2 (basic fibroblast growth factor); HPLC, high-performance liquid chromatography; RSA, rat serum albumin; PBS, phosphate-buffered saline; V_c, volume of the (initial) central compartment.

this is the case. Since the pericardial space is a small fluid-filled closed compartment, facing the heart at one side (Spodick, 1992), it may comprise an ideal compartment for local drug delivery (Smits and Thijssen, 1986; Daemen et al., 1988). This assumption is supported by Lazarous et al. (1997), demonstrating that intrapericardial application was by far the most efficient way to deliver FGF-2 to the heart. They showed that 19% of the intrapericardially applied FGF-2 was recovered in the heart versus only 0.5% after intravenous, 1.3% after left atrial, and 3 to 5% after intracoronary administration. Nevertheless, knowledge of the pharmacokinetics of intrapericardially applied compounds is still rather limited. In particular, it is not yet known whether a potential advantage of local intrapericardial delivery can be maintained over a prolonged period of time. Therefore, in the present study, various substances were applied by intrapericardial or systemic bolus injections (fluorescent macromolecules) as well as by 7 days of infusions (fluorescent macromolecules and steroids). Pharmacokinetic profiles were determined in pericardial fluid and plasma of conscious rats instrumented with intravascular and intrapericardial catheters. Test substances were chosen because of their variable sizes and their potential therapeutic applicability (in the case of heparin, FGF-2, and cortisol) and included the fluorescent macromolecules FITC-rat IgG (155 kDa; assessed by SDS-polyacrylamide gel electrophoresis), Texas Red-RSA (67 kDa), Texas Red-FGF-2 (18 kDa), and (unfractionated) FITC-heparin (mean mol. wt., 18 kDa), the small steroid cortisol (mol. wt. = 362.5), and a carbonic acid derivative thereof (mol. wt. = 348). Finally, rats were intrapericardially or systemically infused for 7 days with ^{125}I-labeled FGF-2 to assess cardiac tissue penetration by autoradiography.

Materials and Methods

Animals. For the experiments, male Wistar rats (Iffa Credo, Someren, the Netherlands) were used, ranging in weight between 280 and 350 g. Animals were housed at the animal facilities of the University of Maastricht with a 12-h light/dark cycle and had free access to standard rat chow and tap water. Experiments were performed according to institutional guidelines and have been approved by the local ethical committee for the use of experimental animals.

Surgical Procedure for Installing a Pericardial Catheter. Construction of the pericardial catheter and the procedure to install the catheter into the pericardial space was conducted using methods adapted from Veelken et al. (1990) and McDermott et al. (1995). The pericardial catheter consisted of silicone tubing (internal diameter: 0.51 mm; external diameter: 0.94 mm; Degania Silicone, Degania Bet, Israel), which, by assembling its endings with a polyolefin shrinking sleeve (Farnell Compounds, Maarssen, the Netherlands), was shaped as a loop (length ~2 cm, width ~1 cm). Silicone glue was applied in the middle of the loop, to create two separate chambers (for fluid injection and pericardial fluid withdrawal) that were provided with two and six holes, respectively, by use of a 25-gauge perforator. The endings of the silicone tubing were connected to polyethylene (PE-10) tubing (i.d. 0.28 mm, o.d. 0.61 mm; Portex Limited, Kent, UK). Before installment, the catheter and the polyethylene extensions were gas sterilized and filled with sterile 0.9% (w/v) NaCl. The extensions were plugged with stainless steel pins.

Rats were anesthetized by i.p. injection of 60 mg/kg sodium pentobarbitone (Nembutal; Sanofi Sante, Maassluis, the Netherlands) and placed on a heating pad, kept at 37°C. A high midline thoracotomy was performed and a retractor was applied. After careful cleavage of the sternohyoid muscle, thymus lobules were separated to expose the upper part of the pericardium. A small incision in the

pericardial sac was made with iris scissors, and the loop catheter was inserted. The pericardial sac was closed by sealing it to the thymus with histoacryl tissue glue (Braun, Melsungen, Germany). After removing the retractor, the polyethylene extensions were guided to the neck and externalized via a small incision in the skin. Wounds were sutured with 3-0 silk (Ethicon, Norderstedt, Germany).

Preparation and Analyses of Compounds. Fluorescein-labeled heparin (unfractionated with a mean molecular weight of 18,000) was obtained from Molecular Probes (Eugene, OR). Rat serum albumin (Sigma-Aldrich, St Louis, MO) and human recombinant FGF-2 (Research Diagnostics, Flanders NJ) were labeled by reaction with the succinimidyl ester of Texas Red (Molecular Probes). Rat IgG (Sigma-Aldrich) was labeled with fluorescein by reaction of fluorescein isothiocyanate (Sigma-Aldrich). Labeling, reagent inactivation, and removal of noncovalently bound fluorescence (the latter resulting in <0.5% unbound fractions of the total fluorescence as assessed by dialysis and ultrafiltration), were conducted by standard procedures (see, e.g., Haugland, 1996).

Fluorescence of pericardial fluid samples and (red blood cell-free) plasma samples was determined (SPF-500 C SLM; Aminco Bowman, Silver Spring, MD) at 595 nm (excitation) and 613 nm (emission) for Texas Red-labeled substances and at 495 nm (excitation) and 523 nm (emission) for fluorescein-labeled compounds, at bandwidths of 5 nm. For some samples, acid precipitation of the proteins was performed to ensure that the fluorescence could not be attributed to noncovalently bound small fluorescent molecules. No indication was found for the presence of free fluorescent molecules in pericardial fluid and plasma of rats treated with fluorescent proteins by either infusion or bolus injection.

Cortisol (Sigma-Aldrich) was modified at its side chain by oxidative cleavage of the α-hydroxy-ketone with periodic acid (Malaprade reaction; see, e.g., Vogel, 1956). Briefly, cortisol was dissolved in acetone to obtain a 10 mg/ml solution and mixed with 0.4 volume of sodium *meta*-periodate dissolved in 0.3 M HCl (chemicals obtained from Merck, Darmstadt, Germany). After a reaction time of 2 h at 40°C, organic compounds were extracted into HPLC grade methylene chloride (Biosolve, Valkenswaard, the Netherlands), the acid was separated from unmodified cortisol by extracting it into 0.1 M NaOH and the aqueous phase washed with methylene chloride. The acid was precipitated by adding HCl, extensively washed with 0.1 M HCl to remove inorganic reagents, filtered and dried. Purity was checked by thin-layer chromatography and HPLC; mass spectrometry revealed a molecular weight of 348 of the product.

For analysis, samples were acidified with 0.1 M HCl, and internal standard was added to the samples. Cortisol served as internal standard when samples were to be analyzed for the carbonic acid of cortisol, whereas for cortisol analysis, the carbonic acid of cortisol was used as internal standard. After extraction of the steroids into HPLC grade methylene chloride, the organic phase was collected and evaporated under a stream of nitrogen. Subsequently, steroids were converted into fluorescent products with sulfuric acid and analyzed on HPLC as described (Mason et al., 1992).

Pharmacokinetic Studies after Bolus Injection. Immediately after implantation of the pericardial catheter, rats (still under anesthesia) were provided with a catheter in the right femoral artery essentially as described (Smits et al., 1982). Rats were allowed to recover at least 2 days before experimentation. One hour before the start of the experiment, 20 μl of pericardial fluid were withdrawn using a Hamilton 1705 (Hamilton Bonaduz AG, Bonaduz, Switzerland) syringe, and 50 μl of saline were injected into the pericardial space to check the integrity of the pericardial catheter. Injections of volumes up to 0.2 ml were previously shown to be without hemodynamic effects (Veelken et al., 1990). Blood (0.15–0.25 ml) was collected in a syringe containing a minimal volume of heparin (Organon Teknika, Boxtel, the Netherlands). These samples served as blanks for later analyses. Experiments in which substances were applied intrapericardially were started by a 50-μl bolus injection of the test substances into the pericardial space, followed by 20 μl of saline to

flush the catheter. If substances were applied systemically, experiments were started by a 100-μl bolus injection of the substances and subsequent injection of 300 μl of saline into the femoral artery catheter. FITC-rat IgG, (10 mg/ml), Texas Red-RSA (10 mg/ml), and FITC-heparin (1 mg/ml) were dissolved in PBS. Texas Red-FGF-2 (20 μg/ml) was dissolved in a 10 mg/ml solution of RSA in PBS.

Pericardial fluid (20 μl) and blood samples were taken at various time points after injection. To substitute withdrawn pericardial fluid, 20 μl of saline were injected into pericardial space immediately after sampling. After every sample, the femoral artery catheter was flushed with 0.3 to 0.4 ml saline and filled with heparinized (5–10 IU/ml) saline. Plasma and pericardial fluid samples were stored at -20°C until analysis.

Data were standardized for body weights. Pharmacokinetic analysis of the data for each animal was conducted using the GPAD (GraphPad Software, San Diego, CA) software package. Data were fitted to the exponential equation $Ct = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$ of one (i.e., A is fixed at 0)- and two-compartment models. Fits were compared using F-tests and data were log transformed for model judgement.

Infusion Studies. Directly after installment of the pericardial catheter, still-anesthetized rats were provided with a catheter in the left jugular vein (Kleinjans et al., 1984). Rats were allowed to recover for 2 days before subcutaneous implantation (under ketamine/xylazine anesthesia) of osmotic minipumps (Alzet 2001; Alza, Palo Alto, CA). Minipumps filled with solutions of the substances to be tested were primed in saline at 37°C at least 4 h before connection to the catheter. Before installing pumps, pericardial fluid and orbital sinus blood were sampled, to serve as blanks. Seven days after pump installment, rats were sacrificed by exsanguination under pentobarbitone and pericardial fluid and blood collected. To check for possible loss of substances during infusion, the remaining pump contents were analyzed. No significant changes in the concentration of the substances in the infusion fluid were found after 1 week of pumping. Infusion rates of the substances were 10 μg/h for FITC-rat IgG and Texas Red-RSA, 20 ng/h for Texas Red-FGF-2, 100 ng/h for FITC-heparin, 684 ng/h for cortisol, and 984 ng/h for the side chain-modified acid analog of cortisol. Doses were chosen to achieve concentrations that were readily measurable but without pharmacological effects (risk of bleeding in the case of heparin); similar doses were applied systemically and intrapericardially to be able to make a good comparison between the two routes of administration. The solvent was PBS, except for Texas Red-FGF-2 and cortisol, which were dissolved in a 10 mg/ml solution of RSA in PBS.

Cardiac Tissue Penetration of Intrapericardially or Systemically Infused ^{125}I-FGF-2. Rats were infused with ^{125}I-labeled FGF-2 (PerkinElmer Life Sciences, Boston, MA) at a dose rate of 25 nCi/h, similar to that described above. After 7 days of infusion, rats ($n = 2$ for intravenous and $n = 3$ for intrapericardial infusion) were sacrificed to remove the hearts. Hearts were weighed and rinsed in saline for 5 min. At the center of the hearts, transverse 2-mm slices were cut, using a razor blade. Slices were rinsed in saline after fixation by 5 min submersion in a 10% (v/v) formaldehyde solution in PBS and 5 min in 70% (v/v) ethanol in water. Slices were used for autoradiography by phosphorimaging. Cardiac tissue concentrations of ^{125}I-FGF-2 were determined by use of a gamma counter.

Results

Bolus Injection Studies. Pericardial fluid concentration-time profiles of intrapericardially applied and plasma concentration-time profiles of systemically applied FITC-rat IgG, Texas Red-RSA, Texas Red-FGF-2, and FITC-heparin are shown in Fig. 1.

Pharmacokinetic parameters are summarized in Table 1. Pharmacokinetics of the fluorescent macromolecules generally appear to be best described using two-compartment models, indicating (rapid) distribution and (slower) elimination phases for the compounds. However, for intrapericardially

applied FITC-rat IgG in pericardial fluid as well as systemically applied Texas Red-FGF-2 in plasma, one-compartment models seem to be most appropriate. Calculated (initial) central compartment volumes (V_c, representing the volume of the compartment to which the substance is applied) do not vary widely between the substances and range between 33 and 46 ml/kg body weight in plasma and between 0.5 and 0.9 ml/kg body weight in pericardial fluid. Pericardial clearances of the macromolecules are 10.6- to 83-fold smaller than plasma clearances. In addition, the difference between the substances regarding their clearances appears to be smaller in pericardial fluid than in plasma.

Figure 2 depicts the ratios of pericardial fluid and plasma concentrations of fluorescent macromolecules after bolus injections into the pericardial space or into blood. The data show that after intrapericardial bolus injections, pericardial fluid concentrations of the compounds exceed plasma concentrations over a prolonged period of time. On the other hand, after systemic bolus injections, pericardial fluid concentrations are lower than plasma concentrations over an approximately similar period of time, but concentration differences between plasma and pericardial fluid generally are less pronounced than after intrapericardial application. No data are shown for FITC-heparin after intra-arterial injection because concentrations in pericardial fluid were below the detection limit.

Infusion Studies. Pericardial fluid and plasma substance concentrations after 7 days of infusion into pericardial space or blood are given in Table 2. Based on pilot experiments in which concentrations were determined on a daily basis, as well as on terminal half-lives (Table 1), it seems reasonable to assume that after 7 days of infusion, steady state has been reached. Following continuous infusion of fluorescent macromolecules into pericardial space, concentrations in plasma are at least 7-fold lower than in pericardial fluid (Table 2). This is also the case for the small compounds cortisol and its carbonic acid analog (Table 2). In contrast, following continuous infusion of macromolecules into blood, pericardial fluid and plasma concentrations are approximately similar.

Calculated clearances derived from steady-state concentrations (i.e., clearance = infusion dose rate/steady-state concentration) in pericardial fluid upon intrapericardial infusion are 5.54 ± 1.98 (FITC-rat IgG), 4.23 ± 0.75 (Texas Red-RSA), 6.86 ± 3.75 (Texas Red-FGF-2), 3.91 ± 0.31 (FITC-heparin), 105 ± 29.3 (cortisol), and 30.8 ± 3.52 μl/kg $\cdot$ min (cortisol carbonic acid). Calculated clearances from plasma steady-state concentrations upon systemic infusion are 30.3 ± 8.3 (FITC-rat IgG), 54.2 ± 18.4 (Texas Red-RSA), and 36.1 ± 3.64 μl/kg $\cdot$ min (Texas Red-FGF-2). In some cases, these clearances are substantially lower than those calculated after bolus injection of the compounds (Table 1). This probably can be attributed to the existence of distribution processes that are saturated after long-term infusion but not after bolus injection of the compounds, which results in an overestimation when calculating clearances for the bolus injections. Regarding FITC-heparin, it should be kept in mind that the pharmacokinetics of heparins are known to be nonlinear (Boneu et al., 1990), so that comparison between concentration profiles after bolus injections or infusions is difficult.

Studies on the Cardiac Tissue Penetration of ^{125}I-FGF-2. To obtain information about the penetration of intra-
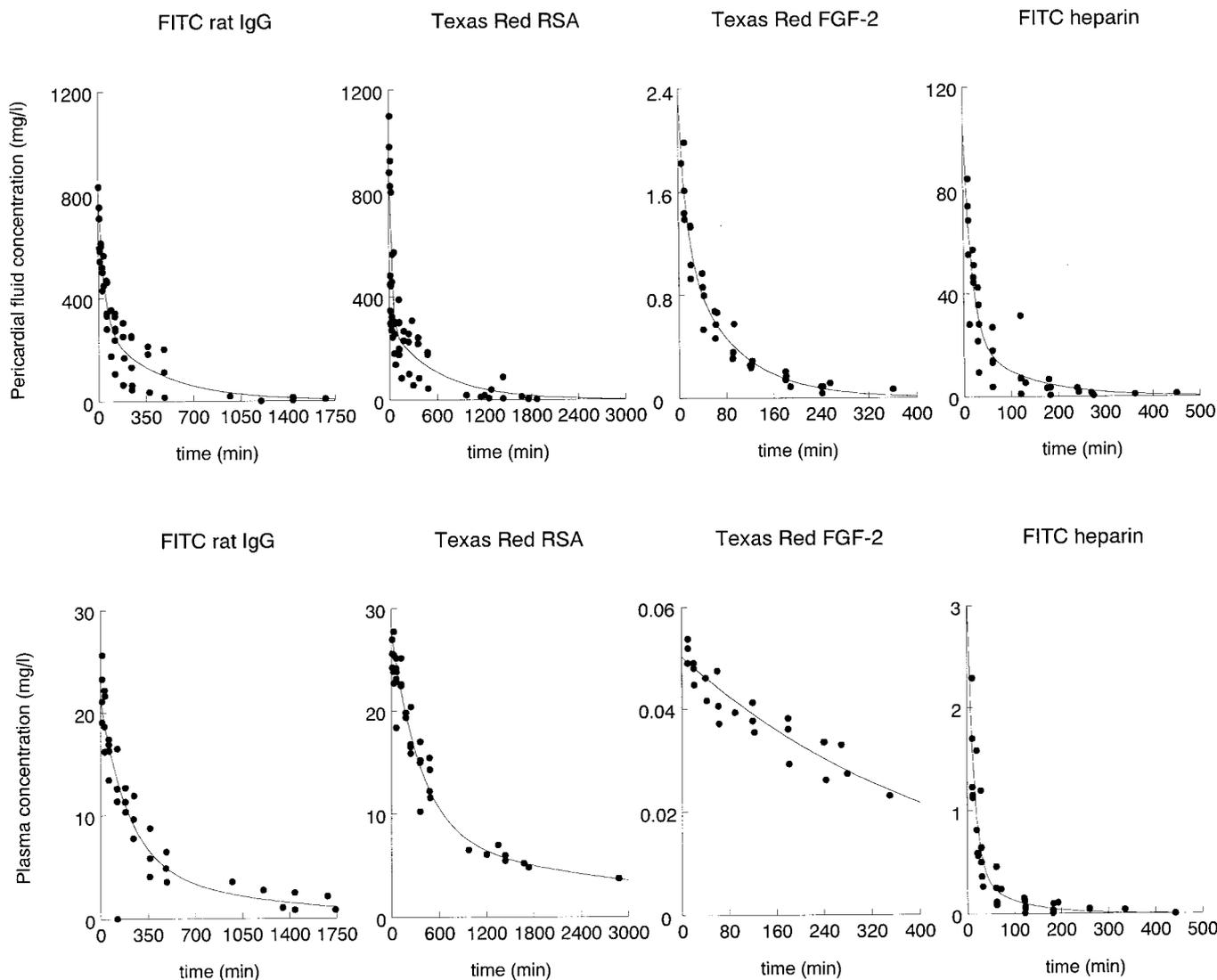
Fig. 1. Concentration-time profiles of fluorescent macromolecules in rat pericardial fluid after intrapericardial bolus injection (top) or in plasma after intra-arterial bolus injection (bottom). Rats were given bolus injections of 50 μl intrapericardially or 100 μl intra-arterially, and pericardial fluid and blood samples were collected at various time points for analysis as described. Data in each graph (three to seven rats) represent concentrations, standardized for body weights [i.e., body weight (kg) $\times$ concentration].

TABLE 1

Pharmacokinetic parameters of fluorescent macromolecules

Parameters were derived by fitting standardized data of every individual animal to the equation $Ct = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$ of one (i.e., A = 0)- and two-compartment models and are expressed as mean $\pm$ S.E.

	A as Fraction of C_0	B as Fraction of C_0	$t_{1/2\alpha}$[a]	$t_{1/2\beta}$[a]	V_c[b]	Cl[c]	Number of Rats
			min		$\mu l/kg$	$\mu l/min \cdot kg$	
Pericardial fluid							
FITC-rat IgG	0.00	1.00	NA	167 $\pm$ 66	893 $\pm$ 114	5.30 $\pm$ 1.10	6
Texas Red-RSA	0.66 $\pm$ 0.11	0.34 $\pm$ 0.11	46.8 $\pm$ 14	589 $\pm$ 133	892 $\pm$ 207	3.72 $\pm$ 0.90	7
Texas Red-FGF-2	0.85 $\pm$ 0.06	0.15 $\pm$ 0.06	17.3 $\pm$ 5.5	102 $\pm$ 19	497 $\pm$ 70	8.05 $\pm$ 0.33	4
FITC-heparin	0.82 $\pm$ 0.06	0.18 $\pm$ 0.06	12.8 $\pm$ 3.9	87 $\pm$ 18	513 $\pm$ 86	16.8 $\pm$ 5.62	5
Plasma							
FITC-rat IgG	0.77 $\pm$ 0.07	0.23 $\pm$ 0.07	116 $\pm$ 18.4	657 $\pm$ 125	46248 $\pm$ 3838	128 $\pm$ 6.38	5
Texas Red-RSA	0.59 $\pm$ 0.12	0.41 $\pm$ 0.12	89 $\pm$ 14.8	1132 $\pm$ 300	34734 $\pm$ 1761	40.5 $\pm$ 2.76	5
Texas Red-FGF-2	0.00	1.00	NA[d]	338 $\pm$ 31	39990 $\pm$ 923	84.2 $\pm$ 10.3	3
FITC-heparin	0.83 $\pm$ 0.06	0.17 $\pm$ 0.06	10.2 $\pm$ 2.3	79.7 $\pm$ 23.2	33175 $\pm$ 5939	1400 $\pm$ 303	5

[a] $t_{1/2\alpha}$ and $t_{1/2\beta}$ were calculated from ln 2/α and ln 2/β.
[b] V_c = Dose/C_0 is the (initial) central compartment volume (i.e., the volume of the compartment to which the agent is applied); C_0 = A + B is the intercept of the C-t curve.
[c] Cl (clearance) as dose/AUC (area under the C-t curve).
[d] NA, not applicable (best fit using one-compartment model).
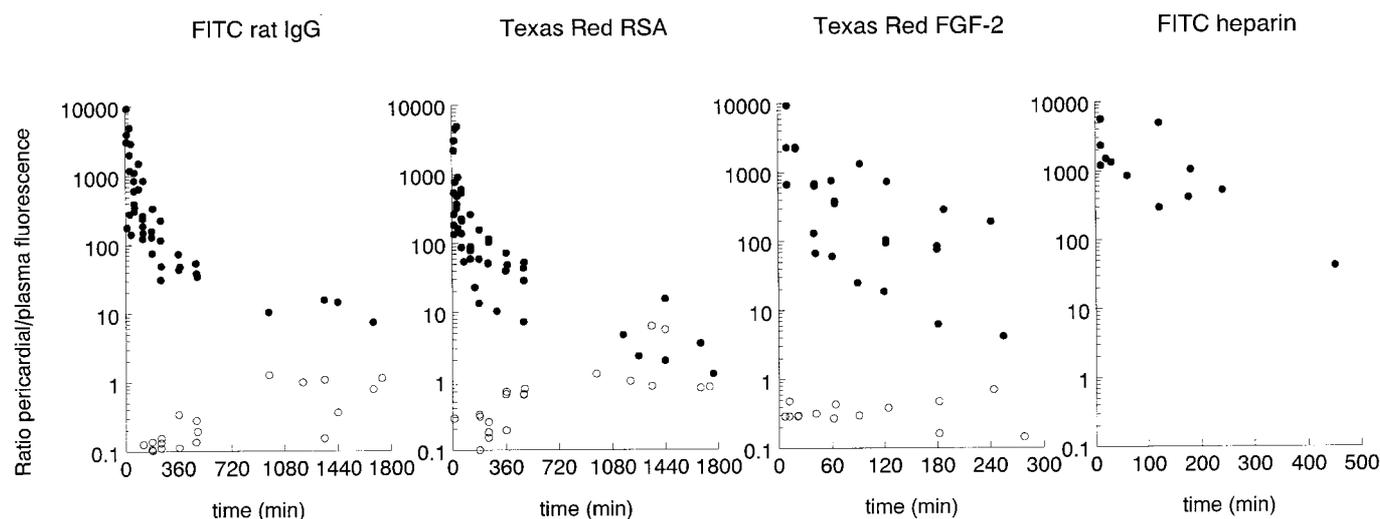
Fig. 2. Ratios of fluorescence measured in pericardial fluid and plasma after intrapericardial (●) or intra-arterial bolus injections of fluorescent macromolecules. Rats were given bolus injections of 50 μl intrapericardially or 100 μl intra-arterially, and pericardial fluid and blood samples were collected at various time points and analyzed as described. Data in each graph represent three to six rats. For FITC-heparin, data are lacking for systemic administration because fluorescence of pericardial fluid could not be detected.

pericardially versus systemically applied substances into cardiac tissue, rats were infused with ^{125}I-FGF-2 for 1 week into pericardial space or blood.

Examples of autoradiograms are shown in Fig. 3. From this figure, it becomes apparent that cardiac tissue levels of ^{125}I-FGF-2 are higher when infused intrapericardially than when infused systemically. Indeed, gamma-counting showed that cardiac tissue concentrations of ^{125}I-FGF-2 are 8-fold higher in intrapericardially infused rats ($n = 3$) than in systemically infused animals ($n = 2$). Inspection of Fig. 3 also shows that intrapericardially (like systemically) infused ^{125}I-FGF is detected in the entire rat heart.

Discussion

Various delivery strategies have been applied to target therapeutic agents to the diseased heart, such as local installation of slow releasing polymers and intramyocardial, intracoronary, transvascular, and intrapericardial applications (Sellke and Simons, 1999; Kornowski et al., 2000). The purpose of these strategies is to improve therapeutic efficiencies and to reduce the risks of side effects inherent to systemic application. The issue, which of the application methods is superior regarding safety, feasibility, and efficiency, is still not resolved (Sellke and Simons, 1999; Kornowski et al., 2000). In this study, we investigated whether intrapericardial application offers pharmacokinetic advantages, by comparing pharmacokinetic profiles of compounds that were intrapericardially and systemically applied by bolus injection or by continuous infusion.

The results show that after intrapericardial injection of macromolecules, pericardial fluid concentrations are substantially higher than plasma concentrations over a prolonged time (Fig. 2). Similarly, if macromolecules or steroids are infused into pericardial space for 7 days, this results in relatively high pericardial fluid concentrations and low plasma concentrations (Table 2). The ratio of local and systemic steady-state concentrations after local application is termed the local advantage. Thus, for the intrapericardially infused substances, local advantages range between 7 and

420 (Table 2). The magnitude of the local advantage depends on substance exchange between the local and the blood compartments and on substance elimination from these compartments. Assuming that the clearance of a substance from the pericardial space is solely due to diffusion into plasma (without differences between back- and forward diffusion rate constants), local advantage of intrapericardial drug application would be determined by the ratio of the systemic and local clearances (Smits and Thijssen, 1986). Although this assumption is oversimplified, data derived from steady-state concentrations (*Results*) or bolus injection data (Table 1) indicate that the local advantages of intrapericardially applied agents can be mainly explained by their low clearances in pericardial space compared with the relatively high plasma clearances. Whether particular physicochemical properties such as size and charge of substances play a role in the local advantage of intrapericardial application is at present unclear. Although the number of agents that were tested is too small to draw any firm conclusions, the data in Table 2 do not reveal a clear relationship between molecular size and the obtained local advantage. The relatively high local advantages of the negatively charged heparin and cortisol carbonic acid may indicate that charge does play a role. On the other hand, recent studies in our laboratory demonstrated a high local advantage (of 97) for the positively charged (small) molecule sotalol as well (data not shown). That there is no clear structure-advantage relationship is not surprising, if it is taken into account that one major determinant of the local advantage of a compound is its systemic clearance, for which no simple general relationship with the structure of the compound is known.

The high local advantages that are found imply that high local drug concentrations can be obtained, whereas drug concentrations in the plasma remain low if substances are applied intrapericardially. For that reason, intrapericardial application of drugs may be expected to lead to higher therapeutic efficiencies and lower risks of side effects. However, if agents do not exert their beneficial effects by interaction with epicardial surface receptors, but act deeper in

TABLE 2
Pericardial fluid and plasma concentrations of various substances after 7 days of continuous pericardial or systemic infusion

Concentrations are given as a fraction of the substance concentration, relative to its concentration in the infusate (infusion rate was 1 μl/h) and are corrected for body weights [i.e., body weight (kg) $\times$ 10,000 $\times$ measured concentration/infusate concentration]. Data are expressed as mean $\pm$ S.E. Concentration ratios were calculated for each animal, and the number of animals is given in parentheses.

	Intrapericardial Infusion			Systemic Infusion		
	Pericardial Fluid	Plasma	Pericardial/Plasma Ratio (Rats)	Pericardial Fluid	Plasma	Pericardial/Plasma Ratio (Rats)
FITC-rat IgG	30.1 ± 10.7	3.17 ± 1.13	9.83 ± 3.67 (5)	3.78 ± 1.11	5.50 ± 1.51	1.36 ± 0.68 (5)
Texas Red-RSA	39.4 ± 6.93	6.35 ± 2.18	8.11 ± 2.60 (4)	2.39 ± 0.58	3.07 ± 1.04	0.98 ± 0.26 (4)
Texas Red-FGF-2	24.3 ± 13.3	4.10 ± 2.36	6.85 ± 1.61 (4)	4.85 ± 2.20	4.62 ± 0.47	1.01 ± 0.38 (2)
FITC-heparin	42.6 ± 3.4	n.d.[a]	>30[b] (4)		n.d. (4)	
Cortisol	1.59 ± 0.44	0.11 ± 0.03	14.4 ± 0.55 (2)		Not determined	
Cortisol carbonic acid	5.42 ± 0.62	0.01 ± 0.002	420 ± 81 (3)		Not determined	

[a] n.d., below detection limit.

[b] No FITC-heparin could be detected in plasma; the value of 30 was calculated by dividing the mean pericardial FITC-heparin concentration by the detection limit of FITC-heparin in plasma.

myocardial tissue or in coronary vasculature, therapeutic efficacy will depend on local tissue concentrations rather than pericardial fluid concentrations. Therefore, we assessed the cardiac tissue penetration of ^{125}I-labeled FGF-2 by autoradiography, since determination of cardiac tissue levels of fluorescent macromolecules was hampered by autofluorescence of the rat hearts. ^{125}I-labeled FGF-2 was selected because of the potential use of (intrapericardially applied) FGF-2 to achieve therapeutic angiogenesis in the heart. This experiment not only showed that total cardiac tissue concentrations of ^{125}I-FGF-2 are 8-fold higher in intrapericardially versus systemically infused rats, but also that if the substance was infused intrapericardially, it appeared to be present at high concentrations in most parts of the rat heart (Fig. 3). The resolution of the autoradiograms that we obtained for ^{125}I-FGF-2 is too low to exactly determine regional differences (e.g., epicardial versus endocardial) in ^{125}I-FGF-2 levels. Nevertheless, the current experiments indicate that the intrapericardial infusion of FGF-2 really seems to be beneficial in that high cardiac tissue levels of the agent can be reached. In line with these observations, ongoing studies in our laboratory indicate that in a rat model, a dose of FGF-2 improves cardiac perfusion only when infused intrapericardially, not when infused systemically (our observations), pointing to high efficiency of the intrapericardial drug infusion. The above observations also correspond with findings

by others (Lazarous et al., 1997; Stoll et al., 1998), comparing various administration routes to deliver FGF-2 by bolus injection. These authors found that the intrapericardial route was the most effective to obtain high cardiac FGF-2 levels.

That the concept of the high local advantage by intrapericardial drug application can indeed be translated into high therapeutic efficiencies (and low risk of peripheral side effects) has been demonstrated not only for FGF-2 but for other substances as well (e.g., Labhasetwar et al., 1994; Landau et al., 1995; Uchida et al., 1995; Ayers et al., 1996; Willerson et al., 1996; Waxman et al., 1999; Laham et al., 2000; Spodick, 2000). For instance, nitric oxide-donors are more effective in exerting regional effects, i.e., reduction of cyclic flow variations of mechanically injured coronary arteries (Willerson et al., 1996) or induction of coronary vasodilatation (Waxman et al., 1999), but produce smaller blood pressure decreases (systemic effects), when applied intrapericardially.

As already discussed, we found that if substances are infused into the pericardial space, their concentration in pericardial fluid will be relatively high, due to low clearances of the substances in the pericardial fluid compared with the higher clearances in the blood. In pericardial fluid, concentrations of various endogenous agents have been shown to exceed those in plasma. This is observed, for instance, for endothelin-1 (Horkay et al., 1995), FGF-2 (Corda et al., 1997), and atrial natriuretic peptide (Amano et al., 1993; Corda et al., 1997) in heart surgery patients. Also, in rats, concentrations of atrial natriuretic peptide in pericardial fluid are higher than in plasma (Klemola et al., 1995). The present study shows that these high pericardial fluid/plasma concentration ratios not only are a matter of local syntheses of the agents in the heart or in the mesothelial cells of the pericardium (Eid et al., 1994; Mebazaa et al., 1998), but can also be attributed to the relatively slow clearances of these agents, once "trapped" into pericardial space.

From the pharmacokinetic data, plasma volumes as well as pericardial volumes of the rats can be derived. Upon systemic bolus injections, central compartment volumes (V_c) (Table 1) range between 33 and 46 ml/kg. These volumes probably represent plasma volumes and are well within the range of previously determined plasma volumes of male Wistar rats (Lee and Blaufox, 1985; Wauquier and Devynck, 1989). Given the almost instantaneous mixing of injected compounds in pericardial space (Santamore et al., 1990), it seems reasonable to assume that calculated V_c values indicate pericardial fluid volume. Hence, pericardial fluid volumes of
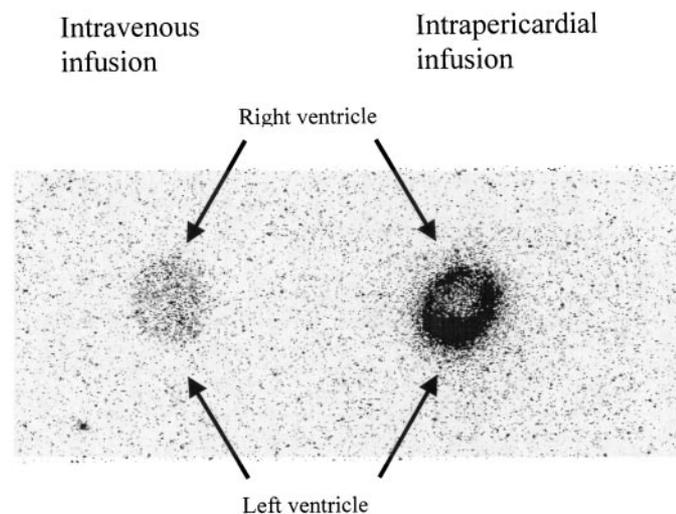

Intravenous infusion   Intrapericardial infusion

Right ventricle

Left ventricle

Fig. 3. Autoradiograms of cardiac tissue slices of rats that received a 1-week infusion of ^{125}-I-FGF-2 into pericardial space or blood.

catheter-instrumented rats would be 0.5 to 0.9 ml/kg. Since the catheter will create additional space in the pericardial cavity (estimated to be about 0.1 ml), which presumably is fully filled with fluid, "real" rat pericardial fluid volumes will probably be 0.25 to 0.35 ml/kg lower. Previous studies showed that mean pericardial fluid volumes are 0.23 ml/kg in greyhounds (Gibson and Segal, 1978) and 0.29 ml/kg in mongrel dogs (Santamore et al., 1990). In non-heart-diseased humans, pericardial fluid volume ranges between 15 and 50 ml (Spodick, 1992), which at a body weight of 75 kg would correspond to 0.2 to 0.67 ml/kg.

Conclusion

After intrapericardial application, high local drug concentrations can be obtained in the heart, whereas plasma drug concentrations remain low. This can be explained by the fact that the clearances of substances in pericardial fluid are low, relative to substance clearances in plasma. Because of this pharmacokinetic advantage, a desirable local drug concentration may be achieved at lower doses, while the potential risk of peripheral side effects is reduced by intrapericardial drug application. Therefore, intrapericardial application of therapeutic agents provides a promising tool to obtain site-specific treatment of heart or coronary diseases.

References

Amano J, Suzuki A, Sunamori M, Shichiri M, and Marumo F (1993) Atrial natriuretic peptide in the pericardial fluid of patients with heart disease. *Clin Sci* **85:**165–168.
Ayers GM, Rho TH, Ben-David J, Besch HR, and Zipes DP (1996) Amiodarone instilled into the canine pericardial sac migrates transmurally to produce electrophysiologic effects and suppress atrial fibrillation. *J Cardiovasc Electrophysiol* **7:**713–721.
Boneu B, Caranobe C, and Sie P (1990) Pharmacokinetics of heparin and low molecular weight heparin. *Baillieres Clin Haematol* **3:**531–544.
Buselmeier TJ, Davin TD, Simmons RL, Najarian JS, and Kjellstrand CM (1978) Treatment of intractable uremic pericardial effusion. Avoidance of pericardiectomy with local steroid instillation. *J Am Med Assoc* **420:**1358–1359.
Corda S, Mebazaa A, Gandolfini M-P, Fitting C, Marotte F, Peynet J, Charlemagne D, Cavaillon J-M, Payen D, Rappaport L, and Samuel J-L (1997) Trophic effect of human pericardial fluid on adult cardiac myocytes. Differential role of fibroblast growth factor-2 and factors related to ventricular hypertrophy. *Circ Res* **81:**679–687.
Daemen MJAP, Smits JFM, Thijssen HHW, and Struijker Boudier HAJ (1988) Pharmacokinetic considerations in target-organ directed drug delivery. *Trends Pharmacol Sci* **9:**138–141.
Eid H, Kuroski de Bold ML, Chen JH, and de Bold AJ (1994) Epicardial mesothelial cells synthesize and release endothelin. *J Cardiovasc Pharmacol* **26:**715–720.
Gibson AT and Segal MB (1978) A study of the composition of pericardial fluid, with special reference to the probable mechanism of fluid formation. *J Physiol* **277:**367–377.
Haugland RP (1996) Fluorophores and their amine reactive derivatives, in *Handbook of Fluorescent Probes and Research Chemicals,* 6th ed. (Spence MTZ, ed.), Molecular Probes, Eugene, OR.
Horkay F, Laine M, Szokodi I, Leppäluoto J, Vuolteenaho O, Ruskoaho H, Juhász-Nagy A, and Toth M (1995) Human pericardial fluid contains the highest amount of endothelin-1 of all mammalian biological fluids thus far tested. *J Cardiovasc Pharmacol* **26 (Suppl 3):**S502–S504.
Klemola R, Laine M, Weckström M, Vuolteenaho O, Ruskoaho H, Huttunen O, and Leppäluoto J (1995) High concentrations of atrial natriuretic peptide and brain natriuretic peptide in rat pericardial fluid and their reduction by reserpine in vivo. *Naunyn-Schmiedeberg's Arch Pharmacol* **352:**331–336.
Kleinjans JC, Smits JFM, van Essen H, Kasbergen CM, and Struijker-Boudier HAJ (1984) Hemodynamic characterization of hypertension induced by chronic intrarenal or intravenous infusion of norepinephrine in conscious rats. *Hypertension* **6:**689–699.

Kohnoe S, Maehara Y, Takahashi I, Saito A, Okada Y, and Sugimachi K (1994) Intrapericardial mitomycin C for the management of malignant pericardial effusion secondary to gastric cancer. *Chemotherapy* **40:**57–60.
Kornowski R, Fuchs S, Leon MB, and Epstein SE (2000) Delivery strategies to achieve therapeutic myocardial angiogenesis. *Circulation* **101:**454–458.
Labhasetwar V, Underwood T, Gallagher M, Murphy G, Langberg J, and Levy RJ (1994) Sotalol controlled-release systems for arrhythmias: in vitro characterization, in vivo drug disposition, and electrophysiologic effects. *J Pharm Sci* **83:**156–164.
Laham RJ, Rezaee M, Post M, Novicki D, Sellke FW, Pearlman JD, Simons M, and Hung D (2000) Intrapericardial delivery of fibroblast growth factor-2 induces neovascularization in a porcine model of chronic myocardial ischemia. *J Pharmacol Exp Ther* **292:**795–802.
Landau C, Jacobs AK, and Haudenschildt CC (1995) Intrapericardial basic fibroblast growth factor induces myocardial angiogenesis in a rabbit model of chronic ischemia. *Am Heart J* **129:**924–931.
Lazarous DF, Shou M, Stiber JA, Dadhania DM, Thirumurti V, Hodge E, and Unger EF (1997) Pharmacodynamics of basic fibroblast growth factor: route of administration determines myocardial and systemic distribution. *Cardiovasc Res* **36:**78–85.
Lazarous DF, Shou M, Stiber JA, Hodge E, Thirumurti V, Gonçalves L, and Unger EF (1999) Adenoviral-mediated gene transfer induces sustained pericardial VEGF expression in dogs: effect on myocardial angiogenesis. *Cardiovasc Res* **44:**294–302.
Lee HB and Blaufox MD (1985) Blood volume in the rat. *J Nucl Med* **25:**72–76.
March KL, Woody M, Mehdi K, Zipes DP, Brantly M, and Trapnell BC (1999) Efficient in vivo catheter-based pericardial gene transfer mediated by adenoviral vectors. *Clin Cardiol* **22 (1 Suppl 1):**I23–I29.
Mason SR, Ward LC, and Reilly PEB (1992) Fluorimetric detection of serum corticosterone using high-performance liquid chromatography. *J Chromatogr* **581:**267–271.
McDermott DA, Meller TM, Gebhart GF, and Guttermann DD (1995) Use of an indwelling catheter for examining cardiovascular responses to pericardial administration of bradykinin in rat. *Cardiovasc Res* **30:**39–46.
Mebazaa A, Wetzel RC, Dodd-o JM, Redmond EM, Shah AM, Maeda K, Maistre G, Lakatta EG, and Robotham JL (1998) Potential paracrine role of the pericardium in the regulation of cardiac function. *Cardiovasc Res* **40:**332–342.
Santamore WP, Constantinescu MS, Bogen D, and Johnston WE (1990) Nonuniform distribution of normal pericardial fluid. *Basic Res Cardiol* **85:**541–549.
Sellke FW and Simons M (1999) Angiogenesis in cardiovascular disease. Current status and therapeutic potential. *Drugs* **58:**391–396.
Smits JFM, Coleman TG, Smith TL, Kasbergen CM, van Essen H, and Struijker-Boudier HAJ (1982) Antihypertensive effect of propranolol in conscious spontaneously hypertensive rats: central hemodynamics, plasma volume and renal function during β-blockade with propranolol. *J Cardiovasc Pharmacol* **4:**904–914.
Smits JFM and Thijssen HHW (1986) Spatial control of drug action: theoretical considerations on the pharmacokinetics of target-aimed drug delivery, in *Rate-Controlled Drug Administration and Action* (Struijker Boudier HAJ, ed) pp 83–114, CRC Press, Boca Raton, FL.
Spodick DH (1992) Macrophysiology, microphysiology and anatomy of the pericardium: a synopsis. *Am Heart J* **124:**1046–1051.
Spodick DH (2000) Intrapericardial therapeutics and diagnostics. *Am J Cardiol* **85:**1012–1014.
Stoll HP, Szabo A, and March KL (1998) Sustained transmyocardial loading with bFGF following single intrapericardial delivery: local kinetics and tissue penetration. *Circulation* **98:**I-2100.
Uchida Y, Yanagisawa-Miwa A, Nakamura F, Yamada K, Tomaru T, Kimura K, and Morita T (1995) Angiogenic therapy of acute myocardial infarction by intrapericardial injection of basic fibroblast growth factor and heparin sulfate: an experimental study. *Am Heart J* **130:**1182–1188.
Veelken R, Sawin LL, and Dibona GF (1990) Epicardial serotonin receptors in circulatory control in conscious Sprague-Dawley rats. *Am J Physiol* **258:**H466–H472.
Vogel AI (1956) *A Text-Book of Practical Organic Chemistry Including Qualitative Organic Analysis,* 3rd ed. Longman Group Ltd, London, UK.
Wauquier I and Devynck MA (1989) Body fluid variations and endogenous digitalis-like compounds during chronic NaCl loading in Wistar rats. *Clin Exp Hypertens* **A11:**1217–1234.
Waxman S, Moreno R, Rowe KA, and Verrier RL (1999) Persistent primary coronary dilation induced by transatrial delivery of nitroglycerin into the pericardial space: a novel approach for local cardiac drug delivery. *J Am Coll Cardiol* **33:**2073–2077.
Willerson JT, Igo SR, Yao S-K, Ober JC, Macris MP, and Ferguson JJ (1996) Localized administration of sodium nitroprusside enhances its protection against platelet aggregation in stenosed and injured coronary arteries. *Texas Heart Inst J* **23:**1–8.
Zhang JCL, Woo YJ, Chen J-A, Swain JL, and Sweeney HL (1999) Efficient transmural cardiac gene transfer by intrapericardial injection in neonatal mice. *J Mol Cell Cardiol* **31:**721–732.

Address correspondence to: Dr. J. J. Rob Hermans, Department of Pharmacology and Toxicology, Cardiovascular Research Institute Maastricht, Univ. Maastricht, P.O. Box 616, 6200 MD Maastricht, The Netherlands. E-mail: r.hermans@farmaco.unimaas.nl