Digoxin Uptake, Receptor Heterogeneity, and Inotropic Response in the Isolated Rat Heart: A Comprehensive Kinetic Model

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ABSTRACT

The cardiac pharmacokinetics of digitalis glycosides is not well understood. In the present study, a mechanism-based pharmacokinetic/pharmacodynamic model was developed to describe the uptake kinetics, receptor interaction, and positive inotropic effect of digoxin in the single-pass isolated perfused rat heart. Three doses of digoxin (0.1, 0.2, and 0.3 μmol) were administered to the heart (n = 12) as consecutive 1-min infusions followed by 15-min washout periods. Outflow concentration and left ventricular developed pressure were measured and analyzed by the model. The uptake of digoxin by the heart was limited by capillary permeability with a permeation clearance of 2.35 ml/min/g (about one-third of perfusate flow). Binding kinetics was determined by a mixture of two receptor subtypes, a low-affinity/high-capacity binding site (K_D,1 = 20.9 nmol, 89% of total receptors) and a high-affinity/low-capacity binding site (K_D,2 = 1.5 nmol, 11%). The time course of inotropic response was linked to receptor occupation, with higher efficiency of the high-affinity receptor population. The results suggest that, in the rat heart, consecutive inhibition of first the α_2^- and then the α_1^-isoform of Na^+/K^+-ATPase mediates the positive inotropic effect of digoxin with increasing dosage.

Cardiac glycosides are widely used in the treatment of congestive heart failure because the inhibition of Na^+/K^+-ATPase (sodium pump), which serves as a functional receptor for digitalis, results in an increase in positive inotropy. Binding of digitalis drugs, such as digoxin, to the catalytic α-subunit inhibits the sodium pump and increases intracellular Ca^{2+} availability for contractile proteins. The cardiac actions of digitalis glycosides and the “pump-inhibition hypothesis” have been critically reviewed (Eisner and Smith 1992; Levi et al., 1994). In the rat, consecutive inhibition of the α_2^- and the α_1^-isoforms of Na^+/K^+-ATPase with high and low affinity, respectively, for ouabain, induces positive inotropism over a wide dose range (Graup et al., 1985; Sneed, 1993; MacPherson et al., 1995; Schwartz and Petraschevskaya, 2001). Despite fundamental new insights obtained in the last decades at the enzyme and cellular level by biochemical and electrophysiological studies, however, there is limited knowledge about the functional role of Na^+/K^+-ATPase isoforms in the intact heart. Although recent evidence suggests that the α_2^-isoform may have a special function in the regulation of intracellular Ca^{2+} (Blaustein and Lederer, 1999; James et al., 1999), implying that only this single isoform mediates the glycoside action, this view is still controversial (Gao et al., 1995; Kometiani et al., 2001; Schwartz and Petraschevskaya, 2001). To understand the role of uptake kinetics and the contribution of Na^+/K^+-ATPase isoforms to cardiotonic effects of cardiac glycosides, we investigated the pharmacokinetics and pharmacodynamics of digoxin in the isolated perfused rat heart. Although findings from an intact, perfused organ are more likely to reflect processes occurring in vivo than are results from isolated cells or membrane preparations, to our knowledge, a kinetic analysis of uptake and receptor binding of cardiac glycosides with the aim to explain the time course of the positive inotropic effect in the intact heart has so far not been reported. Specific questions are: how is the transient kinetics of receptor binding related to that of the positive inotropic effect? Is functional receptor heterogeneity detectable in the intact heart? What are the characteristics of the transcapillary and trans-sarcolemmal transport processes?

A prerequisite to understanding the kinetics and dynamics of cardiac glycosides is a mechanistic and quantitative description of the processes involved. We therefore developed an integrated pharmacokinetic/pharmacodynamic (PK/PD) model of digoxin uptake, receptor binding, and inotropic response in the heart. The model included the barriers represented by the capillary and sarcolemmal membranes. One

ABBREVIATIONS:: PK, pharmacokinetic(s); PD, pharmacodynamic(s); LV, left ventricular; LVDP, LV developed pressure; DMSO, dimethyl sulfoxide.
unique property of drug-receptor interaction is saturability, which implies the nonlinearity of the system. By using both the information provided by the outflow concentration-time profile and the time course of left ventricular pressure following three doses of digoxin in the isolated perfused rat heart, the results of PK/PD analysis support the view that two receptor populations are involved and provide evidence for a permeability-limited, transcapillary exchange process.

**Experimental Procedures**

**Perfused Rat Heart.** Hearts (wet weight, 1.05 ± 0.04 g) from adult male Sprague-Dawley rats (300–350 g; n = 12) were perfused with a Krebs-Henseleit bicarbonate buffer at 37°C with 60 mm Hg pressure (Weiss and Kang, 2002). Left ventricular (LV) pressure was monitored by means of a water-filled latex balloon placed in the left ventricle and connected to a pressure transducer. The chest was opened, an aortic cannula filled with perfusate was rapidly inserted into the aorta, and retrograde perfusion was started with an oxygenated perfusate. The perfusate consisted of Krebs-Henseleit buffer solution, pH 7.4, containing 118 mM NaCl, 4.7 mM KCl, 2.52 mM CaCl₂, 1.66 mM MgSO₄, 24.88 mM NaHCO₃, 1.18 mM KH₂PO₄, 5.55 mM glucose, and 2.0 mM sodium pyruvate, including 0.1% bovine serum albumin. A catheter was inserted into the pulmonary artery and connected to an autosampler. The balloon was inflated with water to create a diastolic pressure of 5 to 6 mm Hg. After stabilization, the system was changed to constant flow condition, maintaining a coronary flow of 8.8 ± 0.3 ml/min. The hearts were beating spontaneously at an average rate of 275 beats/min. Coronary perfusion pressure, left ventricular pressure, and heart rate were measured continuously. A physiological recording system (Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) was used to monitor left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and maximum and minimum values of rate of left ventricular pressure development (dP/dtmax and dP/dtmin). Left ventricular developed pressure was calculated as LVEDP = LVSP − LVEDP. This investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (National Institutes of Health Publication 85-23, revised 1996). Experiments were approved by the Animal Protection Body of the state of Sachsen-Anhalt, Germany.

**Materials.** [³H]Digoxin (17 Ci mmol) and digoxin were purchased from PerkinElmer Life Sciences (Boston, MA) and Sigma Chemie (Deisenhofen, Germany), respectively. All other chemicals and solvents were of the highest grade available.

**Experimental Protocol.** Hearts were allowed to equilibrate for 20 min with Krebs-Henseleit solution. Digoxin was dissolved in dimethyl sulfoxide (DMSO) and diluted with the same amount of 70% ethanol. The mixture was attenuated with 0.45% NaCl solution. The final concentration of DMSO and ethanol in vehicle was 0.5 and 0.35%, respectively. Digoxin solution was made by mixing a labeled (5 μCi/ml) compound with an unlabeled (0.32 μCi/ml) one. The final doses (0.1, 0.2, and 0.3 μmol) were administered as 1-min infusions, permutating the sequence of doses with an interval of 15 min (six permuted blocks in 12 hearts). Infusion was performed into the perfusion tube close to the aortic cannula using an infusion device. Outflow samples were collected every 5 s for 3 min and every 30 s for the next 7 min (total collection period, 10 min). The outflow samples were kept frozen at −20°C until analysis. In each heart, control-matched experiments were performed with the vehicle: 0.3, 0.6, and 0.9 ml of vehicle (19, 38, and 57 μmol of DMSO and 22, 44, and 66 μmol of ethanol) were infused in the same way, and the cardiac response was measured. For determination of [³H]digoxin, the outflow sample (50 μl) was transferred to a vial and 4 ml of cocktail was added. After mixing vigorously, the radioactivity was measured with a liquid scintillation counter (PerkinElmer Instruments, Shelton, CT).

**PK/PD Model and Data Analysis.** The cardiac distribution spaces of digoxin, i.e., the vascular, interstitial, and cellular, were represented by compartments. The model structure is shown in Fig. 1; the corresponding differential equations that describe changes in the amounts of digoxin in the mixing, capillary, interstitial, and cellular compartments as well as the two compartments representing the two saturable binding sites after infusion of digoxin (dosing = RATE) at the inflow side of the heart perfused at flow Q (single-pass mode) are given by eqs. 1 to 6. As suggested by independent experiments with the vascular marker Evans blue (data not shown), a lag time t₀ and an additional compartment with volume V₀ (in series with the capillary compartment) were introduced to account for the delayed drug appearance and mixing in nonexchanging elements of the system, respectively. [Initial estimates of the parameters t₀ and V₀ were obtained by fitting the outflow curve of Evans blue (1-min infusion) to a model consisting of only one additional compartment representing the vascular space.] The subscripts are “vas” for the vascular, “is” for the interstitial, and “cell” for the cellular compartments. Note that the measured outflow concentration Cout(t) = Dout(t)/Vout is the concentration in the vascular compartment.

\[
D_{\text{b}}(t)/dt = -(Q/V_{\text{b}})D_{\text{b}}(t) + \text{RATE}\tag{1}
\]

\[
D_{\text{vas}}(t)/dt = -(Q/V_{\text{vas}} + k_{\text{f}})D_{\text{vas}}(t) + k_{\text{o}}D_{\text{b}}(t) + (Q/V_{\text{b}})D_{\text{b}}(t)\tag{2}
\]

\[
D_{\text{is}}(t) = k_{\text{i}}D_{\text{vas}}(t) - [k_{\text{r}} + k_{\text{RD}_1}]D_{\text{is}}(t) + k_{\text{RD}_2}(R_{\text{tot},2} - RD_{\text{b}}(t))D_{\text{is}}(t) + k_{\text{RD}_1}RD_{\text{b}}(t) + k_{\text{RD}_2}RD_{\text{b}}(t)\tag{3}
\]

\[
RD_{\text{b}}(t)/dt = k_{\text{[Rtot,1]}}RD_{\text{b}}(t) - k_{\text{RD}_1}RD_{\text{b}}(t) + k_{\text{RD}_2}RD_{\text{b}}(t)\tag{4}
\]

\[
RD_{\text{is}}(t) = k_{\text{[Rtot,2]}}RD_{\text{is}}(t) - k_{\text{RD}_1}RD_{\text{is}}(t) + k_{\text{RD}_2}RD_{\text{is}}(t)\tag{5}
\]

\[
D_{\text{cell}}(t)/dt = k_{\text{RD}_1}RD_{\text{b}}(t) - k_{\text{RD}_2}RD_{\text{b}}(t)\tag{6}
\]

(Note that the lag time t₀ was part of the FORTRAN program used in ADAPT II.)

The volume of the vascular compartment, V_{\text{vas}} = 0.06 ml/g, is taken from anatomic data (Dobson and Cieslar, 1997). The rate constants, k_{\text{f}} and k_{\text{i}} describe passive transport across the capillary wall (apparent permeability surface area or permeation clearance, C_{\text{is,app}} = k_{\text{i}}V_{\text{vas}}) and sarcolemmal membrane, respectively. The binding probability of free digoxin in the interstitial space, D_{\text{is}} to two saturable binding sites (s = 1, 2) is dependent on the association rate constant.

**Fig. 1.** Kinetic model of cardiac digoxin uptake and inotropic response with vascular, interstitial, and cellular compartments and two distinct sarcolemmal binding sites, R₁ and R₂.
constants, \( k_i \) (in units of 1/min per nanomole), and concentrations of free membrane receptors, which are equal to \( R_i = |R_{i,\text{tot}}| - RD_i(t) \), where \( R_{i,\text{tot}} \) is the unknown amount of available receptor sites, and \( RD_i \) denotes the receptor-digoxin complexes (i.e., amount of bound digoxin). The rate constants for the dissociation of the bound ligand were denoted by \( k_{-i} \) (in units of 1/min); \( K_{D,i} = k_{-i}/k_i \), and \( K_{A,i} = 1/K_{D,i} \) represent the equilibrium dissociation and affinity constants, respectively, of the two receptor systems.

Integrated PK/PD modeling was performed, linking drug-receptor interaction with the positive inotropic effect of digoxin, \( E(t) \). The latter was defined as the increase in LVDP with respect to the vehicle response \( LVDP_{\text{veh}} \), i.e., the normalized difference \( \Delta LVDP = LVDP - LVDP_{\text{veh}} \):

\[
E(t) = \frac{LVDP(t) - LVDP_{\text{veh}}(t)}{LVDP_{\text{veh}}(t)}
\]

(7)

Assuming that the pharmacological response is proportional to the number of receptors occupied by drug (ligand-receptor complexes, \( RD_i \)), the time course of positive inotropic effect is given by:

\[
E(t) = e_1RD_1(t) + e_2RD_2(t)
\]

(8)

According to occupation theory, the sensitivity to inotropic stimulation is given by (Kenakin, 1993):

\[
e_i = \frac{E_{\text{max},i}}{R_{i,\text{tot}}(1 - p_i)}
\]

(9)

where \( E_{\text{max},i} \) and \( p_i \) represent the maximum response and the fraction of the spare receptor population, respectively, of the \( i \)th receptor system. Since \( p_i \) remains unknown, and the maximum response could not be measured (due to the increase in coronary vascular resistance at higher doses), the steady-state concentration-effect curve corresponding to eq. 8 cannot be predicted using our parameter estimates. However, for comparison with in vitro data, one should note the difference between digoxin steady-state concentrations in the perfusate, \( C_{\text{per}} \), and the interstitium (biophase), \( C_{\text{int}} \):

\[
C_{\text{per}} = C_{\text{int}} \frac{V_{\text{per}}}{V_{\text{int}}}
\]

(10)

On the theory that providing more data to the modeling process would best ensure the development of an appropriate model structure and the derivation of accurate and meaningful parameter estimates, we used as many as six data sets in the parameter estimation process: three outflow, \( C(t) \), and three response data, \( E(t) \), measured for three doses in each heart were simultaneously fitted by a single set of parameter values. This method, also known as global or simultaneous nonlinear regression, has recently become practical for fitting large sets of parameter values (Kakkar et al., 2000). The differential eqs. 1 to 6 were solved numerically and fitted to the data using the ADAPT II software package (D’Argenio and Schumitzky, 1997). Two approaches were applied to this analysis. First, fitting was performed with the maximum likelihood estimation, assuming that the measurement error has a standard deviation that is a linear function, of the measured quantity. The model development was an iterative process, and more than 40 model structures were tested until the best model was selected. Preference was given to those with low Akaike information criterion and those whose pattern of residuals better approximated a random scatter. However, not unexpectedly, because of the system nonlinearity, the dependence of parameter estimates from their initial values and the singularity of the Fisher information matrix pointed to the fact that the system was not uniquely identifiable on the basis of the measured data (Jacquez and Perry, 1990). Since sensitivity analysis indicated that the parameters of trans-sarcolemmal transport process \( (k_{\text{in}}, k_{\text{out}}) \) cannot be reliably estimated with the present experimental design, this process was omitted. To ensure model identifiability, we made use of a priori knowledge of the ratios of the receptor affinities \( K_{A,2}/K_{A,1} = 13.7 \) and capacities \( R_{\text{tot},1}/R_{\text{tot},2} = 8.7 \) of digoxin obtained from rat ventricle microsomal preparations (Noel and Godfraind, 1984). Since the Bayesian approach allows a theoretically sound incorporation of a priori information into the estimation process, we used the maximum a posteriori Bayesian estimator of the program ADAPT II, release 4 (D’Argenio and Schumitzky, 1997), setting the fractional dispersion of these ratios to 30%. Model selection was made according to the following criteria. Any model showing a noninvertible Fisher’s information matrix was discarded as nonidentifiable. The assessment of numerical identifiability was based on the fractional standard deviations (CV), which represent the uncertainty in parameter estimates resulting from the fit and correlation coefficients. Since the short delay \( t_0 \) and mixing volume \( V_0 \) needed to fit the initial appearance of outflow concentration (due to the perfusion system and large vessels) had little influence on the estimation of the other parameters, they were set to fixed values, \( t_0 = 0.036 \) min and \( V_0 = 0.30 \) ml, in the final Bayesian model identification. (Although the time scale of Fig. 4 does not show the effects of \( t_0 \) and \( V_0 \), both parameters were necessary to avoid systematic deviations of the model predictions in the infusion phase.)

The results are expressed as average ± S.D. of the parameters estimated in the 12 hearts. The paired \( t \) test was used to evaluate differences between the parameters \( \epsilon_1 \) and \( \epsilon_2 \), and the significance of changes in the time course of effects was tested by one-way repeated measurement analysis of variance. \( P \) values of less than 0.05 were considered statistically significant.

### Results

**Outflow Concentration.** The averaged outflow concentration-time curves after infusion of three doses of digoxin for 1 min are shown in Fig. 2. The curves reached a plateau within about 20 s, and the rapid decay upon cessation of infusion was followed by a slower terminal phase. The recoveries of digoxin in the outflow perfusate (up to 10 min) were 97.8 ± 2.4, 96.5 ± 4.4, and 96.6 ± 5.7% for doses of 0.1, 0.2, and 0.3 µmol, respectively.

**Cardiac Performance.** Figure 3 shows the average inotropic response-time profiles as a percentage of the difference to the vehicle effect corresponding to the outflow curves (Fig. 2). Treatment with digoxin resulted in an increase in LVDP at 1 min (i.e., end of infusion) to 9.6 ± 5.1, 16.2 ± 3.6, and 27.0 ± 3.4% of the vehicle level for doses of 0.1, 0.2, and 0.3 µmol, respectively.

**Fig. 2.** Outflow concentration-time profiles in rat hearts for a 1-min infusion of three doses (0.1, 0.2, and 0.3 µmol) of digoxin (average ± S.E.M.; \( n = 12 \)).
μmol, respectively, and recovered within about 10 min. Consistent with the increase in LVDP, the maximal rate of pressure development (dP/dt_max) increased to 9.3 ± 6.2, 15.4 ± 4.2, and 26.2 ± 4.2% of the vehicle level, respectively. These inotropic effects of digoxin were significant at the p < 0.05 level by one-way repeated measurement analyses of variance. The decrease in heart rate (−1.7 ± 0.3, −3.5 ± 2.0, and −4.2 ± 1.9%) was not significant, whereas coronary vascular resistance significantly increased with maximum values of 11.2 ± 4.1, 23.8 ± 4.2, and 44.0 ± 7.6% of the vehicle level, respectively, at the end of infusion. There was no change in left ventricular end-diastolic pressure. The corresponding response to vehicle infusion was as follows: LVDP and coronary vascular resistance were increased by 9.2 ± 3.7, 10.5 ± 4.3, and 15.7 ± 3.5%, and 9.2 ± 3.4, 13.2 ± 7.3, and 18.3 ± 5.6% at the end of infusion of 0.3, 0.6, and 0.9 ml of vehicle [mixture of DMSO (63.3 μmol/ml) and ethanol (73.3 μmol/ml)], respectively.

PK/PD Analysis. Figure 4 shows a representative set of outflow and response data (three consecutive doses in one heart) together with the lines obtained by a simultaneous model fit. It is apparent that the PK/PD model perfectly fitted the data; the PD predictions are concordant with the observed time course of positive inotropy. The model was conditional identifiable, and parameter estimates (mean ± S.D.; n = 12), obtained with ADAPT II, are reported in Table 1 together with the precision of the estimates. Cardiac kinetics of digoxin was characterized by transport across the capillary barrier and specific binding to two distinct extracellular sites, R₁ and R₂. Cellular uptake of digoxin was not detectable under the present experimental conditions.

The transcapillary permeation clearance of digoxin (CLvi = 2.35 ± 0.58 ml/min/g) was lower than perfusate flow (8.7 ml/min/g). In our model, the cellular uptake of digoxin was ignored, since it did not have any effect on the fitting of data. The distribution kinetics in interstitium was determined by binding to a low-affinity receptor with high capacity (R₁Kᵣ₁ = 20.9 ± 8.4 nmol) and a dissociation time constant, 1/kᵣ₁ = 1.80 ± 0.38 min, as well as binding to a high-affinity receptor with low capacity (R₂Kᵣ₂ = 1.5 ± 0.6 nmol) and a “fast” dissociation process (time constant, 1/kᵣ₂ = 0.10 ± 0.07 min). The time course of inotropic action of digoxin (∆LVDP) was successfully described as the weighted sum of drug-receptor complexes R₁D₁ and R₂D₂ (eq. 8), whereby the parameters e₁ and e₂ can be regarded as the respective effects per unit of digoxin-receptor complex. The high-affinity receptor R₂ is characterized by a higher (~2-fold greater) sensitivity of the stimulus-response mechanism, e₂ (p < 0.05). Based on the predicted digoxin receptor occupancy, R₁D₁ and R₂D₂, at the end of infusion, Fig. 5 illustrates that the positive inotropy is predominantly mediated by the high-affinity pumps (R₂); whereas their effect decreases with increasing dose, the contribution of the low-affinity pumps (R₁) increases. To test whether the response could be solely mediated by high-affinity receptor binding (R₂), we refitted the data, assuming that binding to R₁ would not contribute to E(t) (i.e., e₁ = 0 in eq. 8). The shape of the best fitted curve in Fig. 6 clearly demonstrates that the model then fails to describe the high-dose (0.3 μmol) response. A significant reduction of the generalized information criterion for the maximum a posteriori estimator (D’Argenio and Schumitzky, 1997) indicates that this result is also statistically supported by the current data.

Discussion

To quantify the PK and PD of digoxin in the present work, a mathematical model of transcapillary exchange, receptor interaction, and effectuation of digoxin in the intact rat heart was built. Although it was necessary to postulate two sarcolemmal binding sites to explain the PK data, the identification of the ratio of high-to low-affinity binding sites was only possible by simultaneous fitting of both PK and PD data. Note that in contrast to conventional PK/PD modeling, in which drug-receptor interaction does not influence PK (i.e., mass balance) (Breimer and Danhof, 1997; Mager and Jusko, 2001), specific binding was an important determinant of digoxin distribution kinetics in the rat heart. To obtain reliable parameter estimates, we utilized a priori information on digoxin receptor binding determined in vitro (Noel and Godfraind, 1984). Incorporating the ratios of binding parameters, Kₐ₂/Kₐ₁ and Rtot₁/Rtot₂, the results of Bayesian modeling were satisfactory both in terms of the capability of the model to describe the data (Fig. 1) and in terms of parameter estimation (Table 1). Only the rate constants of high-affinity binding (k₂ and k₋₂) are poorly estimated (CV >50%). (Note that a formal proof of identifiability is a complicated mathe-
from the values of 13 and 18% found for ouabain binding in microsomes from normal rat heart (Ver et al., 1997). Although our results are in accordance with the general view that the positive inotropic action of cardiac glycosides is primarily mediated by inhibition of the $\alpha_2$-isoform (McDonough et al., 1995), the PK and PD data could not be explained without the assumption of a low-affinity/high-capacity receptor ($\alpha_1$). For the maximum effect at the end of infusion, the dominating role of the $\alpha_2$-isoform ($R_2$) decreases with increasing dosage, whereas the contribution of the low-affinity system ($\alpha_1$) increases (Fig. 5). If the parameters $\epsilon_1$ and $\epsilon_2$ in eq. 8 are interpreted as effects per unit of digoxin-receptor complex, this effect-related sensitivity is significantly higher for the high-affinity receptor population ($R_2$). Our PD model (eq. 8) is analogous to the weighted sum of low- and high-affinity-mediated effects used by Gao et al. (1995) to describe Na$^+$/K$^+$-pump currents in cardiac ventricular myocytes. Interestingly, in these experiments, the onset of receptor blockade was also much more rapid than the offset. Thus, in agreement with the latter results and the recent discussion (Kometiani et al., 2001; Schwartz and Petrashevskaya, 2001), our findings do not support the hypothesis (James et al., 1999) that only the inhibition of the $\alpha_2$-isoform by cardiac glycosides would induce the positive inotropic effect. In view of the underlying discussion regarding the dose dependence of the specific role of these two isoforms (Schwartz and Petrashevskaya, 2001), we further tested the performance of an alternative model in which only one receptor, $R_2$, mediates the positive inotropic effect ($R_1$ remains part of the PK model). As shown in Fig. 6, such a model fails to describe the high-dose PD data (0.3 $\mu$mol), suggesting that the low-affinity/high-capacity isoform ($\alpha_1$) significantly contributes to the effect at higher dose levels ($p < 0.05$).

In view of the fact that we have obtained only indirect information on digoxin binding processes, the term “receptor” as used here is mainly based on the ability of the model to predict the time course of the inotropic effect. The proportionality between effect and receptor occupation (eq. 8) suggests that the time dependence of signal transduction (Mager and Jusko, 2001) was negligible. Finally, it is important to note that our model explains the PD of digoxin without postulating a cellular mechanism of action (Sagawa et al., 2002). Although such an effect cannot be excluded, a significant

### Table 1

<table>
<thead>
<tr>
<th>Process</th>
<th>Parameters</th>
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<tbody>
<tr>
<td>Transport</td>
<td>$k_{v1}$ (1/min)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>(4.4 ± 10.2)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>(14.4 ± 5.4)</td>
</tr>
<tr>
<td>Binding</td>
<td>$R_{tot,1}$ (nmol/g)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>(8.5 ± 12)</td>
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<tr>
<td>CV (%)</td>
<td>(19.9 ± 4.0)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>(20.9 ± 8.4)</td>
</tr>
</tbody>
</table>

$k_{v1}$ and $k_{v2}$, first-order rate constants of transcapillary transport; CL$_{vi}$, transcapillary clearance; $K_{D,1}$, $K_{A,1}$, $R_{tot,1}$, $k_1$, and $k_{v1}$, dissociation constants, affinity constants, maximal capacities, and association and dissociation rate constants of low- ($i = 1$) and high- ($i = 2$) affinity binding sites, respectively.

$^a$ Asymptotic coefficient of variation of parameter estimates (individual fits).

$^b$ $e_{Va}$, % Increase in LVDP per unit of drug-receptor complex.

### Figure 5

Contribution of high-affinity ($R_2$) and low-affinity ($R_1$) pumps to the maximal effect at the end of infusion, i.e., $e/R_D/E$ (percentage) (cf. eq. 8).
contribution appears unlikely in view of the negligible cellular digoxin uptake during the 1-min infusion period. It should be noted that the positive inotropic and vasoconstrictive effects of the vehicle are probably produced by DMSO (Shlafer et al., 1974), since ethanol does not influence cardiac function in this dose range (Kojima et al., 1993).

The new information provided by this study about cardiac uptake kinetics of digoxin is also of importance for a critical appraisal of the pharmacodynamic results discussed above. The myocardial uptake was barrier-limited due to the relatively low transcapillary permeation clearance of digoxin (compared with perfuse flow). If entry into the heart is by passive diffusion through interendothelial clefts, one would expect a $CL_{int}$ value $\sim 0.7$-fold less than that for sucrose (the square root of the ratio of molecular weights of sucrose and digoxin, 0.7, accounts for the different diffusion coefficients). Although our estimate appears concordant with a value of 5.1 ml/min measured for sucrose in rats (Caldwell et al., 1998), a bias due to model mis-specification (compartment approximation versus distributed model) has to be taken into account. However, the digoxin-to-sucrose ratio of capillary permeation clearances should be less biased when the clearances are estimated with the same model (i.e., assuming a well mixed vascular compartment). Applying a reduced model consisting only of a vascular and interstitial compartment to sucrose impulse data (bolus injection), a value of $2.90 \pm 0.37$ ml/min ($n = 5$) was obtained (W. Kang and M. Weiss, unpublished results). This suggests that transcapillary exchange of digoxin is primarily via diffusion through gaps between the endothelial cells. A steady-state ratio of interstitial-to-vascular concentration of 0.53 is predicted by eq. 10, assuming a volume ratio, $V_{vass}/V_{int}$ of 0.38 (Dobson and Cieslar, 1997). This concentration gradient has to be taken into account when comparing in vitro results with those obtained in the intact heart. No quantitative information could be extracted from the data on the trans-sarcolemmal transport process of digoxin. The nearly complete recovery of injected dose and the sensitivity analysis indicate that the cellular accumulation of digoxin in the 1-min infusion experiment was too small to be detectable with our method. Note that such a low uptake rate appears consistent with receptor-mediated endocytosis as a possible uptake mechanism (Núñez-Durán et al., 1988; Eisner and Smith, 1992).

The PK/PD model was selected according to the principle of parsimony as a minimal mechanistic model (Fig. 1), which is in accordance with the information content of the outflow and effect data. The mathematical model, although greatly simplified as a description of a complex process, offers a means to pose hypotheses concerning cardiac PK and PD of digoxin. Although a satisfactory fit to the experimental data is not proof of its correctness, the predictive power of the model is encouraging in view of the ability to accurately predict the time course of inotropic response from receptor occupancy and the principal consistency with previous results on receptor binding obtained in vitro. We suggest that theoretical models of this kind are valuable tools to bridge the gap between studies at the molecular level and the functioning of organ systems. Nevertheless, one must be cautious in its interpretation and extrapolation. The assumption of homogeneous compartments is a limitation of the model (e.g., it does not account for flow heterogeneities). However, the integration of a pharmacological effect excludes the use of a spatially distributed model.

In summary, the present PK/PD modeling approach provides information about the mechanism of drug action, which is unavailable from equilibrium studies. This methodology for the nondestructive measurement of membrane transport and receptor binding kinetics in intact hearts provides, for the first time, an integrated description of cardiac kinetics and dynamics of digitalis drugs. It is possible that a model such as this may resolve some of the controversy regarding the functional role of Na+/K+-ATPase isoforms. Passive transcapillary uptake followed by binding to distinct sarcolemmal receptor populations determines cardiac kinetics and, in accordance with the pump inhibition hypothesis, also the inotropic effect of digoxin.

References


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