British Journal of Pharmacology (2009), 156, 764–773

0007-1188/09
www.brjpharmacol.org

RESEARCH PAPER

Modelling of α_1-adrenoceptor-mediated temporal dynamics of inotropic response in rat heart to assess ligand binding and signal transduction parameters

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Background and purpose: In order to use the transient response to an antagonist (prazosin) to evaluate properties of agonist interactions with the α_1-adrenoceptor system, an integrative mechanistic model of cardiac uptake of prazosin and its competitive interaction with phenylephrine at the receptor site was developed. Based on the operational model of agonism, the aim was to evaluate both the receptor binding and signal transduction process as determinants of the inotropic effect of phenylephrine.
Experimental approach: In Langendorff-perfused rat hearts, prazosin outflow concentration and left ventricular developed pressure were measured, first in the presence of 12.3 $\mu mol \cdot L^{-1}$ phenylephrine following a 1 min infusion of 1.27 nmol [^{3}H]-prazosin, and second, when after 30 min the phenylephrine concentration in perfusate was reduced to 6.1 $\mu mol \cdot L^{-1}$, the 1 min infusion of 1.27 nmol [^{3}H]-prazosin was repeated.
Key results: The kinetic model accounted for cardiac uptake and receptor binding kinetics of prazosin (dissociation constant, mean ± SD: 0.057 ± 0.012 $nmol \cdot L^{-1}$), assuming that the competitive displacement of phenylephrine (dissociation constant: 101 ± 13 $nmol \cdot L^{-1}$) reduced the receptor occupation by the agonist and, consequently, contractility. This competitive binding process appeared to be the rate-determining step in response generation. The relationship between receptor occupancy and inotropic response was described by an efficacy parameter (τ, ratio of receptor density and coupling efficiency) of 4.9.
Conclusions and implications: Mechanistic pharmacodynamic modelling of the kinetics of antagonism by prazosin allows quantitative assessment of the α_1-adrenoceptor system both at the receptor and post-receptor levels.
British Journal of Pharmacology (2009) **156**, 764–773; doi:10.1111/j.1476-5381.2008.00013.x; published online 13 February 2009

Keywords: phenylephrine; prazosin; perfused rat heart; inotropy; α_1-adrenoceptors; drug-receptor interaction; operational model; transducer function

Abbreviations: A, agonist; AR, agonist-receptor complex; B, antagonist; BR, antagonist-receptor complex; C, concentration; CVR, coronary vascular resistance; E, inotropic effect; HR, heart rate; K_A and K_B, equilibrium dissociation constants of agonist and antagonist, respectively; K_E, transducer constant; LVDP, left ventricular developed pressure

Introduction

α_1-Adrenoceptors are essential for a normal cardiac contraction (McCloskey *et al.*, 2003). Acute stimulation of α_1-adrenoceptors leads to a positive inotropic effect in most mammalian species, including rats and humans, and this α_1-adrenoceptor-mediated response increases in the failing heart when β-adrenoceptors are down-regulated (Sjaastad *et al.*, 2003). A recent review has summarized the role for α_1-signalling in myocardial preconditioning and cardiac adaptation (Woodcock *et al.*, 2008).

Interactions between cardiac α_1-adrenoceptors and agonists (or antagonists) have traditionally been studied using pharmacological steady-state methods such as dose–response curves and receptor binding studies, methods that do not allow a direct evaluation of receptor kinetics and the stimulus–response relationship that characterizes the signal transduction process. Data measured in transient kinetic studies, however, contain more mechanistic information. Furthermore, due to receptor desensitization, parameter estimates based on measurements at equilibrium conditions may be different from those of transient response experiments. Potential shortcomings of equilibrium models based on

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Received 16 July 2008; revised 14 August 2008; accepted 21 August 2008

consecutive cumulative dosing experiments have already been pointed out (Christopoulos *et al.*, 1999; Corsi and Kenakin, 2000; Shea *et al.*, 2000; Vauquelin *et al.*, 2002). The usefulness of studying the temporal dynamics of inotropic response to adrenoceptor agonists has been shown recently by McConville *et al.* (2005) using perfused rat hearts.

For a better understanding of the functional role of α_1-adrenoceptors, it appears important to get further insights into the relationship between ligand–receptor binding kinetics, cellular signalling and transient inotropic response in the intact heart. Mathematical models provide the basis for such an integrated dynamic approach (Kenakin, 1997; Woolf and Linderman, 2001; Weiss *et al.*, 2004; Danhof *et al.*, 2007). Through an analysis of transient response data, it becomes possible to estimate the ligand binding rate constants and thus the receptor affinity separately from the parameters of the stimulus–response relationship (Weiss *et al.*, 2004; Yassen *et al.*, 2005). In the Langendorff-perfused heart, model identification is facilitated when the effect of receptor binding can be detected in the outflow concentration of ligand. This cannot be expected to hold for the α_1-adrenoceptor agonist phenylephrine due to the dominating influence of uptake into vesicles of sympathetic nerve terminals (Raffel and Wieland, 1999). As the high degree of specific binding of the α_1-adrenoceptor antagonist prazosin appears promising (Edwards *et al.*, 1988; 1989), we thought it worth attempting to develop a method that uses the measurement of prazosin outflow concentration–time profile after short-term injection of prazosin in the presence of phenylephrine together with the time course of prazosin-induced reduction of inotropy. The approach is based on modelling the kinetics of the competition of receptor binding between prazosin and phenylephrine, given the fact that phenylephrine and prazosin are a selective agonist and antagonist, respectively, for α_1-adrenoceptors relative to α_2-adrenoceptors (Alexander *et al.*, 2008). The overall method of combining the kinetics of cardiac uptake and saturable ligand–receptor binding with response dynamics is similar to that used for digoxin (Weiss *et al.*, 2004).

To this end, we developed, first, a mathematical model that describes transcapillary transport of prazosin, its competitive receptor binding leading to a transient decrease in α_1-adrenoceptor occupation by phenylephrine and the induced inotropic response dynamics and, second, an experimental design that enables the estimation of binding rate parameters of prazosin and phenylephrine together with α_1-adrenoceptor concentration.

Together with the estimation of receptor concentration and ligand binding rate constants, this approach allows, for the first time, a quantification of the stimulus–response relationship as a drug-independent property of the cardiac α_1-adrenoceptor signalling system.

Methods

Perfused rat heart

This investigation conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996). Prior approval was obtained from the Animal Protection Body of the State of Sachsen-Anhalt, Germany. Assessment of left ventricular function was performed utilizing a Langendorff isovolumic heart preparation as described previously (Weiss and Kang, 2002). Briefly, male Wistar rats were heparinized (heparin, 500 IU) and anaesthetized (pentobarbital, 60 $mg \cdot kg^{-1}$) by intraperitoneal injection. Hearts were retrogradely perfused with a Krebs–Henseleit buffer containing NaCl (118 $mmol \cdot L^{-1}$), KCl (4.7 $mmol \cdot L^{-1}$), $CaCl_2$ (1.5 $mmol \cdot L^{-1}$), $MgSO_4$ (1.66 $mmol \cdot L^{-1}$), $NaHCO_3$ (24.88 $mmol \cdot L^{-1}$), KH_2PO_4 (1.18 $mmol \cdot L^{-1}$), glucose (5.55 $mmol \cdot L^{-1}$), Na-pyruvate (2 $mmol \cdot L^{-1}$) and bovine albumin (0.1% w/v) and equilibrated with 95% O_2 and 5% CO_2 at 37°C. After stabilization, the system was changed to constant flow condition, maintaining a coronary flow of 9.7 ± 0.5 $mL \cdot min^{-1}$. Coronary perfusion pressure, left ventricular pressure and heart rate (HR) were measured continuously and a recording system (Hugo Sachs Elektronik, March-Hugstetten, Germany) was used to monitor left ventricular systolic pressure (LVSP) and left ventricular enddiastolic pressure (LVEDP). Left ventricular developed pressure (LVDP) is defined as LVDP = LVSP – LVEDP. Coronary vascular resistance (CVR) is calculated from perfusion pressure divided by coronary flow.

Experimental protocol

Transient kinetics Temporal dynamics of inotropic response and outflow concentration of prazosin was studied in five hearts. After a 20 min equilibration period, at time 0, perfusion was switched to a solution containing 12.3 $\mu mol \cdot L^{-1}$ phenylephrine. After 15 min, a 1.27 nmol dose of [^{3}H]-prazosin was administered as 1 min infusion. Infusion was performed into the perfusion tube close to the aortic cannula with the use of an infusion device. Outflow samples were collected every 5 s for 1.5 min, every 10 s for next 1.5 min and every 30 s for next 7 min, and the cardiac response was measured. At time 30 min, perfusion was switched to a solution containing 6.1 $\mu mol \cdot L^{-1}$ phenylephrine and 15 min after the beginning of this lower concentration of phenylephrine, a second 1.27 nmol dose of labelled prazosin was administered. The perfusate samples were collected as after the first dose. The outflow samples were kept frozen at –20°C until analysis (within 3 days). For determination of [^{3}H]-PRZ, the outflow sample (200 µL) was transferred to a vial and 2 ml of cocktail was added. After being vigorously mixed, the radioactivity was measured with a liquid scintillation counter (Perkin Elmer Instruments; Shelton, CT).

Equilibrium concentration–response curve In order to obtain an appropriate initial estimate for the phenylephrine parameters EC_{50}, ΔE_{max} and the dissociation constant K_B of prazosin, in six perfused hearts a stepwise cumulative infusion of prazosin with 15 min time interval was conducted in the presence of 12.3 $\mu mol \cdot L^{-1}$ phenylephrine in perfusate. The input concentrations of prazosin were 0.1, 1, 10, 100 and 1000 $nmol \cdot L^{-1}$. The drug effect, LVDP, was measured at the end of each infusion time interval.

Modelling

The pharmacokinetic/pharmacodynamic (PK/PD) model of prazosin in the presence of phenylephrine is shown in Figure 1.

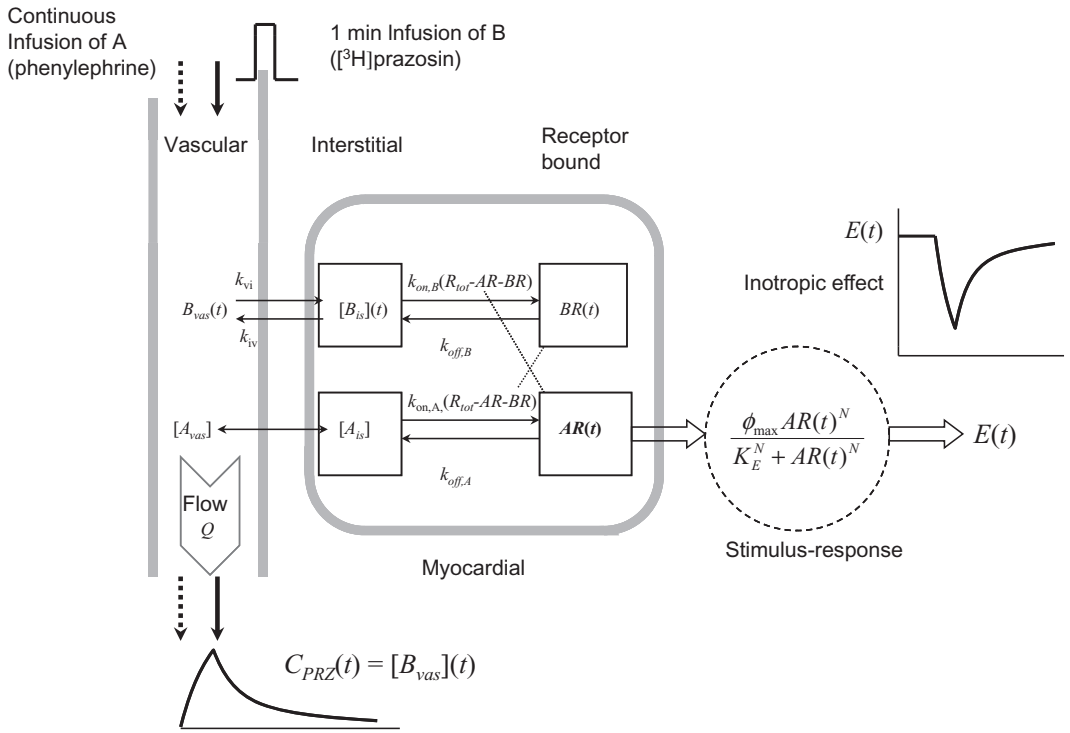

Figure 1 Integrated model of cardiac kinetics of prazosin (*B*) and the competition of prazosin and the agonist phenylephrine (*A*) for the same receptor (*R*) (α_1-adrenoceptor) used in analysis of [^{3}H]-prazosin outflow data and prazosin-induced decrease in inotropic response. *B* is transported from the vascular to the interstitial compartment (k_{vi}) and vice versa (k_{iv}). The vascular and interstitial concentrations of *A* (denoted by [*A*]) are assumed to be in equilibrium at the time of injection of *B*. The fractional rates of saturable receptor binding are $k_{on,B}(R_{tot}$-AR-BR) and $k_{on,A}(R_{tot}$-AR-BR) for *B* and *A* respectively, where R_{tot} is the total number (amount) of α_1-adrenoceptors. The association and dissociation rate constants are denoted by k_{on} and k_{off} respectively. The competitor *B* leads to a reduction in receptor occupation AR(*t*), which is linked via a non-linear stimulus–response relationship to the reduction in the inotropic effect induced by *A*. The measured outflow concentration $C_{PRZ}(t)$ is the vascular concentration $[B_{vas}] = B_{vas}(t)/V_{vas}$ and $E(t)$ is the observed change in left ventricular developed pressure. Dotted lines indicate the competition in receptor binding.

Cardiac uptake and receptor binding kinetics

The competitive interaction between agonist phenylephrine (*A*), antagonist prazosin (*B*) and receptor (*R*) can be written as

$$\mathrm{BR} + A \underset{k_{on,B}}{\overset{k_{off,B}}{\rightleftarrows}} B + R + A \underset{k_{on,A}}{\overset{k_{off,A}}{\rightleftarrows}} B + \mathrm{AR} \rightarrow \mathrm{Response} \quad (1)$$

where k_{on} and k_{off} are the association and dissociation rate constants respectively. In the present experimental design, the agonist is infused at constant rate and it is assumed that binding AR is at steady state when the antagonist is injected at time t_1:

$$\mathrm{AR}_{ss} = \frac{R_{tot}[A]}{[A] + K_A}, \quad \mathrm{BR}(t_1) = 0 \quad (2)$$

where [*A*] denotes the agonist concentration, R_{tot} is the amount of receptor sites available for binding,

$$R_{tot} = \mathrm{AR} + \mathrm{BR} + R \quad (3)$$

and K_A the equilibrium dissociation constant (ratio of the dissociation rate constant $k_{off,A}$ to the association rate constant $k_{on,A}$).

As α_1-adrenoceptors are located at the cell surface, *A* and *B* are the drug amounts in the interstitial space, and we need a kinetic model that describes transport of the ligands from the perfusate to this free ligand compartment (transcapillary uptake) to predict the time course of receptor binding after drug infusion in the single-pass perfused heart. For phenylephrine we have a steady-state situation, that is, the free interstitial concentration $[A_{is}]$ is identical to the free vascular concentration $[A_{vas}]$. A compartmental model was used to describe the changes in the amounts of prazosin in the capillary and interstitial space (Figure 1). Perfusate flow (*Q*) (including drug) enters the vascular space (distribution volume V_{vas}) where transcapillary transport of the unbound drug between vascular and interstitial space is described by rate constants k_{vi} and k_{iv} respectively, and the apparent permeability surface area or permeation clearance $CL_{vi} = k_{vi}V_{vas}$ is determined by k_{vi} and V_{vas}. Assuming passive transport processes, we have $k_{vi}V_{vas} = k_{iv}V_{app,is}$ where $V_{app,is}$ denotes the apparent volume that governs initial distribution of prazosin in the interstitial space; that is, exceeding the distribution space V_{is} due to quasi-instantaneous non-specific tissue binding. The free concentration in the interstitial space that governs receptor binding is then given by $[B_{is}](t) = B_{is}(t)/V_{app,is}$, with $V_{app,is} = (k_{vi}/k_{iv})V_{vas}$. The time-dependent fractional binding rates of prazosin and phenylephrine to free membrane receptors are given by $k_{on,B}[R_{tot} - \mathrm{AR}(t) - \mathrm{BR}(t)]$ and $k_{on,A}[R_{tot} - \mathrm{AR}(t) - \mathrm{BR}(t)]$ respectively, where AR(*t*) and BR(*t*) are the amounts of phenylephrine and PRZ respectively, bound at time *t* to receptor *R* (receptor occupancy). The phenylephrine concentration, $[A_{is}](t)$, is constant before and after the stepwise reduction from 12.3 to 6.1 μmol·L^{-1} at *t* = 30 min. As a receptor occupa-

tion by endogenous ligands cannot be excluded, the only non-zero initial condition in equations (A1) to (A5) is the receptor occupation before phenylephrine infusion ($t = 0$):

$$\mathrm{AR}(0) = \mathrm{AR}_0 \tag{4}$$

By solving the differential equations corresponding to the model in Figure 1 [equations (A1)–(A5) in the Appendix], one obtains the time course of prazosin outflow concentration, $C_{PRZ}(t) = [B_{vas}](t) = B_{vas}(t)/V_{vas}$, and the receptor occupation by the agonist PE, AR(t).

Cellular effects and concentration–response relationship

The relationship between agonist receptor occupation AR(t) and the positive inotropic effect is described by the stimulus–response relationship $\Delta E(t) = \phi[\mathrm{AR}(t)]$, where ϕ refers to the chain of cellular processes that convert the stimulus into response (Kenakin, 1997). According to the operational model of receptor agonism (Black and Leff, 1983; Kenakin, 1997; 2004), we use a hyperbolic function for the transduction of receptor occupancy into response ϕ

$$E(t) = E_{base} + \frac{\phi_{max}\,\mathrm{AR}(t)^N}{K_E^N + \mathrm{AR}(t)^N} \tag{5}$$

where E_{base} is the baseline effect when AR = 0, ϕ_{max} is the maximum achievable effect, the transducer constant K_E denotes AR producing 50% of ϕ_{max}, and N is the Hill coefficient that determines the sigmoidicity of the curve. With decreasing K_E, the response efficiency increases. The LVDP(t) data were used as a measure of inotropic effect, $E(t) = \mathrm{LVDP}(t)$. Note that the inotropic effect before phenylephrine infusion results of the (unknown) inotropy without receptor stimulation, E_{base} and a possible effect of an endogenous ligand, $E_0 = E_{base} + \phi[\mathrm{AR}_0]$ and the temporal displacement of agonist from the receptor [decrease in AR(t)] by the competitor prazosin lead to a transient decrease in LVDP(t).

Equilibrium concentration–response curve

The concentration–response curve at steady state is obtained by substituting AR = AR_{ss}, as given by equation (2), into equation (5) using $N = 1$ and introducing $\tau = R_{tot}/K_E$:

$$E_{ss} = E_{base} + \frac{\phi_{max}\,\tau[A]}{K_A + [A](1+\tau)} \tag{6}$$

This is the basic equation of the operational model of drug action (Kenakin, 2004). In order to obtain the change in the positive inotropic effect (ΔE_{PE}) induced by cumulative infusion of phenylephrine, the agonist concentration [A] must be substituted by $[A] = [A_0] + C_{PE}$, where $[A_0]$ and C_{PE} are the concentrations of the endogenous agonist (leading to AR_0) and phenylephrine respectively:

$$\Delta E_{PE} = \frac{\phi_{max}\,R_{tot}}{K_E + R_{tot}}\left(\frac{[A_0] + C_{PE}}{EC_{50} + C_{PE}} - \frac{[A_0]}{EC_{50}}\right) \tag{7}$$

where

$$EC_{50,PE} = K_A\left(\frac{K_E}{K_E + R_{tot}} + \frac{1}{R_{tot}/\mathrm{AR}_0 - 1}\right) \tag{8}$$

is the concentration C_{PE} that corresponds to 50% of $\Delta E_{PE,max}$ (see equation below) and

$$[A]_0 = \frac{\mathrm{AR}_0 K_A}{R_{tot} - \mathrm{AR}_0} \tag{9}$$

The maximal effect induced by phenylephrine is then given by

$$\Delta E_{max,PE} = \frac{\phi_{max}\,R_{tot}}{K_E + R_{tot}}\left(1 - \frac{(R_{tot}/\mathrm{AR}_0 - 1)K_E}{K_E + R_{tot}}\right) \tag{10}$$

The steady-state response curves obtained in separate experiments by cumulative infusion of phenylephrine (concentration C_{PE}), in the presence of a competitive reversible antagonist (increasing concentrations C_{PRZ} of prazosin), were analysed by (e.g. Kenakin, 1997)

$$\Delta E_{PE}^{PRZ} = \frac{\Delta E_{max,PE} C_{PE}}{EC_{50}\,(1 + C_{PRZ}/K_B) + C_{PE}} \tag{11}$$

where the subscript PRZ denotes the presence of prazosin in concentration C_{PRZ}.

Data analysis

Equations (A1) to (A5) were solved numerically and prazosin outflow concentration and inotropic response [equations (A6) and (A7)] were fitted simultaneously to the data measured in all five hearts using population analysis. The latter was performed with maximum likelihood estimation via the EM algorithm (Schumitzky, 1995; Walker, 1996) using the software ADAPT 5 (D'Argenio and Schumitzky, 2008) where the program (MLEM) is implemented. The program provides estimates of the population mean and inter-subject variability as well as of the individual subject parameters (conditional means). Prazosin outflow concentration and inotropic response data LVDP(t), measured in the presence of 12.3 and 6.1 $\mu mol \cdot L^{-1}$ phenylephrine respectively, were simultaneously fitted by the model functions, $C_{PRZ}(t)$ and $E(t)$, to estimate the parameters V_{vas}, k_{vi}, k_{iv}, K_B, $k_{off,B}$, K_A, $k_{off,A}$ R_{tot}, K_E, ϕ_{max}, N and AR_0 (using the reparameterization $k_{on,A} = k_{off,A}/K_A$ and $k_{on,B} = k_{off,B}/K_B$). In fitting the responses to the double-dose regimen (two consecutive prazosin doses), the phenylephrine concentration, C_{PE} (t), was used as a model input. We assumed normally distributed model parameters and that the measurement error has a standard deviation that is a linear function of the measured quantity. Initial values for the population means in the MLEM analysis were obtained from the literature and previous experiments, while initial values for parameter inter-subject variability were set at 60% of their mean values. The initial estimate for V_{vas} was 0.06 $mL \cdot g^{-1}$ (Dobson and Cieslar, 1997). The estimates of $EC_{50,PE}$, $\Delta E_{max,PE}$ and K_B obtained from equilibrium inhibition curves in independent experiments were used to determine initial values. The Hill coefficient N [equation (5)] was fixed to 1, as the estimates of N were not significantly different from unity. 'Goodness of fit' was assessed using the Akaike Information Criterion and by plotting the predicted versus the measured responses. The estimated population means and variances of the parameters K_A, K_E, R_{tot}, AR(0) and ϕ_{max} were used to perform a population simulation with the simulation

module of ADAPT in order to predict the steady-state concentration–response curve for phenylephrine [equations (7) to (9)].

The recovery of prazosin was calculated from outflow concentration versus time data using a numerical integration method as the amount recovered at 10 min after prazosin infusion.

MLEM was also used to analyse the steady-state inhibition curves, that is, the reduction of response to 12.3 $\mu mol \cdot L^{-1}$ phenylephrine after stepwise cumulative infusion of prazosin in six hearts. These data were fitted by equation (11) for $[A] = 12.3\ \mu mol \cdot L^{-1}$ as a function of prazosin concentration $[B]$.

Chemicals

Prazosin hydrochloride and phenylephrine hydrochloride were obtained from Sigma-Aldrich-Chemie (Steinheim, Germany), [7-methoxy-^{3}H]-prazosin (85 $Ci \cdot mmol^{-1}$) from PerkinElmer (Boston, USA), and dimethyl sulphoxide (DMSO) from Carl Roth (Karlsruhe, Germany). All other chemicals and solvents were of highest grade available.

A concentrated prazosin stock solution was prepared by dissolving 2 mg prazosin in 1 mL DMSO. Then, a 1.4 $\mu mol \cdot L^{-1}$ prazosin solution was prepared by adding 15 µL of the stock solution to the perfusate buffer, and adjusting final volume to 50 mL. Finally, 3 mL of 1.4 $\mu mol \cdot L^{-1}$ prazosin were mixed with 5 µL of [7-methoxy-^{3}H]-prazosin. This labelled prazosin (1.4 $\mu mol \cdot L^{-1}$) was later infused into the heart. In order to prepare 12.3 and 6.1 $\mu mol \cdot L^{-1}$ phenylephrine in the perfusate buffer, 12 $mg \cdot mL^{-1}$ phenylephrine in water was prepared, and then 200 and 100 µL of the solution were added to 1 L of the perfusate buffer.

Results

Model independent results

The baseline values of LVDP, HR and CVR observed before starting perfusion with the phenylephrine-containing solution were LVDP = 96.3 ± 16.4 mm·Hg, HR = 256 ± 18 min^{-1} and CVR = 4.42 ± 1.25 $mm \cdot Hg^{-1} \cdot min \cdot mL^{-1}$ respectively. The inotropic response to phenylephrine infusion was triphasic, an initially positive inotropic effect was followed by a transiently negative effect (not shown), before finally a sustained positive response was reached (only this final response is shown in Figure 2). The 15 min infusion of 12.3 $\mu mol \cdot L^{-1}$ phenylephrine increased LVDP by 22.3 ± 9.5% ($P < 0.01$); the reduction of phenylephrine concentration to 6.1 $\mu mol \cdot L^{-1}$ did not change this value significantly. However, the reduction in agonist (phenylephrine) concentration increased the transient negative inotropic response to the antagonist prazosin, with maximum values of 14.1 ± 1.1% versus 22.6 ± 2.1%, for the first and second doses respectively ($P < 0.001$). No significant changes in HR and CVR were observed after 15 min phenylephrine infusion. While no changes in HR was observed following the prazosin doses, CVR significantly decreased by 11.0 ± 3.7% and 6.6 ± 4.6% for the first and second doses respectively ($P < 0.05$). Recoveries of prazosin up to 10 min were 100.5 ± 3.5% and 104.0 ± 4.7%, respectively, for the two doses.

Modelling results

We developed a kinetic model describing the time course of prazosin outflow, $C_{PRZ}(t)$, and prazosin-induced reduction of the phenylephrine-mediated inotropic effect, LVDP(t). The results of the simultaneous fitting of inotropic response and prazosin outflow data are depicted in Figure 2 for all five hearts. In general, a good agreement between the model and experiments was obtained. Specifically, the model accurately predicted the increase in the negative inotropic response to the second prazosin dose due to the halving of phenylephrine concentration. The parameter estimates are presented in Table 1. The two injection protocols provided conditional estimates of individual parameters with low approximate coefficients of variation (<5%). The meaning of the parameter estimates characterizing the inotropic effect of phenylephrine is illustrated by the hypothetical steady-state concentration–response curve as predicted by population simulation (Figure 3).

The result of the equilibrium inhibition experiments (individual data and predicted mean population profile) are shown in Figure 4. The MLEM analysis led to the following estimates (mean and inter-individual variability in parentheses): $EC_{50,PE} = 29.1\ nmol \cdot L^{-1}$ (3%), $\Delta E_{max,PE} = 39.1$ mm Hg (28%) and $K_B = 0.011\ nmol \cdot L^{-1}$ (54%). These estimates served as initial values for fitting the transient data.

Discussion

Our goal was to determine whether a mathematical model of transport, receptor binding and signal transduction can quantitatively explain α_1-adrenoceptor-mediated inotropic response dynamics in the perfused rat heart. By using the transient response to an antagonist (prazosin) to evaluate properties of agonist interactions with the α_1-adrenoceptor system, it was possible to estimate the system parameters functionally. The mathematical model provided adequate fits for inotropic response and outflow data of [^{3}H]-prazosin and allowed an estimation of the binding rate parameters of prazosin and the unlabelled agonist phenylephrine. In other words, suggesting a direct relationship between the time courses of agonist binding and response, the results showed that also under transient conditions the operational model of drug action (Black and Leff, 1983) is in accordance with experimental data. The temporal dynamics of the α_1-adrenoceptor antagonist effect (decrease in LVDP) was determined by receptor binding kinetics; that is, signal transduction is not the rate-limiting step in response generation. As it is generally difficult to reliably estimate parameters of complex non-linear models using data from individual experiments, it was important to use population analysis (MLEM estimation) that models both inter-study variability and within-study variability. To increase the information content available for parameter estimation, the response was measured at two different levels of phenylephrine.

The transcapillary uptake clearance of prazosin (CL_{vi} = 18 $mL \cdot min^{-1}$) was not much different from that reported for sucrose or digoxin (Weiss *et al.*, 2004). The cardiac distribution kinetics of prazosin was mainly determined by specific binding to α_1-adrenoceptors with complete recovery in the

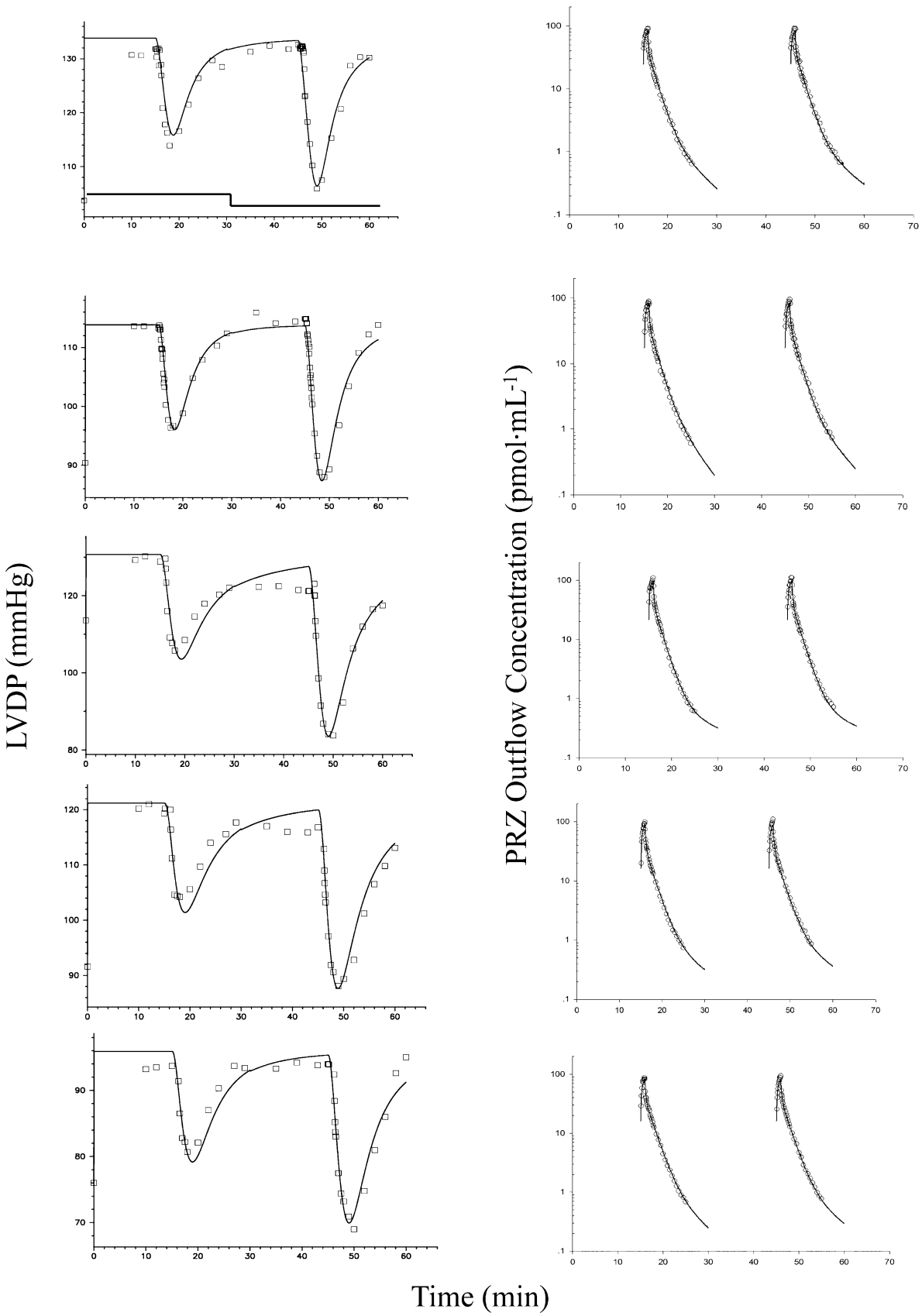

Figure 2 Fits of time course of negative inotropic response (left) and prazosin outflow concentration (right) following two consecutive 1.27 nmol doses of prazosin in the presence of 12.3 μmol·L^{-1} (first prazosin dose) and 6.1 μmol·L^{-1} phenylephrine (second prazosin dose), respectively, of the five hearts using conditional estimation. The step function in the upper right graph indicates the change of the phenylephrine infusion from 12.3 to 6.1 μmol·L^{-1} at time t = 30 min. LVDP, left ventricular developed pressure; PRZ, prazosin.

Table 1 Parameters from curve-fitting of prazosin outflow concentration and inotropic response data following two consecutive 1.27 nmol doses of prazosin in the presence of 12.3 and 6.1 μmol·L^{-1} phenylephrine, respectively, using MLEM (n = 5)

Parameters	*Average value*	*Inter-individual variability (%)*
Distribution		
V_{vas} (mL)	0.064	10
k_{vi} (min^{-1})	200.1	4
k_{iv} (min^{-1})	1.29	7
Binding		
K_B (nmol·L^{-1})	0.057	21
$k_{off,B}$ (min^{-1})	0.193	42
R_{tot} (pmol)	68.2	12
K_A (nmol·L^{-1})	101	13
$k_{off,A}$ (min^{-1})	1.001	22
Response		
ϕ_{max} (mm·Hg)	98.6	12
K_E (pmol)	13.9	22
E_{base} (mm·Hg)	37.0	21
AR(0) (pmol)	19.5	17
$\Delta E_{max,PE}$ (mm·Hg)[a]	24.3	23
$EC_{50,PE}$ (nmol·L^{-1})[a]	57.7	27

[a]Derived parameters [equations (8) and (9)].

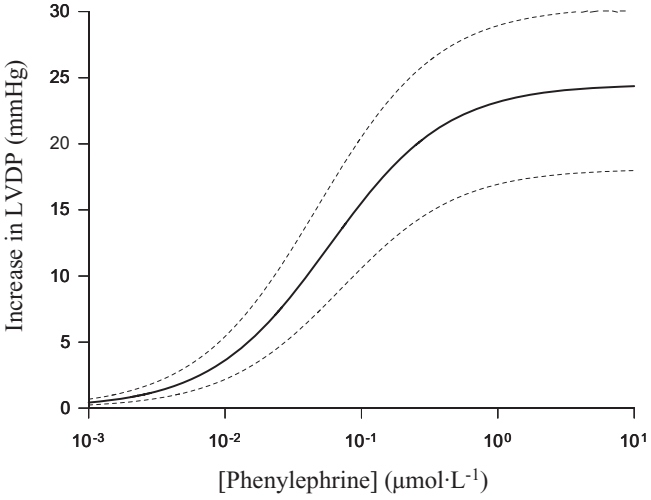

Figure 3 Simulated steady-state concentration–response curve (mean ± SD) of phenylephrine to increase inotropy in rat heart [equation (7)]. The population simulation was based on the means and variances of model parameters estimated in the transient state. LVDP, left ventricular developed pressure.

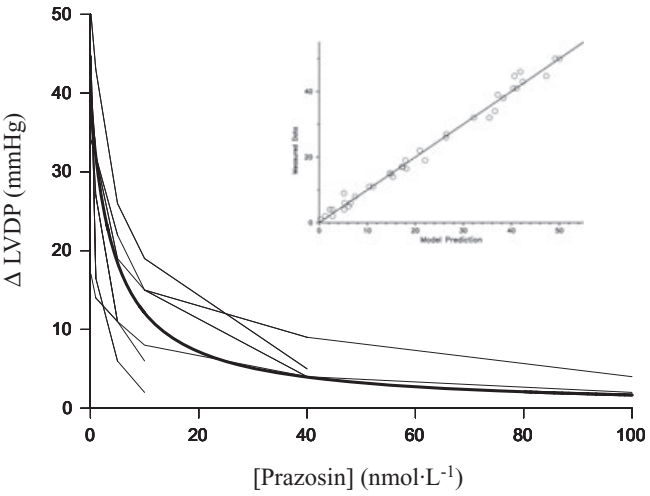

Figure 4 Effect of increasing concentrations of prazosin on the inotropic response (ΔLVDP) to 12.3 μmol·L^{-1} phenylephrine (steady-state inhibition curve). Fine lines indicate individual data and the solid line is the population prediction. The inset shows data versus model prediction up to 50 mm Hg. LVDP, left ventricular developed pressure.

outflow perfusate. The dissociation constant of prazosin (K_B = 0.057 nmol·L^{-1}) is in accordance with the range (0.07–0.15 nmol·L^{-1}) measured by radioligand binding in rat ventricular membrane preparations (Leon-Velarde *et al.*, 2001, Zhang *et al.*, 2002; Jalali *et al.*, 2006). Also, the dissociation time constant for prazosin ($k_{off,B}$) of 0.2 min^{-1} was similar to that of 0.1, 0.08 and 0.04 min^{-1} observed by radioligand binding in rat myocytes (Skomedal *et al.*, 1984), arterial tissue (Tanaka *et al.*, 2004) and perfused rat heart (Edwards *et al.*, 1988) respectively. A somewhat lower estimate of K_B (0.01 nmol·L^{-1}) was obtained from the equilibrium inhibition curves. Assuming that the weight of the left ventricle amounts to 80% of the heart weight, the estimated amount of functionally active α_1-receptor (R_{tot}) of 68.2 pmol corresponds to a value of about 52 pmol·g^{-1} wet weight. This estimate is comparable with the values of 13.2 pmol·g^{-1} wet weight measured by equilibrium radioligand binding assay in perfused rat heart (Edwards *et al.*, 1988) and of 12.2 pmol·g^{-1} wet weight measured by Positron Emission Tomography (Law *et al.*, 2000). That much lower values were reported in the literature based on radioligand binding studies might be explained by a substantial loss of receptor due to a low yield of receptor-bearing membranes after homogenization and fractionation (Edwards *et al.*, 1988; Tanaka *et al.*, 2004). Note that receptor binding sites in non-myocytes represent only a small portion relative to the binding by ventricular myocytes (Skomedal *et al.*, 1984; Law *et al.*, 2000).

When the $EC_{50,PE}$ (57.7 nmol·L^{-1}) and ΔE_{max} (24.3 mm·Hg) of the positive inotropic effect of phenylephrine, predicted by our model analysis of the transient negative inotropic effect of prazosin, is compared with those of 29.1 nmol·L^{-1} and 39.1 mm·Hg obtained by the steady-state inhibition experiment, one has to take into account that in the latter AR_0 = 0 was assumed [equation (11)] and that equilibrium experiments have some other shortcomings (Christopoulos *et al.*, 1999). Note that with AR_0 = 0 in equation (8), we would obtain a similar $EC_{50,PE}$ value (23.8 nmol·L^{-1}). An EC_{50} of 8.3 nmol·L^{-1} was reported by Silva *et al.* (2001) in the perfused rat heart using cumulative infusion of phenylephrine (these authors observed an increase to 63 nmol·L^{-1} in the presence of propranolol). The apparent dissociation constant K_A of phenylephrine and the K_E of α_1-adrenoceptors cannot be estimated directly from single equilibrium concentration–response curves [equation (6)]. The present kinetic modelling approach, in contrast, allows discrimination between the effects of receptor occupation and signal transduction, that is, a separate determination of dissociation constant K_A and the transducer parameter K_E. Note that $1/K_E$ acts as a measure of the efficiency with which the system transduces receptor occupancy into inotropic effect. To our knowledge, these are

the first estimates of K_A and K_E for the cardiac α_1-adrenoceptor system and therefore no published values are available for comparison. For the measure of the efficiency of transduction of receptor occupancy into inotropic effect, $\tau = R_{tot}/K_E$, the value 4.9 is obtained; that is, 20% of the receptors need to be occupied to achieve half-maximal response. Under steady-state conditions, one can estimate K_A and τ in equation (6) by using a multiple concentration–response curve design (Leff *et al.*, 1990). Estimation of K_B can be based on steady-state inhibition curves [equation (11)]. The latter has been recently used in PK/PD modelling of HR response to atenolol after β-adrenoceptor stimulation in the rat *in vivo* (van Steeg *et al.*, 2008). As shown here and in previous applications of this approach (e.g. Weiss *et al.*, 2004), additional information can be extracted from the transient response by modelling the kinetics of receptor binding to estimate K_A (as $k_{off,A}/k_{on,A}$) and R_{tot}.

The reduction of LVDP below baseline values observed in three of the five hearts (Figure 2) was accounted by the presence of a receptor occupation before phenylephrine administration (AR_0) due to an endogenous agonist (noradrenaline). An analogous hypothesis has been advanced to explain the decrease of HR below baseline due to interaction of atenolol with isoprenaline (van Steeg *et al.*, 2008). Note also that noradrenaline release in the isolated rat heart has been reported. The double prazosin dose protocol with two phenylephrine concentrations proved to be a useful experimental design for the estimation of receptor parameters. Although the positive inotropic effect of phenylephrine in the rat heart is normally measured in the presence of propranolol (1 $\mu mol \cdot L^{-1}$) in perfusing buffer to prevent β-adrenoceptor stimulation, we did not use propranolol for the following reasons. First, prazosin is a specific α_1-adrenoceptor antagonist. Second, it cannot be completely excluded that β-adrenoceptor antagonists may interact with α_1-adrenoceptors either at the receptor (Brahmadevara *et al.*, 2004; Leblais *et al.*, 2004) or post-receptor levels (Skomedal *et al.*, 1988; Dzimiri, 2002). Third, we found no significant effect of 1 $\mu mol \cdot L^{-1}$ propranolol on the phenylephrine-induced increase in LVDP (data not shown). This is in accordance with the results by Chess-Williams *et al.* (1990) but in disagreement with those of Silva *et al.* (2001) mentioned above.

Despite some similarities regarding the use of the kinetic version of the operational model of agonism (e.g. the possibility to estimate K_A as the ratio $k_{off,A}/k_{on,A}$), our approach differs from that used in PK/PD studies *in vivo* (Yassen *et al.*, 2005; Danhof *et al.*, 2007). First, the effect of drug receptor binding can be detected in the outflow concentration data, which facilitates model identification. Second, instead of an empirical biophase equilibration model, a physiological model of transcapillary prazosin distribution kinetics is used. Third, it should be noted that the present method would fail under *in vivo* conditions due to the haemodynamic and reflex changes that would result from the systemic vasoconstrictive properties of α_1-adrenoceptor stimulation.

The validity of the model always depends on the available experimental data and the goals of the modelling effort. Based on the classical operational model of drug action, our aim was to construct the simplest model that would account for the observed transient inotropic response kinetics. That we could confirm the predictive power of the operational model of agonism for α_1-adrenoceptor-mediated inotropic response in a kinetic setting is an interesting result, but not a proof of its correctness. The real system is more complex than that implied by this simplified vision (see Shea *et al.*, 2000; Woolf and Linderman, 2001; Kenakin, 2004). However, only inconsistencies with experimental data would provide a basis to further improve the model. As both phenylephrine and prazosin are subtype non-selective ligands, the existence of α_1-adrenoceptor subtypes, α_{1A}- and α_{1B}-, in a ratio of 30:70 (Nagashima *et al.*, 1996) may complicate the interpretation of our results. Although positive inotropism appears to be primarily mediated by α_{1A}-adrenoceptors, there is currently no clarity about the relative contribution of the subtypes (Xiao *et al.*, 2006; Woodcock *et al.*, 2008). It should be also recalled that the validity of the estimated parameter values has to be seen in the light of the *a priori* information used in the data analysis.

In conclusion, we have established a framework that allows quantitative assessment of the α_1-adrenoceptor receptor system in the perfused heart. It was shown that the transient response to an antagonist (prazosin) can be used to evaluate properties of agonist–receptor interaction. The integrative model analysis of the α_1-adrenoceptor-mediated transient inotropic response kinetics can be a useful tool to provide further understanding and may have heuristic value in discovering differences in the results of kinetic and equilibrium experiments. Specifically, it was possible to determine both the receptor properties and the transducer function.

Acknowledgements

PS gratefully acknowledges the support of a scholarship from the Royal Thai Government under the Committee Staff Development Project of Commission on Higher Education. We thank the reviewers for their helpful comments.

Conflicts of interest

The authors state no conflict of interest.

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Appendix

This integrated model of drug uptake and receptor binding (Figure 1) is described by the following differential equations based on mass balance:

$$dB_0(t)/dt = -(Q/V_0)\,B_0(t) + I_B(t) \tag{A1}$$

$$dB_{vas}(t)/dt = -(Q/V_{vas} + k_{vi})\,B_{vas}(t) + k_{iv}B_{is}(t) + (Q/V_0)B_0(t) \tag{A2}$$

$$dB_{is}(t)/dt = k_{vi}B_{vas}(t) - k_{iv}B_{is}(t) - [k_{on,B}(R_{tot} - AR(t) - BR(t))]$$
$$B_{is}(t)/V_{app,is} + k_{off,B}BR(t) \quad \text{(A3)}$$

$$dBR(t)/dt = [k_{on,B}(R_{tot} - AR(t) - BR(t))]B_{is}(t)/V_{app,is} - k_{off,B}BR(t) \quad \text{(A4)}$$

$$dAR(t)/dt = [k_{on,A}(R_{tot} - AR(t) - BR(t))][A_{is}](t) - k_{off,A}AR(t) \quad \text{(A5)}$$

$$C_{PRZ}(t) = B_{vas}(t)/V_{vas} \quad \text{(A6)}$$

$$E(t) = E_{base} + \frac{\phi_{max}\,AR(t)^N}{K_E^N + AR(t)^N} \quad \text{(A7)}$$

where $I_B(t)$ denotes the input rate of prazosin (1.27 nmol dose of [^{3}H]-prazosin as 1 min infusion) at the inflow side of the heart perfused at flow Q. Then the drug first passes the mixing volume V_0 (tubing and large vessels where no exchange with tissue occurs) before it enters the vascular space [equation (A1)]. Note that the unbound agonist concentration in the interstitial space [A_{is}] is identical with the phenylephrine concentration in perfusate, C_{PE} [equation (A5)], and that the outflow concentration $C_{PRZ}(t)$ is the phenylephrine concentration in the vascular compartment [equation (A6)]. For parameter estimation, $C_{PRZ}(t)$ and $E(t)$ are fitted to the measured outflow concentration and inotropic response (LVDP) respectively. The system is solved with initial conditions $B_0(0) = B_{vas}(0) = B_{is}(0) = BR(0) = 0$ while $AR(0) = AR_0$ is estimated.