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Mechanism-based modeling of reduced inotropic responsiveness to digoxin in endotoxemic rat hearts

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Abstract

The mechanisms by which endotoxemia affects myocardial contractility and responsiveness to inotropic drugs are not well understood. We examined the positive inotropic effect of digoxin in single-pass Langendorff-perfused hearts from rats after in vivo pretreatment with lipopolysaccharide (LPS, 4 mg/kg, i.p., 4 h before heart isolation). Using a mathematical modeling approach that allows differentiation between effects elicited at the receptor and postreceptor level, we studied uptake, receptor binding and effectuation kinetics after three consecutive digoxin doses (15, 30, and 45 μg) in the absence and presence of the reverse mode Na^+/Ca^{2+} exchange (NCX) inhibitor KB-R7943 (0.1 μM) in perfusate. LPS significantly depressed baseline contractility and the inotropic response to digoxin without affecting its uptake mechanism. Compared with the control group, the slope of the functional receptor occupancy (stimulus)-to-response relationship was reduced by 44% in the LPS group. Model analysis revealed a significant correlation between changes in digoxin action and LPS-induced febrile response: digoxin receptor affinity increased and the response/stimulus ratio decreased with rise in body temperature, respectively. In contrast, the diminished responsiveness to digoxin observed after NCX inhibition in the control group was not further attenuated in the LPS group. These results support the hypothesis that postreceptor events may be responsible for the diminished contractile response to digoxin during endotoxemia.

Keywords: Na^+/K^+-ATPase; Endotoxin; Sepsis; Cardiac glycoside; Mathematical modeling

1. Introduction

Depression of myocardial contractility constitutes an important feature of human septic shock (Court et al., 2002; Levy and Deutschman, 2004). Myocardial dysfunction has been also demonstrated in experimental animal models following administration of endotoxin, a lipopolysaccharide (LPS) component of the outer membrane of gram-negative bacteria (e.g., Spiers et al., 2000; Grandel et al., 2000; Khadour et al., 2002; Fauvel et al., 2002). It is hardly surprising for a syndrome as complex as sepsis that despite valuable information recently obtained from experimental studies the underlying cellular mechanisms have not been fully defined. Much less is known, however, about the inotropic response to cardiac glycosides under these conditions. Digoxin is a cardiac glycoside, which is widely used in the treatment of congestive heart failure. The inhibition of Na^+/K^+-ATPase, the functional receptor of digitalis, results in positive inotropy. However, there is limited evidence to indicate that treatment with digoxin may be effective in patients with septic shock, although a positive inotropic effect has been demonstrated (Nasraway et al., 1989).

Therefore, the purpose of this present study was to examine the inotropic effect of digoxin in a rat model of endotoxin-induced myocardial dysfunction. We have applied mathematical systems analysis to determine the mechanism of digoxin action in perfused hearts from LPS-treated rats. As described previously (Weiss et al., 2004), digoxin outflow concentration and left ventricular devel-

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oped pressure data were analyzed to evaluate the role of uptake kinetics, receptor interaction and postreceptor events in determining positive inotropic action of digoxin. It is well established that the increase in intracellular Ca^{2+} underlying the positive inotropic effect of digoxin is mediated by the Na^{+}/Ca^{2+} exchanger (NCX). We hypothesized that the reduction of inotropic digoxin action by KB-R7943 could be explained by inhibition of digoxin-induced calcium influx via the reverse mode of the NCX (Weiss et al., 2004). Given the evidence that NCX function is impaired in endotoxic shock (Liu and Xuan, 1986), we considered whether NCX inhibition by KB-R7943 would influence digoxin action in endotoxemia.

2. Materials and methods

2.1. Drugs

[^{3}H]-Digoxin (37 Ci/mmol) was purchased from Perkin-Elmer Life Sciences Inc. (Boston, USA) and Digoxin and lipopolysaccharide (from *Escherichia coli*: serotype 055:B5) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). KB-R7943 [2-[2-[4-(4-nitrobenzyloxyl)phenyl]ethyl] isothiourea methansulfonate] was kindly donated from Nippon Organon KK. All other chemicals and solvents were of the highest grade available.

2.2. Sepsis model

To induce endotoxic shock, Male Wistar rats (250–300 g) were injected i.p. with lipopolysaccharide (LPS) at a dose of 4 mg/kg weight. Control rats (sham group) received the same volume of saline (0.9% NaCl). Hearts were removed from endotoxemic and control animals 4 h after injection for isolated perfused heart experiments as described below. Before excision, rectal temperature was measured using a digital thermometer.

2.3. Isolated perfused heart preparation

The left ventricular contractile function of hearts excised from sham and LPS group rats was studied using a nonrecirculating Langendorff technique, as described previously (Kang and Weiss, 2002; Weiss et al., 2004). Briefly, excised hearts were subjected to retrograde perfusion with an oxygenated Krebs–Henseleit buffer, pH 7.4, consisting of (in mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.66, $NaHCO_3$ 24.88, KH_2PO_4 1.18, Glucose 5.55, Na-pyruvate 2.0, $CaCl_2$ 1.5 and it contained 0.1% of bovine serum albumin. A latex balloon was placed in the left ventricle of the isovolumetrically contracting heart and connected to a pressure transducer line (diastolic pressure was set to 5 to 6 mm Hg). After stabilization, the system was changed to constant flow condition (controlled by a roller pump) maintaining a coronary flow of 9.5±0.4 ml/min. The hearts were beating spontaneously at an average rate of 270 beats/min. Coronary perfusion pressure, left ventricular pressure, and heart rate were measured continuously. Left ventricular developed pressure was calculated as difference between left ventricular systolic pressure and left ventricular enddiastolic pressure (LVDP=LVSP−LVEDP). This investigation conforms to the European Community guidelines for the use of experimental animals. Prior approval was obtained by the Animal Protection Body of the State of Sachsen-Anhalt, Germany.

2.4. Experimental protocol

The following experiments were performed in hearts of the sham and LPS group, respectively ($n=5$ in each group). After a 20-min period of equilibration, three doses (15, 30, and 45 μg) of [^{3}H]-digoxin were administered as 1-min infusions, with an interval of 10 min. Infusion was performed into the perfusion tube close to the aortic cannula using an infusion device. Outflow samples were collected every 5 s for 2 min and every 30 s for the next 5 min (total collection period, 7 min) and the cardiac response was measured. These experiments were repeated in the presence of KB-R7943 (0.1 μM) in perfusate starting 20 min after perfusion with KB-R7943-containing buffer.

The outflow samples were kept frozen at 20 °C until analysis. For determination of [^{3}H]-digoxin concentration in the perfusate, 200 μl of collected outflow sample was transferred to a scintillation vial and 2 ml of cocktail (Lumasafe™ Plus) was added. After vigorous mixing, the radioactivity was measured with a liquid scintillation counter (Perkin-Elmer Instruments, Shelton, CT).

2.5. Modeling and data analysis

The pharmacokinetic/pharmacodynamic modeling methodology has been previously described in detail (Weiss et al., 2004). It is based on a nonlinear compartmental model that accounts for the changes in the amounts of digoxin in the mixing, capillary, and interstitial space as well as in two compartments representing the two saturable digoxin binding sites, i.e., two receptor classes R_1 and R_2 on the sodium pump as represented by Fig. 1 and described by differential equations in Appendix A. Perfusate flow (Q) that contains digoxin during the infusion period (mass flow QC_{in}) first passes the mixing volume V_0 (tubing and large vessels where no exchange with tissue occurs) before it enters the vascular space (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. The permeation clearance into the interstitial space is given by $CL_{vi}=k_{vi}V_{vas}$. For passive transport processes, $CL_{vi}=k_{vi}V_{vas}$ is equal to $CL_{iv}=k_{iv}V_{app,is}$ where $V_{app,is}$ denotes the apparent volume that governs initial distribution of digoxin in the interstitial space. $V_{app,is}$ exceeds V_{is} due to quasi-instantaneous nonspecific tissue binding. The free concentration in

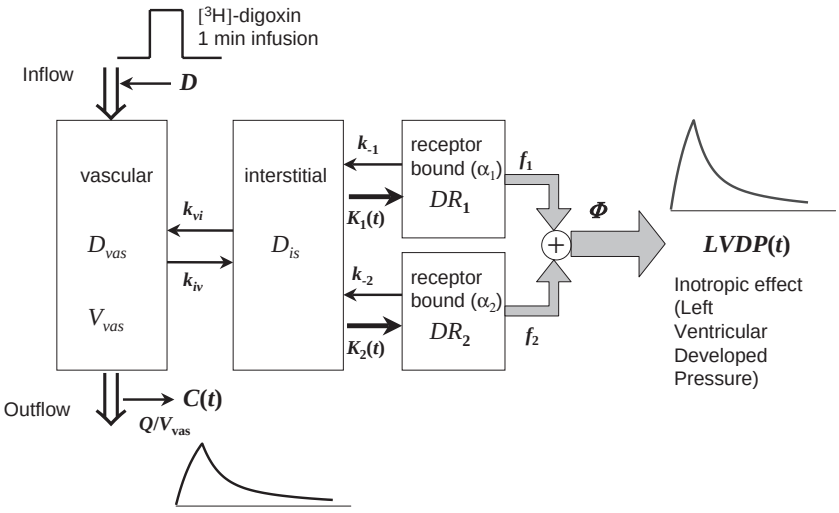

Fig. 1. The compartmental model used to analyze cardiac kinetics and inotropic response of digoxin (Weiss et al., 2004). The model describing distribution and receptor binding of digoxin (boxes) is followed by cellular response generation with fractional receptor occupancy contributions f_i to the stimulus–response relationship Φ (which is a linear or hyperbolic function in the absence and presence of the NCX inhibitor KB-R7943, respectively). V_{vas} and $V_{app,is}$=vascular and interstitial distribution volumes; $C_{is}(t)=D_{is}(t)/V_{app,is}$=unbound interstitial digoxin concentration; k_{vi} and k_{iv}=first order rate constants of transcapillary transport; $K_i(t)=k_i[R_{tot,i}-DR_i(t)]$=fractional rate for saturable receptor binding; k_i and k_{-i}=association and dissociation constants; receptor occupancy=DR_i; the index i=1,2 denotes the receptor population α_i.

the interstitial space that governs receptor binding is then given by $C_{is}(t)=D_{is}/V_{app,is}$. The equilibrium dissociation and affinity constants $K_{d,i}=k_{-i}/k_i$ and $K_{a,i}=1/K_{d,i}$, respectively, are determined by digoxin association and dissociation (k_i and k_{-i}) of the two receptor populations (i=1,2). The time-dependent fractional binding rate of digoxin to free membrane receptors ($R_{tot,i}-DR_i$) is given by $K_i(t)=k_i[R_{tot,i}-DR_i(t)]$, where $DR_i(t)$ is the amount of digoxin bound at time t to receptor i (amount $R_{tot,i}$). The correlation between receptor occupancy and positive inotropic effect $E(t)$ is described by the stimulus–response relationship $E(t)=\Psi[DR_T(t)]$, where Ψ refers to the cascade of cellular processes which convert the stimulus $DR_T(t)$ into response $E(t)$. The stimulus is given by the total functional receptor occupation DR_T, which is the weighted sum of both isoforms, $DR_T(t)=f_1DR_1(t)+(1-f_1)DR_2(t)$, where f_1 is the fraction of R_1-occupancy contribution. The nonlinear stimulus–effect relationship which holds in the presence of KB-R7943 (Weiss et al., 2004)

$$E(t)=\frac{\phi_{max}DR_T(t)}{K_{DR}+DR_T(t)} \quad (1)$$

can be approximated under normal conditions by a linear function (Kang and Weiss, 2002),

$$E(t)=e_TDR_T(t) \quad (2)$$

where the effect per amount of stimulus e_T is referred to as coupling or effect/stimulus ratio. In addition, a first order delay with time constant τ was introduced (Eq. (2) was replaced by Eq. (A6)) to account for the fact that under KB-R7943 the time course of $E(t)$ was delayed with respect to that of $DR_T(t)$ (Weiss et al., 2004). The left ventricular developed pressure LVDP(t) data was used as a measure of inotropic response, i.e., the increase in LVDP with respect to the baseline (predrug) value $LVDP_0$,

$$E(t)=\frac{LVDP(t)-LVDP_0}{LVDP_0} \quad (3)$$

The differential equations (Eqs. (A1)–(A5)) corresponding to the compartmental model described above (Weiss et al., 2004) were solved numerically in analyzing the data using the Bayesian approach of parameter estimation as implemented in the ADAPT II software (D'Argenio and Schumitzky, 1997). To this end, we incorporated a priori knowledge on the ratios of the receptor affinities ($K_{a,2}/K_{a,1}$) and capacities ($R_{tot,1}/R_{tot,2}$) of digoxin, i.e., the ratios $K_{a,2}/K_{a,1}$=45 and $R_{tot,1}/R_{tot,2}$=3 were selected for the control group (Weiss et al., 2004). Since for the LPS group no a priori information was available, we left these values unchanged. The assessment of numerical identifiability was guided by the asymptotic fractional standard deviations (CV) provided by the fitting procedure, which represent the uncertainty in parameter estimates resulting from the fit and correlation coefficients.

Table 1
Effect of endotoxemia on baseline values of left ventricular developed pressure at ($LVDP_0$), left ventricular developed enddiastolic pressure (LVEDP) and coronary vascular resistance (CVR) prior to digoxin administration

	Sham-treated		Endotoxemia	
	Control	KB-R7943	Control	KB-R7943
$LVDP_0$ (mmHg)	76.4±9.9	76.9±10.3	49.3±7.0[a]	50.2±6.9[a]
LVEDP (mmHg)	6.37±1.2	6.50±1.1	6.03±1.7	6.18±1.8
CVR (mmHg min/ml)	5.0±1.4	5.0±1.5	4.1±0.6	4.2±0.8
Body temperature	37.0±0.19		38.5±0.49[a]	

Values are means±S.D. (n=5 in each group).
[a] $p<0.001$ sham vs. LPS group.

Descriptive data are expressed as means±S.D. To get a quantitative estimate of the positive inotropic effect that is independent of the model, we calculated the time integral (over 7 min) of the developed effect ($\int_0^7 E(t)\mathrm{d}t$) using trapezoidal rule. The responses in the two groups were compared using two-way ANOVA, with repeated measures performed on the three dose levels of vehicle and ISO group, followed by Student–Newman–Keuls post hoc test for multiple comparisons. Differences in parameter estimates between the normal and hypertrophied hearts were assessed by student t test. For all analyses, a two-tailed P value of <0.05 was used to indicate statistical significance (Sigma Stat; Jandel, San Rafael, CA).

3. Results

3.1. Baseline parameters

Four hours after LPS injection, rats exhibited signs of sepsis including decreased spontaneous movement and a

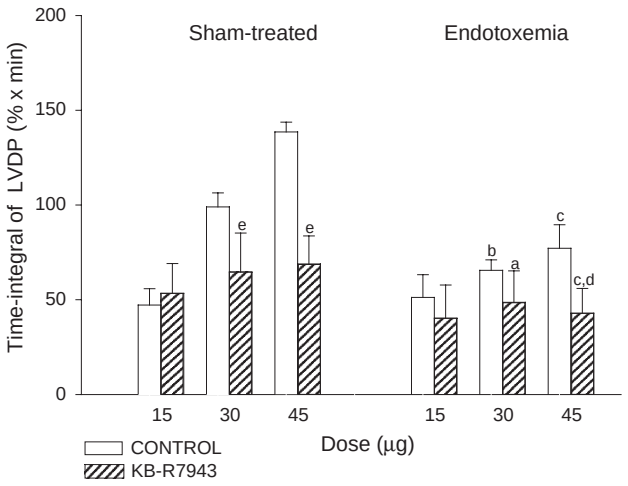

Fig. 3. Effect of endotoxemia and Na^+/Ca^{2+} exchange inhibition by KB-R7943 (0.1 μM) on the time integral of effect (percent increase in developed left ventricular pressure, LVDP). Data are means±S.D. from 5 experiments in each of sham and LPS groups. Significant differences between sham-treated and endotoxemic hearts ($^a p<0.05$, $^b p<0.01$, $^c p<0.001$) and between values before and after before exposure to KB-R7943 ($^d p<0.01$, $^e p<0.001$).

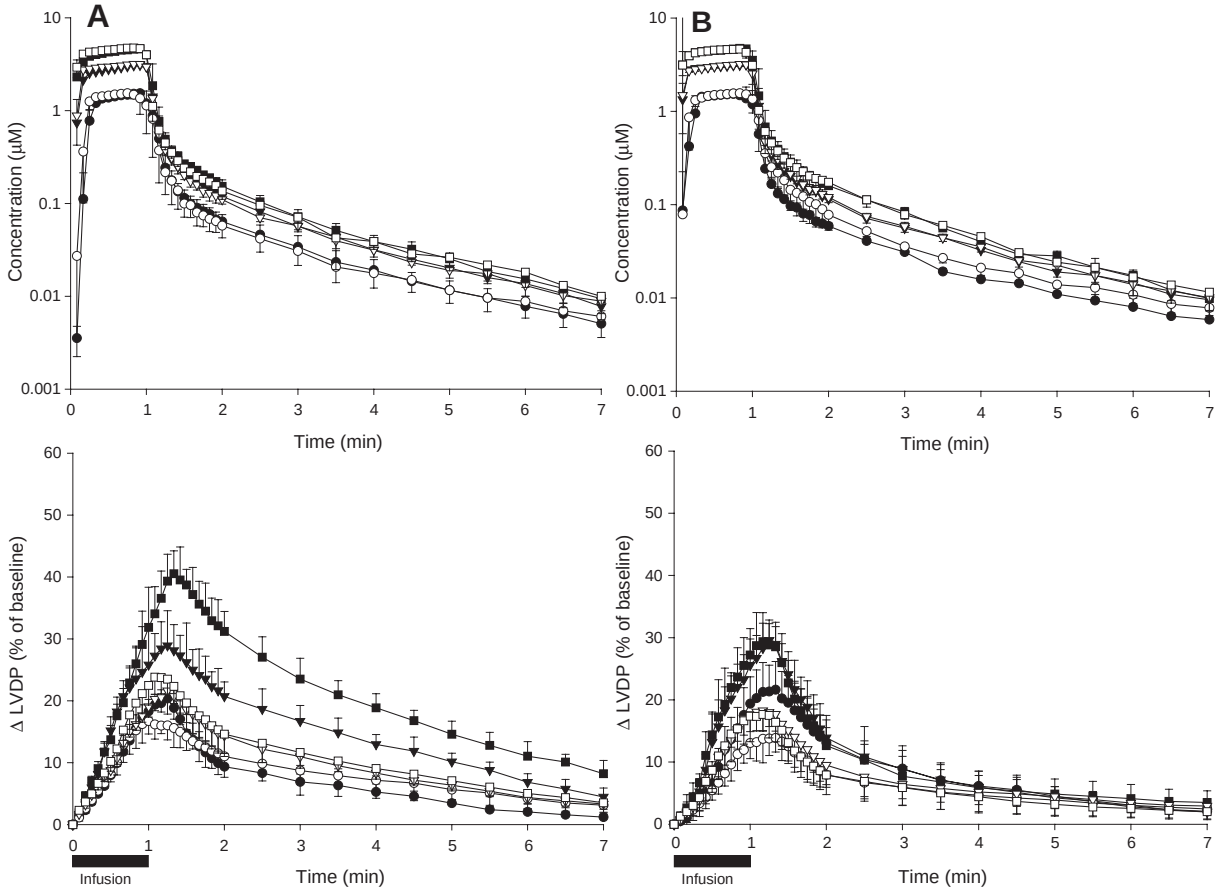

Fig. 2. Average digoxin outflow concentration (upper panel) and percent increase in developed left ventricular pressure (LVDP) (lower panel) for three digoxin doses of 15 (☆), 30 (▼), and 45 (□) μg under control conditions and in the presence of KB-R7943 (0.1 μM) (open symbols) as observed in sham-treated (A) and endotoxemic rats (B), respectively.

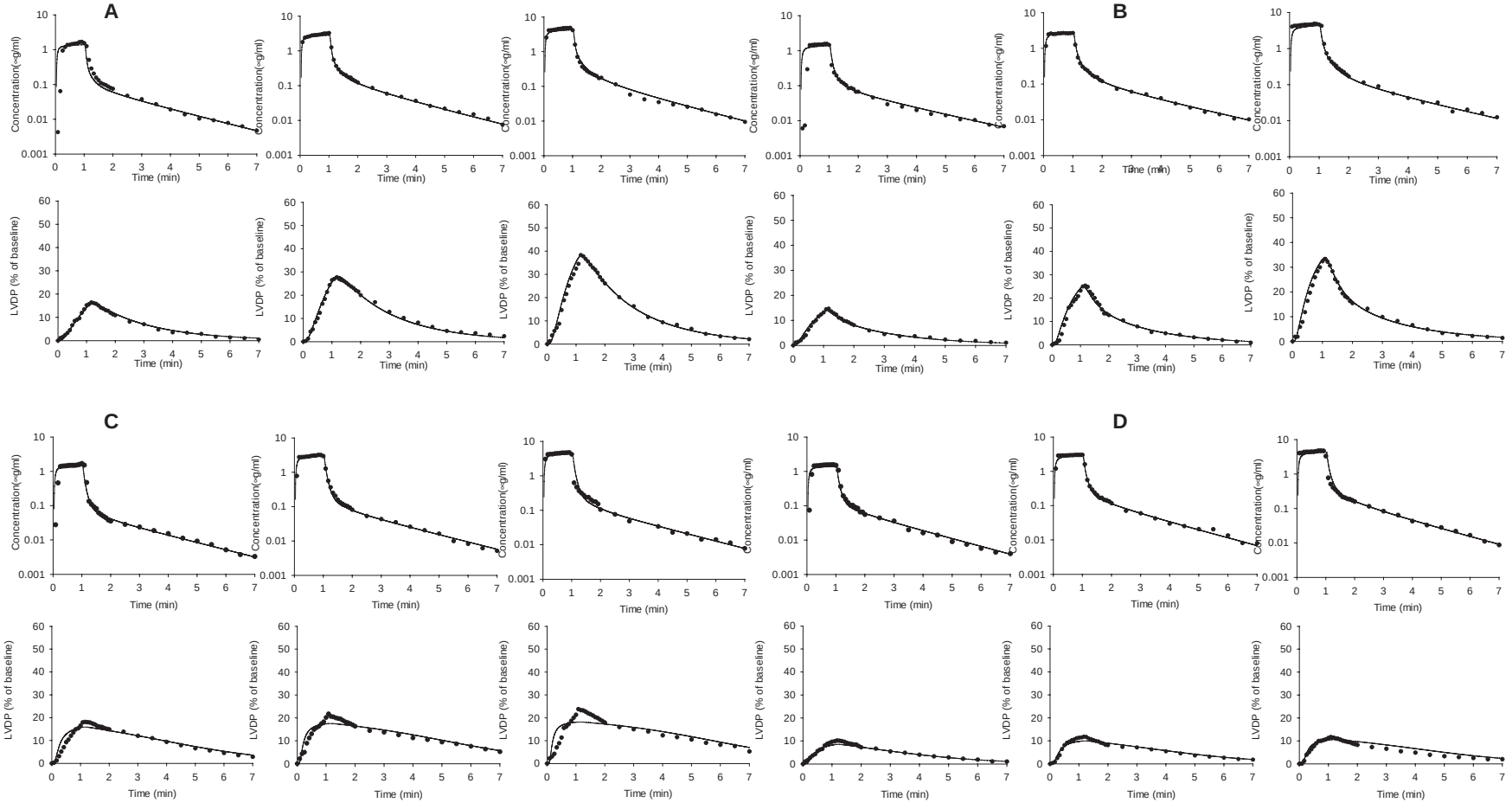

Fig. 4. Representative simultaneous fits of the model (smooth curves) to experimental data (symbols): Digoxin outflow concentration (upper panels) and percent increase in developed left ventricular pressure (LVDP) (lower panels) for three digoxin doses (15, 30, and 45 μg) as observed in normal (A) and endotoxemic hearts (B) under control conditions as well as in the presence of KB-R7943 (C and D), respectively.

significant increase of body temperature from 37±0.2 °C to 38.5±0.5 °C ($p<0.001$). In the LPS group, baseline contractility ($LVDP_0$) was reduced to 65% of sham group values ($p<0.001$). No significant changes in left ventricular enddiastolic pressure and in coronary vascular resistance were observed. In both groups the presence of the NCX inhibitor KB-R7943 in perfusate did not affect baseline function of the heart (Table 1).

3.2. Digoxin outflow concentration and inotropic effect

Fig. 2 shows the averaged digoxin outflow concentration–time curves, $C(t)$, together with inotropic response as percent increase in developed left ventricular pressure after infusion of three doses of digoxin (15, 30, and 45 μg) for 1 min, measured in normal and endotoxemic hearts, respectively, in the absence and presence of KB-R7943. Endotoxemia significantly attenuated the increase in time integral $\int_0^7 E(t)dt$ of percentage increase in LVDP(t) caused by digoxin doses of 30 and 45 μg (Fig. 3). KB-R7943 (0.1 μM) reduced the inotropic response to digoxin at 30 and 45 μg doses in normal hearts ($p<0.001$) but only at the 45 μg dose in endotoxemic hearts ($p<0.01$) as evidenced by a significant decrease in the time integrals of $E(t)$ (Fig. 3).

3.3. Model parameters

Fig. 4 shows typical fits of the model to the data obtained for three consecutive digoxin doses (15, 30, and 45 μg) in normal and endotoxemic hearts. The average model parameters and averaged estimation errors, as a percentage of parameter estimates, are listed in Table 2.

There was no significant change in parameters describing cardiac uptake of digoxin into the interstitial space (CL_{vi} and $V_{app,is}$). As shown previously (Weiss et al., 2004), binding kinetics could be well described by a mixture of two receptor subtypes, a low affinity/high capacity binding site (α_1) and a high affinity/low capacity binding site (α_2). Although there was a tendency for a higher receptor binding affinity ($K_{a,i}$, $i=1,2$) in endotoxemic than in normal rats, this difference did not attain statistical significance, except in the presence of KB-R7943 (see Table 2). However, significant correlations were found between the increase in receptor binding affinities and the LPS-induced rise in body temperature (Fig. 5). The capacity and affinity ratios, $R_{tot,1}/R_{tot,2}$ and $K_{a,2}/K_{a,1}$, estimated in normal and endotoxemic hearts are not much different from the respective a priori values used in the Bayesian estimation procedure. A significant difference between normal and endotoxemic hearts was observed at the postreceptor level: The ratio of increase

Table 2
Parameters estimated by simultaneous fitting of outflow and inotropic response data after 1-min infusions of 15, 30, and 45 μg digoxin in isolated rat hearts in sham and LPS group

	Sham-treated		Endotoxemia	
	Control	KB-R7943	Control	KB-R7943
Cardiac uptake				
CL_{vi} (ml/min/g)	7.755±4.21 (21±10)[a]	8.385±1.55 (28±17)	6.927±1.47 (20±5)	8.174±1.78 (23±3)
$V_{app,is}$ (ml/g)	0.877±0.36 (28±17)	0.819±0.15 (15±2)	0.849±0.21 (22±13)	0.804±0.28 (19±7)
Receptor binding				
$R_{tot,1}$ (nmol/g)	32.70±9.81 (37±17)	29.57±4.30 (37±4)	26.70±12.7 (23±9)	25.87±4.10 (28±16)
k_1 (1/min/nmol/ml)	0.021±0.01 (26±14)	0.020±0.01 (54±14)	0.018±0.01 (66±12)	0.022±0.01 (58±15)
k_{-1} (1/min)	2.870±1.35 (29±12)	2.820±1.46 (51±12)	2.228±1.10 (83±65)	2.118±0.79 (73±34)
$R_{tot,2}$ (nmol/g)	9.423±1.53 (35±17)	6.387±1.11 (33±4)	8.964±4.10 (22±8)	7.084±1.31 (26±16)
k_2 (1/min/nmol/ml)	0.269±0.03 (55±26)	0.282±0.23 (45±6)	0.310±0.17 (39±17)	0.341±0.05 (46±26)
k_{-2} (1/min)	0.872±0.10 (16±6)	0.627±0.10 (7±1)	0.760±0.13 (14±9)	0.720±0.12 (14±6)
$K_{d,1}$ (nmol/ml)	150.6±28.6	131.9±54.2	102.3±47.7	77.49±14.8
$K_{d,2}$ (nmol/ml)	3.289±0.55	2.906±1.14	2.273±1.06	1.669±0.34[b]
$K_{a,2}/K_{a,1}$	45.66±1.63 (2±1)	45.12±1.53 (2±1)	45.05±0.11 (2±1)	46.54±1.23 (2±1)
$R_{tot,1}/R_{tot,2}$	3.761±1.19 (12±3)	3.648±0.42 (11±1)	2.950±0.07 (10±1)	3.708±0.67 (11±1)
Cellular effectuation				
e_T (%/nmol)	19.76±4.14 (47±31)		11.61±2.13[c] (20±8)	
f_1	0.516±0.07 (56±21)		0.659±0.17 (25±11)	
Φ_{max} (%)		15.87±1.06 (5±1)		15.07±3.73 (6±1)
K_{DR} (nmol)		0.195±0.06 (12±1)		0.284±0.18 (13±1)
τ (min)		0.010±0.01 (33±1)		0.010±0.01 (33±1)

Values are means±S.D. ($n=5$ in each group).
[a] Values in parentheses are the asymptotic coefficients of variation of parameter estimates (mean±S.D.) obtained from individual fits.
[b] $p<0.05$.
[c] $p<0.01$ compared with the corresponding value in sham group.

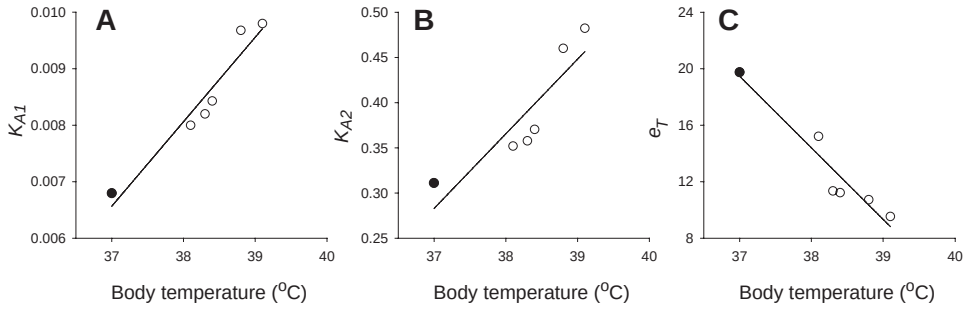

Fig. 5. The correlation between parameters of digoxin action and LPS-induced rise in body temperature: (A) affinity of α_1 receptors (K_{A1}) ($r^2=0.93$, $p<0.01$), (B) affinity of α_2 receptors (K_{A2}) and ($r^2=0.91$, $p<0.01$) and (C) response/stimulus ratio (e_T) ($r^2=0.92$, $p<0.01$). The filled circles are the means of the sham group and the solid curves are the linear regression lines (all slopes different from zero, $p<0.01$).

in inotropic response to the increase in stimulus (functional receptor occupancy) e_T was 19.8±4.1 in control hearts and 11.6±2.1 in LPS hearts ($p=0.01$ versus LPS) (Table 2; Fig. 6).

Furthermore, the effect/stimulus ratio e_T decreases with increasing febrile response in the LPS group (Fig. 5). The attenuated inotropic responsiveness to digoxin under reverse mode NCX inhibition by KB-R7943 was not further diminished by endotoxemia, i.e., no differences in the parameters ϕ_{max} and K_{DR}, characterizing hyperbolic stimulus–response function (Eq. (1)) were found between the LPS and sham group (Table 2). In other words, the differences between both groups disappeared under KB-R7943 as illustrated by the simulated stimulus–response relationships in Fig. 6.

4. Discussion

The observed degree of left ventricular (LV) dysfunction is in accordance with previous reports of impaired contractility of isolated perfused hearts from LPS-treated rats (Spiers et al., 2000; Grandel et al., 2000; Khadour et al., 2002; Fauvel et al., 2002). However, at present no information is available in septic shock about the inotropic effect of digoxin. At doses greater than 15 μg inotropic responsiveness to digoxin (time integral of inotropic effect) was reduced by about 50%. This study provides, to our knowledge, the first evaluation of the diminished inotropic response to digoxin in endotoxemic hearts. The results of the pharmacokinetic–pharmacodynamic modeling approach suggest that this response attenuation is due to a reduced slope e_T of the linear stimulus–response relationship; endotoxemia reduced this coupling ratio to 66±20% of the value estimated in the sham group. Especially in the low concentration range, the increase in receptor affinity could partly offset this decreased responsiveness at the postreceptor level. Although the cellular mechanism underlying this impairment of inotropic response to digoxin remains unclear, some of the explanations discussed for the diminished baseline contractility in LPS-treated rats could be taken into account; as, for example, inhibition of Ca^{2+} transport across the sarcoplasmatic reticulum (Stamm et al., 2001; Wu et al., 2002) or reduced myofibrillar Ca^{2+} sensitivity (Yasuda and Lew, 1997; Tavernier et al., 2001).

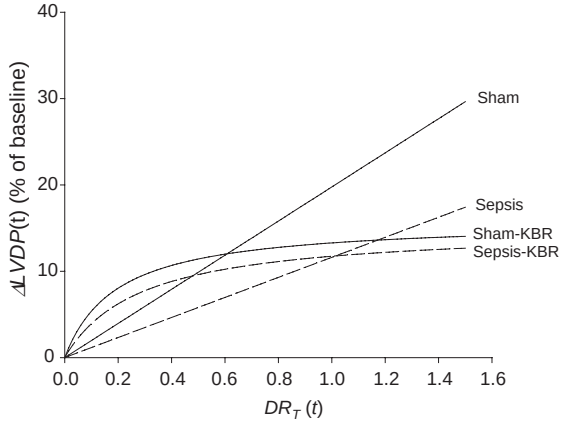

Fig. 6. Model simulations of the relationship between total functional receptor occupancy [stimulus, $DR_T(t)$] and inotropic response under control conditions and after Na^+/Ca^{2+} exchange inhibition by KB-R7943 (KBR) for digoxin in sham-treated (solid lines) and endotoxemic hearts (dashed lines), respectively.

Model analysis revealed that both the increase in receptor binding affinity and the decrease in inotropic potency of digoxin correlated with the LPS-induced rise in temperature (Fig. 5). To the best of our knowledge, this is the first study to demonstrate that cardiac contractile response changes with degree of endotoxemia. One may speculate that this correlation is caused by a common mediator behind the LPS-induced changes in digoxin action and body temperature, e.g., levels of circulating proinflammatory cytokines (for review see Müller-Werdan et al., 1996). Obviously, the inherent variability in the degree of endotoxemia-induced changes in receptor prevented a significant difference between LPS and control group. The differences in inotropic responsiveness between endotoxemic and normal hearts disappeared after NCX inhibition by KB-R7943 where digoxin response was reduced to the same limiting

behavior (Fig. 6) characterized by practically identical (hyperbolic) stimulus–response curves. Taken together, these results are similar to those observed following isoprenaline-induced left ventricular hypertrophy (Baek and Weiss, 2005), where the response/stimulus ratio e_T was reduced to 38% of control values and the parameters ϕ_{max} and K_{DR} characterizing the nonlinear stimulus–effect relationship in the presence of KB-R7943 remained unchanged. Interestingly, Takeuchi et al. (2000) suggested that contractile dysfunction in septic and hypertrophic hearts may be caused by similar abnormalities of calcium handling. On the other hand, the present and previous (Weiss et al., 2004) results on the effect of KB-R7943 on inotropic response to digoxin show that endotoxin induced NCX inhibition (Liu and Xuan, 1986; Wang et al., 2000) could be involved in the reduced digoxin action during septic shock.

The capacity and affinity of the two populations of functional receptors in the vehicle group are in agreement with our earlier findings (Weiss et al., 2004) showing that normal rat hearts express two functionally distinct Na^+ pumps. That both isoforms are involved in mediating the positive inotropic digoxin effect is consistent with recent findings in mice (Dostanic et al., 2003, 2004). The effects of sepsis on Na^+/K^+-ATPase remain unclear, despite numerous investigations. That endotoxemia increased the affinity of Na^+/K^+-ATPases to digoxin (Fig. 5) could be interpreted as the consequence of an increased pump activity (e.g., Clausen, 2003). This would be in accordance with the effect of sepsis on skeletal muscle Na^+/K^+-ATPase activity (O'Brian et al., 1996; Bundgaard et al., 2003), but in contrast to the finding by Schornack et al. (1997) of a reduced transport activity of the Na^+ pump in septic rat hearts (where, however, sepsis was not accompanied by a decrease in contractility).

A limitation of our approach is that due to the underlying identifiability problem the parameter estimation procedure had to be based on a priori values of the receptor affinity and capacity ratios, $K_{a,2}/K_{a,1}$ and $R_{tot,1}/R_{tot,2}$. Since relevant information was lacking for the endotoxemic heart, we left these ratios unchanged. Although this assumption led to a satisfactory fit of the data from the LPS-group, this only means that the resulting set of parameter estimates is in accordance with the measurements, i.e., this analysis does theoretically not provide a unique answer. In other words, our inferences are only as good as the validity of the assumptions underlying the model.

In summary, we attempted to clarify the complex experimental results on endotoxemia-induced changes in inotropic response to cardiac glycosides using a mathematical modeling approach that allows differentiation between effects elicited at the receptor and postreceptor level. The results of this study indicate that endotoxemia reduces the development of inotropic effect per inhibited sodium pump.

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Appendix A

The differential equations corresponding to the compartment model shown in Fig. 1 are listed below. They describe the time course of outflow concentration $C(t)$ and inotropic effect $E(t)$ after infusion of digoxin (dosing=RATE) at the inflow side of the heart perfused at flow Q.

$$dD_0(t)/dt = -(Q/V_0)D_0(t) + \mathrm{RATE} \tag{A1}$$

$$dD_{vas}(t)/dt = -(Q/V_{vas} + k_{vi})D_{vas}(t) + k_{iv}D_{is}(t) + (Q/V_0)D_0(t) \tag{A2}$$

$$dD_{is}(t)/dt = k_{vi}D_{vas}(t) - k_{iv}D_{is}(t) - \left[k_1\left(R_{tot,1} - DR_1(t)\right) + k_2\left(R_{tot,2} - DR_2(t)\right)\right]C_{is}(t) + k_{-1}DR_1(t) + k_{-2}DR_2(t) \tag{A3}$$

$$dDR_1(t)/dt = k_1\left[R_{tot,1} - DR_1(t)\right]C_{is}(t) - k_{-1}DR_1(t) \tag{A4}$$

$$dDR_2(t)/dt = k_2\left[R_{tot,2} - DR_2(t)\right]C_{is}(t) - k_{-2}DR_2(t) \tag{A5}$$

where $k_{vi}=CL_{vi}/V_{vas}$, $k_{iv}=CL_{vi}/V_{app,is}$, and $C_{is}(t)=D_{is}(t)/V_{app,is}$ denotes the unbound digoxin concentration in the interstitial space. The resulting outflow concentration is then obtained as $C(t)=D_{vas}(t)/V_{vas}$. Sodium pump inhibition by digoxin [as quantified by $DR_1(t)$ and $DR_2(t)$ obtained by solving Eqs. (A1)–(A5)] ultimately leads to an increase in the force of contraction as described by Eqs. (1) and (2), respectively. In the presence of KB-R7943 (Eq. (1)) the following differential equation has to be added to account for the delay τ between response and stimulus:

$$dE/dt = \frac{1}{\tau}\left[\frac{\phi_{max}DR_T}{K_{DR} + DR_T} - E\right] \tag{A6}$$

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