Mathematical modelling of the calcium–left ventricular pressure relationship in the intact diabetic rat heart

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Abstract
Aim: The objective was to characterize cross-bridge kinetics from the cytoplasmic calcium ion concentration ([Ca2+]i) and the left ventricular pressure (LVP) in the early-stage diabetic rat heart under baseline conditions and upon β-adrenergic stimulation.

Methods: Four weeks after the induction of diabetes in rats by the injection of streptozotocin, the hearts were perfused according to Langendorff, and [Ca2+]i was obtained by epifluorescence measurements using Indo-1 AM. [Ca2+]i and LVP were measured simultaneously at a temporal resolution of 200 Hz. The input/output relationship between the Ca2+ and the pressure transients was described by a mathematical model representing the chemical binding of Ca2+ to troponin C on the actin myofilament (TnCA), and the subsequent cooperative force-producing cross-bridge formation of the Ca2+-TnCA complex with myosin. The kinetic parameters of this model were evaluated using a numerical optimization algorithm to fit the model equations to the experimental data. β-adrenergic stimulation of the hearts with increasing doses of isoproterenol allowed quantification of the model parameters over an extended dynamic range, because isoproterenol administration increased developed pressure, heart rate, as well as [Ca2+]i amplitude in a dose-dependent manner.

Results: Model analysis of the experimental data indicates that β-adrenergic stimulation of healthy hearts resulted in a decreased sensitivity of TnCA for Ca2+, increased rates of cross-bridge cycling and decreased cooperativity. By contrast, the responses in cross-bridge kinetic parameters to isoproterenol stimulation were blunted in the 4-week diabetic heart.

Conclusion: We conclude from our modelling results that myocardial cross-bridge cycling is impaired at the early stage of diabetes.

Keywords calcium, cooperativity, cross-bridge cycling, diabetic hearts, Indo-1, mathematical model.

Derangement of the chemomechanical coupling between intracellular free calcium ([Ca2+]i) and contraction is believed to play a key role in the diabetes-induced deterioration of heart function (Sharma & McNeill 2006). [Ca2+]i–contraction coupling is governed by interactions between Ca2+ and the contractile proteins troponin C, actin and myosin (Bers 2001). Previous studies on isolated cardiac muscle cells (Malhotra et al. 1997) and papillary muscle (Joseph et al. 2005) indicate that diabetes indeed could induce changes at the subcellular level of the cardiomyocyte and is associated with alterations in the relation between [Ca2+]i and force. A major
limitation of these analyses is that they do not address the dynamic relationship between \([\text{Ca}^{2+}]\) and contractile force in the physiologically relevant environment: the intact beating heart.

The development of fluorescence techniques has enabled the measurement of \([\text{Ca}^{2+}]\), in intact Langendorff-perfused hearts at a high sample rate (Gryniewicz et al. 1985, Brandes et al. 1993). When considering \([\text{Ca}^{2+}]\)–contraction coupling in the intact beating heart as a physiological control system, the transient oscillations in \([\text{Ca}^{2+}]\), can be considered the input signal, whereas the time-course of the left ventricular pressure (LVP) can be regarded as the output of the system. The analysis of the input/output relationship between the obtained data on \([\text{Ca}^{2+}]\), and LVP in terms of kinetics of the underlying biochemical processes requires a deterministic mathematical model of \([\text{Ca}^{2+}]\)–force development, describing the binding of \(\text{Ca}^{2+}\) to troponin C on the actin myofilament and the subsequent formation of a force-producing actinomyosin cross-bridge (Shimizu et al. 2002, Rhodes et al. 2003). This model makes the identification of kinetic parameters, such as the rate of cross-bridge formation and detachment, as well as the nonlinear cooperativity observed in \([\text{Ca}^{2+}]\)–LVP measurements, possible.

In the application of a mathematical model to the experimental data, it should be taken into account that the number of kinetic parameters that can be estimated from the available data is limited. The ability to uniquely recover model parameters from observed measurements depends on the model complexity as well as the information content of the data. The importance of identifiability and estimatability has been previously addressed for biological models (Jacquez & Perry 1990) and for a model of the \(\text{Ca}^{2+}\)–force relationship in skeletal muscle in particular (Shames et al. 1996). Hence, it is desired to develop a mathematical model with minimal number of parameters to ensure their unique quantification from the experimental data. It is uncertain whether current models of \(\text{Ca}^{2+}\)–contraction coupling in \textit{ex vivo} beating hearts comply with the criteria of identifiability and have unique numerical solutions.

The aim of the present study was to characterize the dynamic relationship between \([\text{Ca}^{2+}]\), and LVP in intact Langendorff-perfused hearts of healthy and 4-week diabetic rats. A mathematical model of \([\text{Ca}^{2+}]\)–contraction coupling was developed based on existing cross-bridge models (Shames et al. 1996, Rhodes et al. 2003) and the unique identifiability of the model parameters was investigated. The parameters were then estimated based on \(\text{Ca}^{2+}\) epifluorescence and LVP measurements in intact \textit{ex vivo} hearts of healthy and 4-week diabetic rats. The hearts were challenged by exposure to various concentrations of the \(\beta\)-adrenergic compound isoproterenol to increase developed pressure and \([\text{Ca}^{2+}]\), amplitude, thereby increasing the dynamic range for model parameter estimation. The approach taken in this paper revealed that \(\beta\)-adrenergic stimulation significantly altered the sensitivity of the contractile proteins to \(\text{Ca}^{2+}\), the rate of cross-bridge cycling, and the nonlinear cooperativity in control hearts, while the response to this stimulus was blunted in the diabetic hearts.

**Materials and methods**

**Experimental protocol**

For details on the \([\text{Ca}^{2+}]\), and LVP measurements in isolated diabetic hearts, we refer to one of our previous studies (op den Buijs et al. 2005). In brief, Male Sprague–Dawley rats (200–250 g body mass) were anaesthetized intra-peritoneally (i.p.) with a combination of 37.5 mg/kg ketamine and 7.5 mg/kg xylazine. Subsequently, randomly selected animals were rendered diabetic via an intravenous injection of 70 mg/kg streptozotocin (STZ), dissolved in a disodium citrate buffer (0.01 mm, pH 4.5). Age-matched controls were treated with vehicle only.

Four weeks after the induction of diabetes, the animals were anaesthetized by 48 mg/kg sodium pentobarbital i.p. and the hearts were isolated and perfused according to Langendorff with a Krebs–Henseleit solution at 37 °C and pH 7.4. The perfusate was gassed with 95% O₂–5% CO₂ and the perfusion pressure was set to 70 mmHg. A latex balloon filled with distilled water was positioned into the left ventricle to assess LVP. The hearts were loaded with 6.25 \(\mu\)M Indo-1 and epifluorescence measurements were performed using excitation light from a 100 W DC mercury arc lamp. Emission was measured at 400 nm (\(\text{Ca}^{2+}\)-bound dye) and 506 nm (\(\text{Ca}^{2+}\)-free dye). The signals were recorded with a 200-Hz sampling rate and calibrated (Gryniewicz et al. 1985) to calculate \([\text{Ca}^{2+}]\). A dissociation constant for Indo-1 was used (Bassani et al. 1995). Six healthy and six diabetic hearts were measured under control conditions and upon stimulation with 0.5, 1.0 and 5.0 \(\mu\)M isoproterenol. Data for the analysis were collected as soon as haemodynamic parameters stabilized, which was about 5 min after the administration of each dose of the drug.

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was conducted after prior approval by the Laboratory Animals Committee of the Semmelweis University of Budapest, Hungary.
**Model development**

A schematic diagram of the proposed cross-bridge model is shown in Figure 1. It is based on a previously published mathematical model of cross-bridge cycling (Shames et al. 1996, Shimizu et al. 2002, Rhodes et al. 2003), with some modifications to enable the unambiguous estimation of the model parameters from the experimental data.

The following assumptions were made in the development of the model:

1. Cross-bridge cycling is activated when \([\text{Ca}^{2+}]_i\) exceeds a threshold \([\text{Ca}^{2+}]_T\). The threshold accounts for the absence of pressure generation during baseline \([\text{Ca}^{2+}]\) levels and for the delay between the initial rise of the \([\text{Ca}^{2+}]_i\) transient and the LVP curve (Shames et al. 1996). Although the activation threshold can also be modelled using mass action (Rhodes et al. 2003), the current approach requires only a single parameter which can be obtained from the data and does not need to be estimated by an optimization procedure (see Model parameter estimation), thereby increasing the reliability of the estimation of the remaining model parameters.

2. When the threshold value is exceeded, \([\text{Ca}^{2+}]\) starts binding with troponin C on the actin myofilament [TnCA] with association rate \(k_1\) and dissociation rate \(k_d\). In previous cross-bridge models (Shimizu et al. 2002, Rhodes et al. 2003), [TnCA] is modelled as a separate variable with time-dependent changes as a result of binding and release of \([\text{Ca}^{2+}]\). By contrast, we assume that the binding of \([\text{Ca}^{2+}]\) with [TnCA] can be described using an effective concentration \([\text{CaTnCA}]_{\text{eff}}\), which we modelled as a constant.

3. The \([\text{Ca}^{2+}]\)-activated TnCA ([CaTnCA]) subsequently forms cross-bridges with myosin ([M]) with association rate \(k_a\) and dissociation rate \(k_d\). It is known that chemomechanical coupling in cardiac muscle includes a positive feedback mechanism, termed cooperativity (Bers 2001). This is incorporated in the model by varying \(k_a\) as a function of the cross-bridge concentration: \(k_a = x_a[\text{CaTnCAM}]^2 + \beta_a\) where \(x_a\) is the degree of nonlinear feedback and \(\beta_a\) is the basal association rate of myosin with the CaTnCA complex.

4. In line with our model treatment of [TnCA], the concentration of myosin [M] was modelled as a constant effective concentration \([\text{M}]_{\text{eff}}\). This allowed to combine the following parameters: \(x_a = x_a[\text{M}]_{\text{eff}}\) and \(\beta_a = \beta_a[\text{M}]_{\text{eff}}\).

5. In the model, the contractile state is governed by the concentration of \([\text{Ca}^{2+}]_i\)-activated troponin C on the actin myofilament, bound by myosin. This concentration is described by [CaTnCAM]. It was assumed that LVP minus end-diastolic pressure is proportional to the cross-bridge concentration [CaTnCAM]. This linear proportionality was assumed to be implicitly integrated in the model parameters, such that the model output [CaTnCAM] could be fitted to the experimentally measured LVP.

The two model differential equations describing the reaction kinetics can be written as follows:

\[
\frac{d[\text{CaTnCAM}]}{dt} = k_1([\text{Ca}^{2+}]_i - [\text{Ca}^{2+}]_T) - (k_3 + k_a)[\text{CaTnCA}] + k_d[\text{CaTnCAM}],
\]

\[
\frac{d[\text{CaTnCAM}]}{dt} = k_a[\text{CaTnCAM}] - k_d[\text{CaTnCAM}],
\]

where \(k_a = x_a[\text{CaTnCAM}]^2 + \beta_a\).

Figure 1 **Schematic diagram of the input/output relationship between intracellular \([\text{Ca}^{2+}]\) and LVP.** Cross-bridge cycling is activated when \([\text{Ca}^{2+}]_i\) exceeds a threshold \([\text{Ca}^{2+}]_T\). TnCA represents the troponin C molecule on the actin (A) myofilament, M represents the myosin head. \([\text{Ca}^{2+}]\) binds to TnCA, tropomyosin shifts so myosin and actin can bind forming an actinomyosin cross-bridge, \([\text{Ca}^{2+}]\) dissociates from TnCA with cross-bridge attached, and finally the cross-bridge breaks. The curved arrow indicates positive feedback, 'cooperativity' of the CaTnCAM complex on the binding of M to CaTnCA.
Model parameter estimation

For each data set, a period of 500 samples were analysed with the model during a time interval where haemodynamic parameters and the calcium transient were stable. These 500 samples of the \([\text{Ca}^{2+}]_i\) and LVP signals were filtered with a second-order Butterworth filter at 20 Hz. Using a threshold detection algorithm, the upstroke of the calcium transient was detected and the diastolic calcium level was determined in a small time-window just before the threshold was reached. The \([\text{Ca}^{2+}]_i\) and LVP transients were point aligned and averaged on a point-by-point basis. The threshold value \([\text{Ca}^{2+}]_1\) was determined manually as the \([\text{Ca}^{2+}]_i\) concentration where LVP started to rise. For model fitting, only the time points where \([\text{Ca}^{2+}]_i > [\text{Ca}^{2+}]_1\) were taken into account. The baseline values were subtracted from both signals before initiating the following fitting procedure and added back thereafter.

The kinetic parameters \(k^\prime_1, k_3, z_a, \beta_a\) and \(k_d\) were estimated by fitting the model to the averaged data by minimizing the mean-squared error between the experimentally measured and the simulated LVP with the minimum search simplex algorithm from the software MATLAB (The Mathworks, Natick, MA, USA). Zero initial conditions were used in the simulations. The simplex search algorithm requires an initial guess for the parameters and the following values were taken based on trial-and-error: \(k^\prime_1 = 40 \text{ s}^{-1}\), \(k_3 = 300 \text{ s}^{-1}\), \(z_a = 0.1 \text{ nm}^{-2} \text{ s}^{-1}\), \(\beta_a = 350 \text{ s}^{-1}\) and \(k_d = 200 \text{ s}^{-1}\). It should be noted that all the estimated parameters appeared to have positive values. Therefore, no constraints were implemented.

To establish the uniqueness of the model parameters, we calculated the variation in the estimated parameters because of the nature of the mathematical model and the numerical search algorithm as follows. Fifty separate runs of the search algorithm were performed, while drawing the initial parameter guesses from a uniform distribution with the mean the same as above and a width of 25% of this mean. This resulted in Gaussian-like distributions of the parameter estimates (see Results). This procedure was performed for one control heart under baseline conditions.

Additionally, the correlation between parameter estimates was obtained from the parameter covariance matrix \(C\), which was calculated using the inverse of the second derivative of the cost function \(J\) with respect to the parameter set \(\theta\), evaluated at the optimal parameter set \(\theta_0\):

\[
C = \left( \frac{\partial^2 J}{\partial \theta \partial \theta^T} \right)^{-1}
\]

(2)

The correlation matrix \(R\) was then derived as:

\[
R_{ij} = \frac{C_{ij}}{\sqrt{C_{ii}C_{jj}}}
\]

(3)

Because the measurement of the \([\text{Ca}^{2+}]_i\) transient (the input signal of the model) is subjected to noise (see Potential limitations), we analysed the variation in the parameter estimates with regard to slight alterations in the \([\text{Ca}^{2+}]_i\). This was carried out for a representative control heart under baseline conditions and upon 5.0 nM isoproterenol stimulation by increasing the cut-off frequency of the Butterworth filter in steps of 20 Hz, up to 100 Hz (half the sample frequency). Thereafter, the same parameter estimation procedure was performed. Note that the \([\text{Ca}^{2+}]_i\) transient were still averaged on a beat-to-beat basis.

Statistical analysis

The obtained model parameters in healthy and diabetic hearts, as measured under unchallenged conditions and upon stimulation with 0.5, 1.0 and 5.0 nM isoproterenol, were evaluated with a one-way analysis of variance. Parameters obtained under isoproterenol-stimulated conditions were compared with those under basal conditions by a Tukey–Kramer honestly significant difference test (*\(P < 0.05\)). At each isoproterenol concentration, control and diabetic groups were compared by a two-tailed t-test (*\(P < 0.05\)).

Results

Haemodynamics and \([\text{Ca}^{2+}]_i\), transient

Table 1 summarizes heart rate, developed pressure (i.e. difference between peak systolic and end-diastolic pressure) and \([\text{Ca}^{2+}]_i\), amplitude (i.e. difference between peak systolic and end-diastolic \([\text{Ca}^{2+}]_i\)) as measured in six control and six 4-week diabetic rat hearts. It can be noted that stimulation with isoproterenol increased developed pressure, heart rate and calcium amplitude in a dose-dependent manner in control hearts compared with baseline. By contrast, these responses were blunted in the 4-week diabetic hearts. For a more elaborate analysis of the effects of isoproterenol stimulation and diabetes on \([\text{Ca}^{2+}]_i\) handling in intact rat hearts, we refer to our previous study (op den Buijs et al. 2005).

Mathematical model of \([\text{Ca}^{2+}–\text{contraction coupling}]

To investigate the sensitivity of the model to parameter changes, we varied the kinetic model parameters...
estimated in a control heart under baseline conditions by 20% in both directions. The error between the resulting model simulation and the experimental data were described by the normalized square root of the sum of squared errors, denoted by $J$:

$$J = \sqrt{\frac{\sum (LVP_{\text{model}} - LVP_{\text{data}})^2}{\sum LVP_{\text{data}}^2}} \quad (4)$$

Function $J$ was plotted vs. the model parameters and showed distinct local minima for this particular data set (Fig. 2), which indicates the ability of the numerical search algorithm to accurately calculate the optimal parameters.

To inspect whether the model was capable of recovering parameters in a unique manner, we repeated the parameter estimation algorithm 50 times with initial guesses drawn from a uniform distribution, as described in the Material and methods section. It was found that the mean and standard deviation of the parameters were converged after 50 rounds of optimization. The resulting distribution of the obtained parameter estimates for a control heart under baseline conditions are shown in Figure 3. The resulting distributions appear to be unimodal, which is an indication that parameters can be estimated in a unique manner. The standard deviations of the parameters were less than 15% of the mean value.

The model was subsequently applied to the experimental data. $[\text{Ca}^{2+}]$ and LVP transients of representative control and diabetic hearts are compared with model simulations under baseline conditions and upon stimulation with 5.0 nM isoproterenol (Fig. 4).

A phase plane diagram of measured LVP vs. $[\text{Ca}^{2+}]$ in a representative control and diabetic heart compared with simulation results of the mathematical model indicates the ability of the model to capture the dynamics between $[\text{Ca}^{2+}]$ and LVP under baseline conditions as well as upon stimulation with isoproterenol in hearts of both healthy and diabetic rats (Fig. 5).

The mathematical model was then used to estimate the kinetic parameter using $[\text{Ca}^{2+}]$ and LVP curves of $n = 6$ control hearts and $n = 6$ diabetic hearts under baseline conditions and during the three concentrations of isoproterenol (Table 2). The model predicted that:

### Table 1: Heart rate, developed pressure (systolic minus diastolic left ventricular pressure) and $[\text{Ca}^{2+}]$ amplitude (systolic minus diastolic cytoplasmic $[\text{Ca}^{2+}]$) in control and 4-week diabetic rat hearts upon stimulation with isoproterenol

<table>
<thead>
<tr>
<th>Isoproterenol (nm)</th>
<th>Heart rate (beats min$^{-1}$)</th>
<th>Developed pressure (mmHg)</th>
<th>$[\text{Ca}^{2+}]$ amplitude (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Diabetic</td>
<td>Control</td>
<td>Diabetic</td>
</tr>
<tr>
<td>Baseline</td>
<td>291 ± 29</td>
<td>67 ± 8</td>
<td>172 ± 47</td>
</tr>
<tr>
<td>0.5</td>
<td>307 ± 55</td>
<td>73 ± 7</td>
<td>258 ± 50</td>
</tr>
<tr>
<td>1.0</td>
<td>332 ± 51</td>
<td>82 ± 10</td>
<td>285 ± 71</td>
</tr>
<tr>
<td>5.0</td>
<td>360 ± 57</td>
<td>95 ± 13</td>
<td>374 ± 118</td>
</tr>
</tbody>
</table>

Control vs. diabetic (*$P < 0.05$). Isoproterenol vs. baseline (#$P < 0.05$). Information in this table is in part derived from data published in op den Buijs et al. (2005).
The activation threshold $[\text{Ca}^{2+}]_T$ increased with isoproterenol stimulation in control hearts. This finding is in agreement with the earlier observed decline in Ca$^{2+}$ sensitivity of isometric tension upon isoproterenol stimulation of skinned rat ventricular myocytes (Strang et al. 1994). The

Figure 3 Distributions of the model parameters in a control heart under baseline conditions, obtained after 50 model optimizations. The initial guesses of the parameters were drawn from a uniform distribution. Mean and standard deviation are shown in each graph.

Figure 4 $[\text{Ca}^{2+}]_i$ transients as recorded in a representative control heart (a) and diabetic heart (b) under baseline conditions and upon stimulation with 5.0 nM isoproterenol (iso). Corresponding LVP transients in the same control heart (c) and diabetic heart (d) are compared with model simulations.

1. The activation threshold $[\text{Ca}^{2+}]_T$ increased with isoproterenol stimulation in control hearts. This finding is in agreement with the earlier observed decline in Ca$^{2+}$ sensitivity of isometric tension upon isoproterenol stimulation of skinned rat ventricular myocytes (Strang et al. 1994). The
are compared with model simulations (solid lines).

The increase in \([\text{Ca}^{2+}]_T\) upon isoproterenol stimulation was less pronounced in diabetic hearts than in control hearts and did not reach a statistically significant difference at any concentration of the \(\beta\)-adrenergic drug in the diabetic group.

(3) The rate of cross-bridge cycling increased in control hearts upon \(\beta\)-adrenergic stimulation. This is indicated by increases in parameters \(k_1'\), \(k_3\), \(\beta_a'\) (the rate of dissociation of CaTnCA), \(\beta_a\) (the basal rate of cross-bridge formation) and \(k_d\) (the rate of cross-bridge detachment) after the administration of isoproterenol. These increases were statistically significant at 5.0 nM isoproterenol for \(k_1'\), \(k_3\) and \(k_d\).

(4) In contrast to the control hearts, \(\beta\)-adrenergic stimulation did not significantly alter model parameters \(k_1'\), \(k_3\), \(\beta_a'\) and \(k_d\) when compared with baseline conditions in the diabetic hearts. For parameters \(\beta_a'\) and \(k_3\), this resulted in statistically significant differences between the diabetic and control groups at 1.0 and 5.0 nM isoproterenol.

(5) The nonlinear cooperativity decreased upon \(\beta\)-adrenergic stimulation in both control and diabetic hearts, as indicated by a decrease in model parameter \(x_a'\). Increased force development because of decreased cooperativity has been previously explained using a model of myofilament activation by Campbell (1997) and is in line with the present findings. In the control group, the decrease in \(x_a'\) was statistically significantly different at both 1.0 and 5.0 nM isoproterenol. The decrease in the diabetic group was less steep and resulted in a significant difference in \(x_a'\) at 5.0 nM isoproterenol only.

**Table 2** Estimated parameters of the cross-bridge cycling model in control and 4-week diabetic rat hearts under baseline conditions and upon stimulation with isoproterenol (iso)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>0.5 nm iso</th>
<th>1.0 nm iso</th>
<th>5.0 nm iso</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Ca}^{2+}]_T) (nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>117 ± 71</td>
<td>144 ± 59</td>
<td>201 ± 74</td>
<td>285 ± 89*</td>
</tr>
<tr>
<td>Four-week diabetic</td>
<td>153 ± 30</td>
<td>170 ± 42</td>
<td>181 ± 55</td>
<td>221 ± 55</td>
</tr>
<tr>
<td>(k_1) (s(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>38 ± 13</td>
<td>42 ± 20</td>
<td>43 ± 13</td>
<td>77 ± 28*</td>
</tr>
<tr>
<td>Four-week diabetic</td>
<td>48 ± 22</td>
<td>77 ± 79</td>
<td>68 ± 55</td>
<td>100 ± 94</td>
</tr>
<tr>
<td>(k_3) (s(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>361 ± 87</td>
<td>360 ± 87</td>
<td>376 ± 97</td>
<td>684 ± 239*</td>
</tr>
<tr>
<td>Four-week diabetic</td>
<td>264 ± 78</td>
<td>263 ± 132</td>
<td>207 ± 23*</td>
<td>300 ± 100*</td>
</tr>
<tr>
<td>(x_a') (nm(^{-2}) s(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.29 ± 0.16</td>
<td>0.19 ± 0.10</td>
<td>0.13 ± 0.06*</td>
<td>0.11 ± 0.04*</td>
</tr>
<tr>
<td>Four-week diabetic</td>
<td>0.16 ± 0.05</td>
<td>0.12 ± 0.06</td>
<td>0.08 ± 0.03</td>
<td>0.06 ± 0.04*</td>
</tr>
<tr>
<td>(\beta_a') (s(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>658 ± 293</td>
<td>571 ± 248</td>
<td>610 ± 280</td>
<td>917 ± 429</td>
</tr>
<tr>
<td>Four-week diabetic</td>
<td>382 ± 107</td>
<td>337 ± 160</td>
<td>306 ± 62*</td>
<td>455 ± 263*</td>
</tr>
<tr>
<td>(k_d) (s(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>212 ± 44</td>
<td>242 ± 73</td>
<td>328 ± 137</td>
<td>501 ± 212*</td>
</tr>
<tr>
<td>Four-week diabetic</td>
<td>173 ± 80</td>
<td>207 ± 123</td>
<td>224 ± 79</td>
<td>342 ± 230</td>
</tr>
</tbody>
</table>

Control vs. diabetic (*\(P < 0.05\)). Isoproterenol vs. baseline (\(\#P < 0.05\)).
Model of the \([\text{Ca}^{2+}]\)--LVP relationship in diabetic rat hearts · J Op Den Buijs et al.  

**Figure 6** \([\text{Ca}^{2+}]\), transients in a representative control heart under baseline conditions and upon stimulation with 5.0 nM isoproterenol (iso). Filtered, beat-to-beat averaged transients (solid lines) are compared with unfiltered beat-to-beat averaged transients (dashed lines).

To inspect whether these parameters could be estimated independently, mean and standard deviation of the absolute values of the parameter correlation matrix \(R\) were calculated for control and diabetic hearts under baseline conditions and upon stimulation with 5.0 nM isoproterenol (Table 3). The correlation matrix is symmetric and values range between zero and one, where zero represents absence of correlation and one means high correlation. In agreement with the distributions shown in Figure 3, this table revealed only moderate correlation between parameters, which is another indication that the parameters could be uniquely estimated.

The sensitivity of the parameters with regard to slight alteration in the input \([\text{Ca}^{2+}]\) transient was analysed in a representative control heart by increasing the filter cut-off frequency and subsequently performing the parameter estimation procedure as described in the Materials and methods section. The maximally distorted \([\text{Ca}^{2+}]\), transient was compared with the \([\text{Ca}^{2+}]\), transient filtered using a cut-off frequency of 20 Hz (Fig. 6). The percentage of deviation in the parameter values are shown in Table 4.

The results of our modelling could be used to calculate the steady-state relationship between the pCa and developed pressure, where pCa = \(-\log_{10}(\text{[Ca}^{2+}] / 1 \text{ nM})\). From the model equations (1a) and (1b) the steady-state relation can be derived and is given by:

\[
\frac{\text{[CaTnCAM]}}{\beta'} \left( \frac{1}{k_d} \right) (\text{[Ca}^{2+}] - \text{[Ca}^{2+}]_{\text{Tr}}) = \frac{\beta' k_1}{k_d k_3} (\text{[Ca}^{2+}] - \text{[Ca}^{2+}]_{\text{Tr}})^2
\]

This function was plotted using the average parameter values during baseline and 5.0 nM isoproterenol for both the control and diabetic groups (Fig. 7). Under baseline conditions, the control and diabetic groups show no difference between the steady-state \(\text{Ca}^{2+}\)–force relation. However, \(\beta\)-adrenergic stimulation shifted the curve to the right in both groups, indicating the decreased \(\text{Ca}^{2+}\) sensitivity of the force. Notice that the

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**Table 3** Mean and standard deviations of absolute values of the parameter correlation matrices obtained in the control and diabetic groups under baseline conditions and upon stimulation with 5.0 nM isoproterenol

| \(|\text{Ca}^{2+}]_{\text{Tr}}\) | \(|\text{Ca}^{2+}]_{\text{Tr}}\) | \(|\text{Ca}^{2+}]_{\text{Tr}}\) | \(|\text{Ca}^{2+}]_{\text{Tr}}\) |
|---|---|---|---|
| \(k'_1\) | \(1.00\) | \(0.25 \pm 0.06\) | \(0.29 \pm 0.18\) | \(0.26 \pm 0.06\) | \(0.29 \pm 0.10\) | \(0.15 \pm 0.09\) |
| \(k_3\) | \(1.00\) | \(0.26 \pm 0.15\) | \(0.25 \pm 0.10\) | \(0.23 \pm 0.13\) | \(0.20 \pm 0.12\) |
| \(k_d\) | \(1.00\) | \(0.27 \pm 0.16\) | \(0.24 \pm 0.18\) | \(0.34 \pm 0.23\) |
| \(\beta'\) | \(1.00\) | \(0.24 \pm 0.13\) | \(0.22 \pm 0.13\) |
| \(\alpha'\) | \(1.00\) | \(0.29 \pm 0.21\) |

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**Table 4** Impact of increasing the filter cut-off frequency of the \([\text{Ca}^{2+}]\), transient on the parameter estimates

<table>
<thead>
<tr>
<th>(40 \text{ Hz})</th>
<th>(60 \text{ Hz})</th>
<th>(80 \text{ Hz})</th>
<th>(100 \text{ Hz (unfiltered)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta')</td>
<td>(k'_1)</td>
<td>(k_3)</td>
<td>(k_d)</td>
</tr>
<tr>
<td>(0.6)</td>
<td>(3.5)</td>
<td>(1.7)</td>
<td>(3.6)</td>
</tr>
<tr>
<td>(-3.6)</td>
<td>(4.3)</td>
<td>(11.3)</td>
<td></td>
</tr>
<tr>
<td>(-11.0)</td>
<td>(4.3)</td>
<td>(18.0)</td>
<td>(3.8)</td>
</tr>
<tr>
<td>(-0.7)</td>
<td>(-13.2)</td>
<td>(1.6)</td>
<td>(-11.3)</td>
</tr>
<tr>
<td>(8.1)</td>
<td>(-11.0)</td>
<td>(7.1)</td>
<td>(8.3)</td>
</tr>
</tbody>
</table>

Relative deviation of parameter values are given as a percentage of parameter values using a filter cut-off frequency of 20 Hz. Data were obtained in a representative control heart, under baseline conditions and upon administration of 5.0 nM isoproterenol.
right shift of the curve is less pronounced in the diabetic hearts.

The cross-bridge formation rate $k_0^a = a^0 [\text{CaTnCAM}]^{2} + \beta_0^a$ is plotted as a function of the developed pressure (Fig. 8) for both control and diabetic hearts under baseline conditions and upon 5.0 nM isoproterenol. Because of the nonlinear cooperativity included in the model, the rate of cross-bridge formation increases upon increased pressure. Under baseline conditions, the curve is flattened in diabetic hearts when compared with control hearts. Administration of isoproterenol flattens the curve further in both groups because of the decrease of $\alpha^0$. The decrease in this parameter was similar in both control and diabetic hearts: 62% vs. 63% respectively.

Discussion

The dynamic relation between $[\text{Ca}^{2+}]$ and LVP was analysed by identification of a mathematical model of cross-bridge cycling using experimentally obtained waveforms of $[\text{Ca}^{2+}]$, and LVP in intact healthy and 4-week diabetic rat hearts, under baseline conditions and upon $\beta$-adrenergic stimulation with isoproterenol. The main findings of the model were that: (1) isoproterenol stimulation decreased the sensitivity of the contractile proteins for $\text{Ca}^{2+}$ in control hearts, whereas this response was not present in diabetic hearts; (2) isoproterenol stimulation significantly enhanced the rate of cross-bridge cycling in control hearts, but this response was blunted in the diabetic hearts; (3) isoproterenol stimulation decreased cooperativity in both groups; and (4) the force-dependency of cross-bridge formation was decreased in diabetic hearts when compared with control hearts under baseline conditions and during isoproterenol stimulation (Fig. 8).

Heart contraction is induced by the cyclic influx and efflux of $\text{Ca}^{2+}$ into the cytoplasm of the cardiomyocyte and subsequent activation of the contractile machinery. Changes in the contractile force depend on changes in the intracellular $\text{Ca}^{2+}$ level, the sensitivity of TnCA for $\text{Ca}^{2+}$ and the response of actin and myosin filaments to the $\text{Ca}^{2+}$-activated TnCA, including cooperative effects between neighbouring contractile elements. In a natural physiological environment, such as the intact heart, these three mechanisms are simultaneously active in a dynamic manner. This makes it difficult to identify how these mechanisms contribute to any alterations in the $\text{Ca}^{2+}$–contraction coupling during pharmacological and/or pathophysiological interventions. Using a relatively simple mathematical model of the $\text{Ca}^{2+}$–LVP relationship, we were able to characterize $\text{Ca}^{2+}$ sensitivity of the contractile proteins, kinetics of cross-bridge cycling and the extent of cooperativity in the intact, beating heart.

$\beta$-adrenergic stimulation and $\text{Ca}^{2+}$–contraction coupling

It is well documented that $\beta$-adrenergic stimulation with isoproterenol increases the sarcolemmal $\text{Ca}^{2+}$ current ($I_{Ca}$) via phosphorylation of the sarcolemmal $\text{Ca}^{2+}$ channels, enhances the sarcoplasmic reticulum $\text{Ca}^{2+}$ ATPase (SERCA) through phospholamban phosphorylation and decreases myofilament sensitivity for $\text{Ca}^{2+}$ because of phosphorylation of troponin I (Bers 2001). We have previously shown that the inotropic, lusitropic and chronotropic effects caused by isoproterenol are indeed accompanied by increased $\text{Ca}^{2+}$ amplitude, as well as rates of rise and decline in $[\text{Ca}^{2+}]$, in the intact heart (op den Buijs et al. 2003, Ligeti et al. 2006). In control hearts stimulated with 5.0 nM isoproterenol,
Ca\(^{2+}\) amplitude increased by 117\%, whereas the increase in developed pressure was only 42\%. Our present modelling results suggest that the lesser increase in contractile force may at least in part be caused by the decreased Ca\(^{2+}\) sensitivity of the myofilaments, as indicated by the increase in model parameter \([\text{Ca}^{2+}]_T\) upon isoproterenol administration.

The effects of \(\beta\)-adrenergic stimulation and subsequent protein kinase A (PKA)-mediated phosphorylation of several key proteins involved in Ca\(^{2+}\)-contraction coupling on the kinetics of cross-bridge cycling and cooperativity are less clear. Assessment of the Ca\(^{2+}\)-LVP relationship in intact dopamine-stimulated guinea pig hearts by a mathematical model suggested that rates of cross-bridge cycling are markedly increased when compared with baseline, whereas cooperativity was found to be decreased (Rhodes et al. 2006). In addition, model analysis of the Ca\(^{2+}\)-force relationship in isolated rat trabeculae suggested that isoproterenol reduces cooperativity (Dobrunz et al. 1995), which is consistent with our finding of a decreased \(x'_a\) upon isoproterenol stimulation. Although cooperativity tends to enhance contractile force, especially at low \([\text{Ca}^{2+}]\), it also reduces the rate of force development and relaxation (Campbell 1997). This counter-intuitive effect may explain our findings of a reduced cooperativity accompanied by increased developed pressure and rates of pressure development and relaxation during \(\beta\)-adrenergic stimulation. Reduction in sensitivity changes in a step-wise manner at the higher concentrations of isoproterenol. In line with this observation, a similar step-wise change can be observed in parameters \(k'_1, k_3\) and \(\beta'_a\) (Table 2). Taken together, these data indicate that binding of Ca\(^{2+}\) to Tn and CaTnCA complex to myosin are under \(\beta\)-adrenergic control with high activation threshold.

**Diabetes and Ca\(^{2+}\)–contraction coupling**

Several studies on papillary muscle indicate that the rate of cross-bridge cycling is decreased in the diabetic heart. Using a frequency-domain analysis technique, Ishikawa et al. (Ishikawa et al. 1999) demonstrated that cross-bridge cycling is depressed in papillary muscle of 4- to 7-week STZ-induced diabetic rats. Measurement and model analysis of unloaded shortening velocity and isometric tension in papillary muscle from 13-week STZ-induced diabetic rats indicated that the rates of cross-bridge attachment and detachment were significantly reduced (Joseph et al. 2005). In the intact beating heart, we observed slight but insignificant alterations in the basal rate of cross-bridge attachment \(\beta'_a\) and rate of cross-bridge detachment \(k_d\) under baseline conditions, most likely because our experiments were conducted at an early stage of the disease, namely 4 weeks after induction by STZ. On the other hand, stimulation with isoproterenol revealed a blunted response in the various model rate parameters describing cross-bridge cycling in diabetic hearts when compared with control hearts. These results indicate that the contractile machinery in the diabetic heart may be impaired, which may in part be caused by alterations in myosin heavy chain expression (Rundell et al. 2004).

The role of cooperativity, i.e. the generation of more force-bearing cross-bridges upon the activation of cross-bridges, is less well described in the diabetic myocardium. Our modelling predicts a flatter relationship between developed pressure and the total (i.e. cooperative and basal) rate of cross-bridge attachment in diabetic hearts than in control hearts under both baseline conditions and isoproterenol stimulation (Fig. 8). It may be speculated that deterioration of contractile structure because of STZ-induced diabetes (Dyntar et al. 2006) reduces the probability of cooperative myosin activation.

**Model uniqueness**

The dynamic model formulated here was based on previously described models of cross-bridge cycling in the cardiomyocyte (Rhodes et al. 2003) and skeletal muscle (Shames et al. 1996). Fitting the model to the data requires that the number of model parameters is sufficiently large to satisfactorily describe the experimental data, but sufficiently small to allow for accurate estimation of the parameters (Jacquez & Perry 1990, Shames et al. 1996). We examined the accuracy of the model predictions by performing a Monte Carlo experiment in which the parameters were estimated from various initial guesses sampled from a uniform distribution. The resulting distributions of parameter estimates were normal, unimodal and had small standard deviations when compared with the biological variation among different hearts (Fig. 3). In addition, we found only moderate correlation between parameter estimates, as indicated by the correlation matrix (Table 3). Taken together, these results indicate that the model could indeed be used to accurately calculate its parameters by fitting it to the experimental data. It should be noted that this ‘identifiability’ is a property of the mathematical model, and does not depend on the measurement technique or the animal species used in the experiments (Jacquez & Perry 1990).

**Potential limitations**

The input for the model calculations are local, volume-averaged measurements of Ca\(^{2+}\) transients obtained with an optical probe on the left ventricular epicardial surface with a surface area of approx. 28 mm\(^2\). The
model is then fitted to the LVP wave, a more global measurement obtained with a pressure transducer connected to a water-filled balloon in the left ventricle. As a result of this ‘volume mismatch’ between the measurements of \([Ca^{2+}]_i\) and LVP, there is also a ‘time mismatch’ because of the finite action-potential conduction velocity; the contractile state in different subvolumes of the left-ventricular wall may be different.

It is an implicit assumption of the model that the local measurement of \([Ca^{2+}]_i\), represents the kinetics of \(Ca^{2+}\) in the entire ventricular wall.

As a result of rhythmic cardiac contraction, a motion artefact exists in the fluorescence measurements, which may be augmented upon stimulation with isoproterenol. We kept track of the motion artefact through the measurement of the 340-nm fluorescence (a wavelength unrelated to Indo-1 fluorescence) and found that a deviation of up to 10% could exist in this signal during stimulation with the maximal isoproterenol dosage used. However, if a deviation of 10% is present in the same direction in both the 400-nm (\(Ca^{2+}\)-bound dye) and 506-nm (unbound dye) fluorescence signals, the ratio of these two signals, used in the calculation of \([Ca^{2+}]_i\), remains theoretically the same. Thus, we expect that small motion artefacts in the fluorescence signals have minor impact on the \([Ca^{2+}]_i\) calculations.

Fluorescence in isolated perfused hearts loaded with Indo-1 is generated not only by cardiomyocytes, but also by the endothelial cells and pericytes from the coronary capillaries (Shinozaki et al. 1993). Our model implicitly assumes that the calculated \([Ca^{2+}]_i\), is generated by the cardiomyocytes only. As the cardiomyocytes take up approx. 80–90% of the volume of cardiac tissue (Gerecht-Nir et al. 2006), we expect only a minor quantitative error as a result of fluorescence by other cells. Because of the above-mentioned limitations in the measurement of the \([Ca^{2+}]_i\), transient, we inspected the effect of alterations in the \([Ca^{2+}]_i\), transient on the estimated model parameters by decreasing the degree of filtering of the raw data (Table 4). The results indicate that slight distortions in the model input have only minor impact (up to 15%) on estimated parameters.

Diabetic hearts are characterized by a decrease in heart rate, whereas intervention with isoproterenol is known to have a positive chronotropic effect. To appreciate this effect and obtain measurements in a physiological situation, we chose not to pace the hearts. The resulting variation in heart rate, however, may have influenced the calculations of the various rate constants in the model. In addition, the question may be raised if changes in the model parameters as calculated 4 weeks after diabetes induction by STZ may in part be explained by alterations in passive muscle stiffness rather than abnormalities in \(Ca^{2+}\)-contraction coupling. Indeed, diabetes is known to produce a stiff myocardium 4 months after the injection of STZ (Norton et al. 1996). In the early-stage diabetic rat heart (26 days after the induction of diabetes with STZ), however, Litwin et al. (1990) showed that myocardial stiffness was unchanged, and myocyte morphology was normal. Thus, it appears that any characteristics of an early-dilated cardiomyopathy are related to metabolic rather than structural alterations of the myocardium.

As a result of simplification, the model contains ‘lumped’ parameters, which describe various interactions at the molecular level. This has the consequence that changes in cross-bridge cycling are relative changes, which may have various sources. For example, a change in parameter \(k_i\) could be caused by a change in TnCA levels or a change in the binding affinity of TnCA for \(Ca^{2+}\). On the other hand, we have shown that the lumped-parameter model allowed for accurate quantification of the kinetic parameters.

Conclusion

The mathematical modelling method delineated in this paper, offers a simple solution to characterize alterations in cross-bridge cycling and \(Ca^{2+}\) sensitivity of the contractile machinery in an intact heart set-up. Here, we have shown the effectiveness of this method in the identification of the dynamic relation between \([Ca^{2+}]_i\), and LVP in \(\beta\)-receptor stimulated early-stage diabetic rat hearts. Although more detailed models of cross-bridge cycling exist (Cortassa et al. 2006), the current method offers a means to uniquely and accurately quantify model parameters.

Conflict of interest

The authors declare that there is no conflict of interest.

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\[ \frac{d[Ca^{2+}]}{dt} = k_1[Ca^{2+}] - k_3[CaTnCA] \]

\[ \frac{dTnCA}{dt} = k_1[CaTnCA] - k_3[Ca^{2+}] \]

\[ k_1 = 8 \mu M^{-1} s^{-1} \]

\[ k_3 = 30 s^{-1} \]

\[ [TnCA]_{t=0} = 70 \mu M \]


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**Appendix**

The following numerical experiment shows that modelling \([TnCA]\) as a constant can be justified. Focusing on the binding of \([Ca^{2+}]\) to \([TnCA]\) and leaving out the other components of the model, the following two sets of differential equations can be compared:

**Model 1: time-dependent \([TnCA]\)**

\[ \frac{d[Ca^{2+}]}{dt} = k_1[Ca^{2+}] - k_3[CaTnCA] \]

\[ \frac{dTnCA}{dt} = k_1[CaTnCA] - k_3[Ca^{2+}] \]

\[ k_1 = 8 \mu M^{-1} s^{-1} \]

\[ k_3 = 30 s^{-1} \]

\[ [TnCA]_{t=0} = 70 \mu M \]

**Model 2: constant \([TnCA]\)**

\[ \frac{d[Ca^{2+}]}{dt} = k'_1[Ca^{2+}] - k_3[CaTnCA] \]

\[ k'_1 = 472 s^{-1} \]

\[ k_3 = 30 s^{-1} \]
In model 2 parameter $k'_1$ incorporates the constant [TnCA]. As input calcium transient a sawtooth with an amplitude of 1 μM has been used:

\[
[Ca] = \begin{cases} 
1.0t/0.05, & t < 0.05, \\
1.0 \left(1 - \frac{t - 0.05}{0.35}\right), & t \geq 0.05,
\end{cases}
\]

The fluctuating [TnCA] for model 1 is presented in the left panel of the following figure. The resulting [CaTnCA] for models 1 and 2 (see right panel) are very similar.

From this numerical experiment it can be justified to model [TnCA] as a constant. This eliminates the need for an estimation of the initial value of [TnCA], which would be highly correlated with the binding rate $k_1$. 