Mathematical Modeling of the Relation between Myosin Phosphorylation and Stress Development in Smooth Muscles

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Received May 1, 2005

In this paper the 4-state latch bridge model proposed by Rembold and Murphy is expanded; first by incorporation of the analytical expression of Ca^{2+} dependent MLCK activation from the work of Kato et al. and second, by inclusion of the myosin dephosphorylation based on the Michaelis−Menten kinetics. The analysis of the proposed model and the comparison with the original model results as well as with the experimental data is presented. The model is able to predict the steady-state isometric stress and the myosin phosphorylation in dependence on steady cytosolic $[Ca^{2+}]$ as well as the temporal evolution of the system in dependence on the input Ca^{2+} signal in the form of biphasic transient, whereby our model results are in several aspects in better agreement with experimental observations.

INTRODUCTION

Contraction of skeletal and smooth muscles is caused by the cross bridge cycling between the myosin and actin filaments.[1−4] In smooth muscles this mechanism is regulated by a Ca^{2+} dependent phosphorylation of a myosin light chain (MLC).[3,4] It is widely accepted that phosphorylation is essential for formation of stress generating cross bridges.[3,4]

Phosphorylation of MLC is associated with an increase in cytosolic $[Ca^{2+}]$, binding of four Ca^{2+} ions to calmodulin (CaM), and Ca^{2+}/CaM-dependent activation of myosin light chain kinase (MLCK).[5,6] During the cycling the cross bridges are dephosphorylated by myosin light chain phosphatase (MLCP). Dephosphorylation is associated with a decrease in cytosolic $[Ca^{2+}]$. The extent of phosphorylated MLC and, consequently, the magnitude of stress depend on the balance of the Ca^{2+}-dependent activity of MLCK and MLCP.[7] The activities of both enzymes are modulated by several protein kinases[7] although these signaling pathways are not taken into consideration in this work.

Experimental data of Sieck et al.[8] show that biphasic Ca^{2+} transient is an important cell signal which regulates stress generation and its maintenance in smooth muscles. A sustained stress developed seems to be tightly related to the occurrence of $[Ca^{2+}]$ plateau in a descending phase of Ca^{2+} transient. Development of sustained stress in smooth muscles was explained by the latch bridge hypothesis.[1,9] Reference 8 reports that development of maximal stress is delayed with respect to the peak in biphasic Ca^{2+} transient.

In the previous studies of smooth muscle contraction the 4-state kinetic model of cross bridge cycling was proposed.[1,9] The model enabled authors to evaluate quantitatively experimental observations of Ca^{2+} mediated time-dependent phosphorylation and stress generation. Our proposed model is based on the original model of Hai and Murphy[9] and Murphy and Rembold;[1] however, it is expanded in such a way that it no longer requires a semitheoretical prediction of Ca^{2+}-dependent MLCK activation. The recent model takes into account a Ca^{2+}/CaM dependent MLCK activation proposed by Kato et al.[2] Furthermore, it includes the MLCP dependent dephosphorylation of myosin cross bridges based on the Michaelis−Menten kinetics.

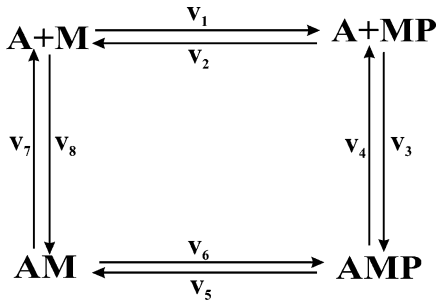

Figure 1. The 4-state kinetic scheme of cross bridge cycling as proposed by Rembold and Murphy.[1]

The present mathematical model is handled in the view of detailed theoretical analysis by considering the stationary as well as dynamic properties of the system. The model is able to predict the steady-state isometric stress and MLC phosphorylation in dependence of a steady cytosolic $[Ca^{2+}]$ as well as the temporal evolution of the system in dependence of time-dependent Ca^{2+} signal. The present study is focused on the comparison between predictions of the original and the present model and, subsequently, on the comparison of both models with the published experimental data.[8,10]

MATERIALS AND METHODS

The mathematical modeling is used for analyzing the Ca^{2+}-dependent development of stress in smooth muscles. The kinetic scheme of major steps in the cross bridge cycling process is presented in Figure 1. Phosphorylated states of

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10.1021/ci050178a CCC: $30.25
Published on Web 10/15/2005

detached myosin cross bridges and myosin cross bridges attached to actin filaments are denoted as MP and AMP, respectively, the corresponding dephosphorylated species are denoted as M and AM, respectively and v_i are the velocities of reactions.

The mathematical model of the species involved consists of the following first-order differential eqs 1−4 proposed by Rembold and Murphy[1], whereby all different states of the myosin are represented by four corresponding variables:

$$\frac{d[M]}{dt} = -v_1 + v_2 + v_7 - v_8 \quad (1)$$

$$\frac{d[MP]}{dt} = v_1 - v_2 - v_3 + v_4 \quad (2)$$

$$\frac{d[AMP]}{dt} = v_3 - v_4 - v_5 + v_6 \quad (3)$$

$$\frac{d[AM]}{dt} = v_5 - v_6 - v_7 + v_8 \quad (4)$$

The velocities of the reactions are defined as

$$v_i = k_i[m_i];\ i = 1...8 \quad (5)$$

where k_i are the rate constants and $[m_i]$ are the concentrations of the myosin species. For $i = 1$ and 8 m_i = M, for $i = 2$ and 3 m_i = MP, for $i = 4$ and 5 m_i = AMP, and for $i = 6$ and 7 m_i = AM. Attachment and detachment rate constants k_3, k_4, k_7, and k_8 are taken the same as in the work of Rembold and Murphy.[1] The rate constants k_1 and k_6 as well as k_2 and k_5 simulate the activities of the enzymes MLCK and MLCP, respectively. The definitions are given below.

In the original model of Rembold and Murphy,[1] the calculation of the rate constants k_1 and k_6 is based on the expression fitted to the experimentally determined dependence of myosin phosphorylation on changes in aequorin-estimated $[Ca^{2+}]$. Instead of this semitheoretical approach, we use a completely theoretical description based on a simple kinetic scheme of interactions between Ca^{2+}, CaM, and MLCK proposed by Kato et al.[2] (Figure 2).

According to the kinetic scheme presented in Figure 2 MLCK is activated in two steps. First, four Ca^{2+} ions bind to CaM and, second, the Ca_4CaM complex interacts with MLCK and activates it. The final product Ca_4CaM·MLCK represents the active form of MLCK (MLCK*).[11] K_1 and K_2 are the equilibrium constants for the binding of Ca^{2+} to CaM and for the binding of the Ca_4CaM complex to MLCK, respectively. Considering the rapid equilibrium approximation for both reactions[2] and assuming that all Ca^{2+} binding sites on CaM are equivalent and independent[2] the eq 3 of ref 2 yields the relative amount of active MLCK

$$\frac{[MLCK^*]}{[MLCK]_{TOT}} = \frac{1}{2}\left(1 + \frac{[CaM]_{TOT}}{[MLCK]_{TOT}} + \frac{K_2}{[MLCK]_{TOT}}\left(1 + \frac{K_1}{[Ca^{2+}]}\right)^4 - \sqrt{\left(1 + \frac{[CaM]_{TOT}}{[MLCK]_{TOT}} + \frac{K_2}{[MLCK]_{TOT}}\left(1 + \frac{K_1}{[Ca^{2+}]}\right)^4\right)^2 - 4\frac{[CaM]_{TOT}}{[MLCK]_{TOT}}}\right) \quad (6)$$

where $[MLCK]_{TOT}$ and $[CaM]_{TOT}$ are the total concentrations

$$4Ca^{2+} + CaM \underset{}{\overset{K_1}{\rightleftharpoons}} Ca_4CaM$$

$$Ca_4CaM + MLCK \underset{}{\overset{K_2}{\rightleftharpoons}} Ca_4CaM{\cdot}MLCK$$

Figure 2. The kinetic scheme of interactions between Ca^{2+}, CaM, and MLCK as proposed by Kato et al.[2] Note that the complex Ca_4-CaM·MLCK represents the active MLCK form (MLCK*). K_1 and K_2 are equilibrium constants.

of MLCK and CaM, respectively. Following eq 6 the rate constants k_1 and k_6 are determined as

$$k_1 = k_6 = k^0\frac{[MLCK^*]}{[MLCK]_{TOT}} \quad (7)$$

where k^0 is the parameter representing the rate constant of the corresponding reactions.

The process of dephosphorylation of species MP and AMP is treated as an enzymatic reaction. The kinetic scheme presented in Figure 3 assumes that both substrates have the same kinetic properties with respect to the enzyme.

The conservation relation for the total concentration of MLCP ($[MLCP]_{TOT}$) reads

$$[MLCP]_{TOT} = 2[MLCP] + [MLCP{\cdot}MP] + [MLCP{\cdot}AMP] \quad (8)$$

where [MLCP·MP] and [MLCP·*A*MP] are the concentrations of the intermediate enzyme in the complex with the substrates MP and AMP, respectively. Differential equations describing the time evolution of [MLCP·MP] and [MLCP·AMP] are written as

$$\frac{d[MLCP{\cdot}MP]}{dt} = k_1'[MLCP][MP] - k_{-1}'[MLCP{\cdot}MP] - k_2'[MLCP{\cdot}MP] \quad (9)$$

$$\frac{d[MLCP{\cdot}MP]}{dt} = k_1'[MLCP][AMP] - k_{-1}'[MLCP{\cdot}AMP] - k_2'[MLCP{\cdot}AMP] \quad (10)$$

By assuming the steady-state condition for the intermediate complexes ([MLCP·MP], [MLCP·AMP]) and by taking into account the conservation relation (8) we obtain a system of three algebraic equations. Solving it for [MLCP·MP], [MLCP·AMP] and by considering eq 5 the rate constants k_2 and k_5 are

$$k_2 = k_5 = k_2'\frac{[MLCP]_{TOT}}{2K_M + [MP] + [AMP]} \quad (11)$$

where K_M is the Michaelis−Menten constant defined as $K_M = (k_2' + k_{-1}')/k_1'$. By considering the conservation relation of all myosin species

$$[M]_{TOT} = [M] + [MP] + [AMP] + [AM] + [MLCP{\cdot}MP] + [MLCP{\cdot}AMP] \quad (12)$$

the system of differential eqs 1−4 reduces to three equations.

$$2\text{MLCP} + \text{MP} + \text{AMP} \underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} \text{MLCP}\cdot\text{MP} + \text{MLCP}\cdot\text{AMP} \xrightarrow{k'_2} \text{M} + \text{AM} + 2\text{MLCP}$$

Figure 3. The kinetic scheme of MLCP mediated myosin dephosphorylation. MLCP is the free enzyme, MP and AMP are the substrates, MLCP·MP and MLCP·AMP are the intermediate complexes, whereas M and AM are the final products. k'_1, k'_{-1}, and k'_2 are the rate constants.

The level of phosphorylated myosin cross bridges is defined as

$$u = u_0 + \frac{[\text{MP}] + [\text{AMP}] + [\text{MLCP}\cdot\text{AMP}] + [\text{MLCP}\cdot\text{MP}]}{[\text{M}]_{\text{TOT}}} \quad (13)$$

Equation 13 differs from the original definition of Rembold and Murphy[1] since the basal, Ca^{2+}-independent myosin phosphorylation (u_0) is also considered in the definition. The existence of this phenomenon is described in the work of Somlyo and Somlyo.[12] A value of the model parameter u_0 was estimated from the experimental work of Kai et al.[13] and Sieck et al.[8] showing the nonzero basal phosphorylation of approximately 20% and zero stress at resting $[Ca^{2+}]$. Furthermore, we consider complexes MLCP·AMP and MLCP·MP in the definition.

The stress is defined as

$$\sigma = \sigma_{\max}\frac{([\text{AMP}] + [\text{AM}] + [\text{MLCP}\cdot\text{AMP}])}{([\text{AMP}] + [\text{AM}] + [\text{MLCP}\cdot\text{AMP}])_{\max}} \quad (14)$$

where $\sigma_{\max}$ is the maximal mean stress and $([\text{AMP}]+[\text{AM}]+[\text{MLCP}\cdot\text{AMP}])_{\max}$ is the maximal concentration of attached cross bridges. Equation 14 also differs from the original definition[1] as the MLCP·AMP complex is considered as the stress-generating specimen.

In addition to the steady-state analysis we study the response of the system on biphasic Ca^{2+} transient signal. A biphasic Ca^{2+} transient is simulated by the mathematical expression

$$[Ca^{2+}](t) = \begin{cases} [Ca^{2+}]_r; & t < t_1 \\ [Ca^{2+}]_r + ([Ca^{2+}]_{\max} - [Ca^{2+}]_r)\dfrac{t - t_1}{t_1 - t_2}; & t_1 \le t \le t_2 \\ [Ca^{2+}]_p + ([Ca^{2+}]_{\max} - [Ca^{2+}]_p)e^{-k(t-t_2)}; & t > t_2 \end{cases} \quad (15)$$

where $[Ca^{2+}]_{\max}$, $[Ca^{2+}]_p$, and $[Ca^{2+}]_r$ are the peak, the plateau, and the resting Ca^{2+} concentrations of the signal, respectively. t_1 is the time at the initiation of Ca^{2+} increase, t_2 is the time at signal's peak value, and k is the rate constant of its descending phase.

Parameter values are listed in Table 1 along with the references consulted in obtaining them.

RESULTS AND DISCUSSION

The carried out calculations are (i) the steady-state isometric stress and MLC phosphorylation as functions of cytosolic $[Ca^{2+}]$, the steady-state isometric stress as a function of MLC phosphorylation, and (ii) the time courses of stress development corresponding to a Ca^{2+} transient in the form of biphasic signal. Software used in our calculations was Berkeley Madonna 8.0.1 (Macey&Oster). The system of ordinary differential equations is numerically integrated by the fourth-order Runge−Kutta integration method. An integration step of 2×10^{-4} s and the initial condition [M] = 30 μM are used in all calculations. The data from ref 8 are obtained by the use of Digitizer tool in Origin 6.1.

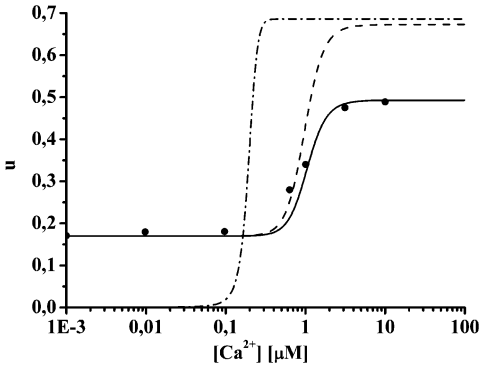

Figure 4. Steady-state MLC phosphorylation (u) versus $[Ca^{2+}]$: results of the present model for $k^0 = 1.6\ s^{-1}$ (solid line) and $k^0 = 3.1\ s^{-1}$ (dashed line); result of the original mode[l1] (dash−dot line); experimental data of Figure 11B in ref 8 (full circles). Other model parameters are given in Table 1.

Table 1. Model Parameters

parameter	value	references
Rate Constants		
k°	(see text)	
k'_2	16 s^{-1}	14
k_3	0.4 s^{-1}	1, 9
k_4	0.1 s^{-1}	1, 9
k_7	0.02 s^{-1}	1, 9
k_8	0.001 s^{-1}	1
Others		
u_0	0.17	13
$\sigma_{\max}$	14.5 N/cm^2	8
$(\text{AMP}+\text{AM}+\text{MLCP}\cdot\text{AMP})_{\max}$	22.8 μM	
$[\text{MLCP}]_{\text{TOT}}$	10 μM	14
$[\text{MLCK}]_{\text{TOT}}$	1.0 μM	2
$[\text{CaM}]_{\text{TOT}}$	10 μM	2
$[\text{M}]_{\text{TOT}}$	30 μM	2
K_1	10 μM	2
K_2	0.001 μM	2
K_M	15 μM	14

In the first case (i) cytosolic $[Ca^{2+}]$ is treated as a parameter within the interval 0.001 μM−100 μM. Step changes of $[Ca^{2+}]$ are calculated from the geometric series interpolated between the boundaries of the interval. For each value of $[Ca^{2+}]$ differential equations are numerically integrated as it is described above, until the steady state is achieved with the tolerance smaller than 10^{-6}. The steady-state values of the system variables [MP], [AMP], [AM], [MLCP·MP], and [MLCP·AMP] are used in the predictions of myosin phosphorylation and stress.

Figure 4 shows our model results for MLC phosphorylation versus $[Ca^{2+}]$ calculated for two different values of the parameter k^0. Both predictions are compared with the experimental data published by Sieck et al.[8] as well as with

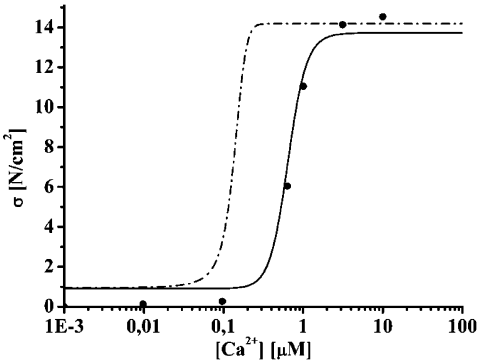

Figure 5. Steady-state stress (σ) versus [Ca^{2+}]: result of the present model for $k^0 = 1.6\ s^{-1}$ (solid line); result of the original model[1] (dash−dot line); experimental data of Figure 11A in ref 8 (full circles). Other model parameters are given in Table 1.

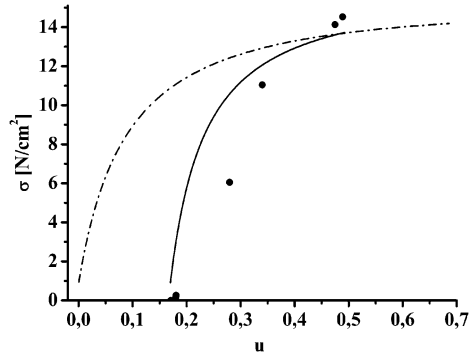

Figure 6. Steady-state stress (σ) versus myosin phosphorylation (u): result of the present model for $k^0 = 1.6\ s^{-1}$ (solid line); result of the original model[1] (dash−dot line); experimental data[8] (full circles). Model parameters are given in Table 1.

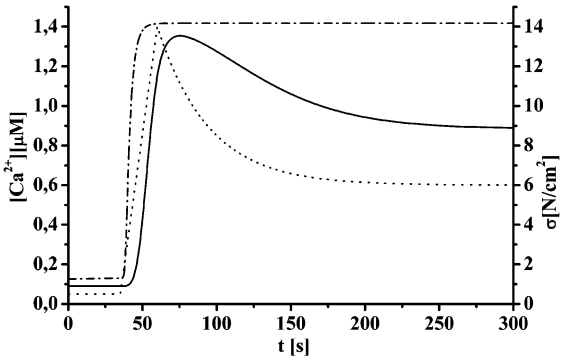

Figure 7. Simulated Ca^{2+} transient and corresponding time-dependent stress development: mathematically generated Ca^{2+} spike (eq 15) (dotted line), time courses of stress development obtained by the original model[1] (dash−dot line) and with our present model for $k^0 = 1.6\ s^{-1}$ (solid line). Values of the model parameters used for simulation of Ca^{2+} transient are $[Ca^{2+}]_r = 0.05\ \mu M$, $[Ca^{2+}]_p = 0.60\ \mu M$, $[Ca^{2+}]_{max} = 1.4\ \mu M$, $t_1 = 35$ s, $t_2 = 60$ s, $k = 0.029\ s^{-1}$. Other values of model parameters are given in Table 1.

the predictions of the original model proposed by Rembold and Murphy.[1] The prediction of the original model[1] differs significantly from experimental data[8] and our model predictions. Two values for parameter k^0 are selected for comparison; first, the value $k^0 = 3.1\ s^{-1}$ is selected to obtain the same maximal level of phosphorylation as predicted by the original model, and second, the value $k^0 = 1.6\ s^{-1}$ is selected for the best fit to experimental data. The discrepancy between our model prediction and the original prediction of Rembold and Murphy originates from the fact that completely different methods are used in the modeling of parameter values k_1 and k_6 describing the activation of MLCK. These differences were recently discussed in our accompanying manuscript in the *Journal of Chemical Information and Modeling*.[15] The study[15] shows that the model prediction of Kato et al.[2] is in better agreement with experimental results of quantitative measurements of Ca^{2+}/CaM-dependent MLCK activation[16] than the semitheoretical prediction of Rembold and Murphy.[1] However, the comparison shows[15] that it is possible to achieve even better agreement with experimental results, albeit with more complex models.[15,17] Summing up, three theoretical refs 2, 15, and 17 and several experimental refs 8, 13, 16, and 18 speak in favor of reasonableness of incorporating the theoretical prediction of MLCK activation into the modeling of stress generation. Taking into account the Ca^{2+}-independent myosin phosphorylation (u_0) improves the model results.

Figure 5 presents the model results of the steady-state stress (σ) versus [Ca^{2+}]. The discrepancy between predictions of original and our present model is evident since the original prediction achieves its half-maximal value at significantly lower [Ca^{2+}] (0.13 μM) as measured by experiment[8] (0.6 μM). However, the complete agreement with experimental results is not achieved. The predicted Ca^{2+}-dependent steady-state stress overestimates known experimental data at low [Ca^{2+}]. This is due to an inherent model property that even at [Ca^{2+}] = 0 the cross bridge cycling system does not relax to only detached myosin cross bridge species.

The diagram in Figure 6 shows that rather small differences between our model predictions and experimental data presented in Figures 4 and 5 implicate relatively significant differences in the diagram where steady-state stress (σ) is plotted versus myosin phosphorylation (u) (Figure 6). However, any final conclusions cannot be made since only 4 adequate experimental data points are available for comparison.

In the second case (ii) a typical Ca^{2+} transient observed in smooth muscle cells[8] is incorporated in the modeling. Figure 7 shows Ca^{2+} transient similar to that presented in Figure 10 of ref 8 only that it is described by the mathematical expression (15) (dotted line). Furthermore, corresponding two simulations of temporal evolution of stress obtained by our present model (solid line) as well as by original model[1] (dash−dot line) are presented. Comparison between both model predictions shows significant difference in the time delay of the temporal evolution of stress. References 8 and 10 speak in favor of our model prediction. Reference 10 reports detailed temporal aspects of excitation-contraction coupling in airway smooth muscles; i.e., the time of 16 s is required for force to reach the half-maximal value after the release of caged Ca^{2+}, on the other hand, approximately 4 times lower $t_{1/2}$ required for force relaxation is reported. Although the direct comparison between these experimental observations[10] and our model predictions is impossible due to different Ca^{2+} signals considered, the results of our model indicate the temporal behavior of the same order of magnitude as reported in the experiments.[8,10] Much slower stress relaxation and generation in comparison to the original model is in our model a consequence of the slow-rate MLCP-mediated myosin dephosphorylation kinetics and of a different approach in the modeling of MLCK activation kinetics. In contrast to our model the stress generation predicted by the original model[1] is triggered at too low [Ca^{2+}] (see also

Figure 5), which is reflected in overestimated stress despite a relatively slow changing [Ca^{2+}] considered in the transient presented in Figure 7.

Comparisons of Figures 4−7 show that our model represents an improvement in the modeling of stress generation in smooth muscle cells with respect to the original model presented by Rembold and Murphy;[1] however, a complete agreement between theoretical model and experiment has not been achieved yet. The recently published complex models of Ca^{2+}/CaM-dependent MLCK activation,[15,17] based on the novel experimental evidences,[19−21] showed that the interactions among these species are much slower as previously thought. Thus, a slow activation/inactivation of MLCK might contribute to an additional time delay in stress generation/relaxation.

CONCLUSIONS

The original model proposed by Hai and Murphy[9] and later adopted by Rembold and Murphy[1] takes into account four different states of cross bridges and represents one of the first attempts of theoretical modeling of Ca^{2+}-mediated stress generation in smooth muscles. We would like to point out that the original model[1] is not stated on the purely theoretical basis since the kinetics of particular reaction steps is based upon the semiquantitative approach; i.e., by fitting the experimental data with mathematical expression and incorporating it in the modeling by using the model constraints at equilibrium. By this approach the applicability of the original model is rather limited.

Here we present and study an expanded 4-state cross bridge cycling model on a theoretical basis. The significant changes between the present model and the original models[1,9] are as follows: (a) The semitheoretical prediction of MLCK activity (k_1 and k_6) used in previous studies[1,9] is in this work replaced by the purely theoretical prediction based on the mathematical model of interactions between Ca^{2+}, CaM, and MLCK, proposed by Kato et al.[2] (eqs 6 and 7). Thus, the model prediction of stress can be controlled and regulated by changing the values of the model parameters. (b) Instead of the constant MLCP activity, i.e., constants k_2 and k_5 previously used in the mathematical description of dephosphorylation,[1,9] this phenomenon is treated as the enzymatic reaction and modeled with the Michaelis−Menten kinetics in the present study (eqs 8−11). According to this novel approach the definitions of phosphorylation (13) and stress (14) have been modified. Moreover, we gained the temporal delay of stress development with respect to the Ca^{2+} transient (Figure 7). (c) Ca^{2+} independent dephosphorylation is also considered and described by the additive constant term in the definition of dephosphorylation (u_0 in eq 13), which shifts the phosphorylation/[Ca^{2+}] relationship upward.

Not only that our model became purely theoretical, even more, the effects of these novel approaches in modeling are visible in nearly all aspects of the steady state and temporal analysis of the model system. Hence, our model can predict experimentally observed properties of Ca^{2+}-dependent smooth muscle contraction, which cannot be predicted by the original model.[1]

The main physiological implication of the present model is the prediction of the temporal delay of stress generation with respect to the Ca^{2+} transient. Namely, excitation-contraction coupling in smooth muscle cells involves a cascade of intracellular events, whereby all of them contribute to the temporal delay that is in an order of seconds and is substantially longer than in striated muscles.[10] It has been hypothesized[10] that the coupling of elevations and reductions in [Ca^{2+}] to stress generation and relaxation is much slower than acetylcholine-induced Ca^{2+} oscillations in airway smooth muscle cells, which may lead to stable force generation. Our preliminary calculations show (results not shown) that the coupling of Ca^{2+} oscillations to the present model results in a stable stress generation, i.e., oscillations of stress with very low amplitude compared to its mean value. Furthermore, we expect that the time delay in contractile response predicted by the mathematical model might be even larger since we have recently shown in our accompanying paper[15] in the *Journal of Chemical Information and Modeling* that the process of Ca^{2+}/CaM-dependent MLCK activation adds an additional significant time delay that has been neglected in the present as well as in the previous studies. In contrast to our recent studies[15,17] it has been previously hypothesized[22,23] that the process of MLCK activation is extremely fast and is not likely to contribute more than a few milliseconds to the overall delay. On the basis of our model predictions and preliminary results (not shown here) we hypothesize that the oscillatory input Ca^{2+} signal might be decoded into rather constant force output, especially in the frequency range of several Hz as observed in porcine[10] and rat[24] airway smooth muscle cells. This will be in accordance with the experimental observations that oscillatory Ca^{2+} signals in single airway smooth muscle cells lead to a stable force generation on the tissue level.[10]

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CI050178A