Combined model of bladder detrusor smooth muscle and interstitial cells

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Abstract

Although patients with lower urinary tract symptoms constitute a large and still growing population, understanding of bladder detrusor muscle physiology remains limited. Understanding the interactions between the detrusor smooth muscle cells and other bladder cell types (e.g. interstitial cells, IC) that may significantly contribute to coordinating and modulating detrusor contractions represents a considerable challenge. Computer modeling could help to elucidate some properties that are difficult to address experimentally; therefore, we developed *in silico* models of detrusor smooth muscle cell and interstitial cells, coupled through gap junctions. The models include all of the major ion conductances and transporters described in smooth muscle cell and interstitial cells in the literature. The model of normal detrusor muscle (smooth muscle cell and interstitial cells coupled through gap junctions) completely reproduced the experimental results obtained with detrusor strips in the presence of several pharmacological interventions (ryanodine, caffeine, nimodipine), whereas the model of smooth muscle cell alone (without interstitial cells) failed to reproduce the experimental results. Next, a model of overactive bladder, a highly prevalent clinical condition in both men and women with increasing incidence at older ages, was produced by modifying several processes as reported previously: a reduction of Ca^{2+} -release through ryanodine receptors and a reduction of Ca^{2+} -dependent K^{+} -conductance with augmented gap junctional coupling. This model was also able to reproduce the pharmacological modulation of overactive bladder. In conclusion, a model of bladder detrusor muscle was developed that reproduced experimental results obtained in both normal and overactive bladder preparations. The results indicate that the non-smooth muscle cells of the detrusor (interstitial cells) contribute significantly to the contractile behavior of bladder detrusor muscle and should not be neglected. The model suggests that reduced Ca^{2+} -release through ryanodine receptors and Ca^{2+} -dependent K^{+} -conductance together with augmented gap junctional coupling might play a major role in overactive bladder pathogenesis.

Keywords: Model, bladder, smooth muscle cell, detrusor muscle, interstitial cell, overactive bladder

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Introduction

Smooth muscles represent complex and heterogeneous tissues that are present in a number of internal organs. These tissues contain a variety of cell types other than muscle cells, including interstitial cells (IC). IC in smooth muscle organs are receiving increasing attention after they were shown to be functionally connected with smooth muscle cells (SMCs), and a number of regulatory functions in both normal and pathophysiological conditions have been suggested.¹ In the bladder, the IC may coordinate detrusor activity by transmitting and amplifying neural signals and/or contribute to spontaneous detrusor activity.²

IC in the bladder carry the same receptor tyrosine kinase c-kit marker as IC in the gut and are located in the suburothelium as well as in a network inside the detrusor muscle.^{3,4} Experiments with a c-kit tyrosine kinase inhibitor indicated that IC may be responsible for generating bursts of action potentials and contractions in detrusor smooth muscle.⁵ IC were also demonstrated to respond to cholinergic stimulation by firing calcium transients.⁶ With regard to the close structural relationship between IC and cholinergic nerves shown by immunohistochemical labeling and confocal microscopy, IC may transmit, disseminate, and amplify cholinergic signals from nerves to detrusor SMCs.⁷

Taken together, IC seem to play a central role in bladder contractile phenomena, both in spontaneous events (spontaneous action potentials, calcium transients and contractions) during filling and in parasympathetic voiding contractions.

Upregulation of IC has been suggested to be involved in bladder pathologies such as overactive bladder (OAB).⁸ OAB induced by bladder outlet obstruction was shown to be associated with an increased population of IC in both guinea-pig and rat models.^{9–11} Cultured IC from OABs showed spontaneous calcium waves with higher frequency and lower amplitude than those from control bladders.¹⁰ Stretch-induced calcium transients were detected in cultured IC, and those generated in IC from OAB were more likely to transfer to adjacent SMC than those generated in IC from control bladders.¹⁰ IC represent a potential therapeutic target for patients with lower urinary tract symptoms and merit further investigation by all available methods. Computer modeling provides a unique opportunity to study the functional interactions of the two cell types, detrusor SMC and IC, which cannot easily be addressed in biological preparations. The presence of IC inside detrusor muscle limits the interpretation of experimental results obtained in bladder muscle strips, although they are peeled from the mucosa and lamina propria.¹² The contributions of each of these cell types (IC and/or SMC) to contractile behavior of the bladder cannot be unequivocally defined and remain unclear.

In this study, the *in silico* models of detrusor SMC and IC were developed and combined together to create a new more realistic model of bladder detrusor activity. The resultant model reproduced previous experimental data obtained in both normal and pathological (OAB) preparations, including several pharmacological interventions.

Materials and methods

The models of normal detrusor muscle consist of models of SMC and IC coupled through gap junctions (Figure 1(a)). The model of SMC (Figure 1(b)) is based on vascular SMC models.^{13,14} The model has four variables, the calcium concentration in the cytosol (c), the calcium concentration in the sarcoplasmic reticulum (s), the membrane potential (v), and the open state probability (w) of calcium-activated potassium channels. The variables c and s correspond to the intracellular oscillator, v and w to the membrane oscillator.¹³

The set of nonlinear differential equations is provided in the Appendix and it includes the following ionic fluxes: the calcium release from IP3-sensitive stores (J_{IP3}) that depends on the concentration of IP3, the calcium-induced calcium release from the endoplasmic/sarcoplasmic reticulum (J_{CICR}) via ryanodine receptors/channels, the calcium influx through the sarcolemmal voltage-operated calcium channels (J_{VOCC}), the leak from endoplasmic/sarcoplasmic reticulum (J_{leak}), calcium influx through the sarcolemma via mechanosensitive channels (stretch-activated channels, $J_{stretch}$), the Na^+/Ca^{2+} exchange ($J_{Na/Ca}$), the sarcoplasmic reticulum uptake ($J_{SRuptake}$), the calcium extrusion from the SMC by the sarcolemmal calcium pump (sarcolemmal Ca^{2+} -ATPase, $J_{extrusion}$),

the sodium pump (Na^+/K^+ -ATPase, $J_{Na/K}$), the chloride (J_{Cl}), and potassium (J_K) current. Equations for these fluxes are provided in the Appendix.

The model of IC (Figure 1(c)) with five pacemaker units is based on the gastrointestinal IC model.^{15,16} The model variables may be divided into the pacemaker unit state variables, the whole cell current gating variables, and the global variables.^{15,16} The pacemaker unit state variables include – calcium concentration in subspace 1 (C_{S1}), calcium concentration in subspace 2 (C_{S2}), calcium concentration in endoplasmic reticulum (C_{ER}), mitochondrial calcium concentration (C_{MT}), sodium concentration in subspace 1 (N_{S1}), IP3R control variable (H), and IP3R recovery variable (ζ). The whole cell current gating variables include $I_{Ca(T)}$ activation rate (d_T), $I_{Ca(T)}$ inactivation rate (f_T), $I_{Ca(T)}$ open probability (O_T), $I_{K(v1.1)}$ activation ($d_{v1.1}$), $I_{K(v1.1)}$ inactivation ($f_{v1.1}$), $I_{K(ERG)}$ activation (d_{ERG}). The global variables are the membrane potential (V_m) and the bulk cytoplasmic calcium concentration (C_{Cy}). The set of nonlinear differential equations for calculating the variables is provided in the Appendix.

The model conductances may be divided into the pacemaker unit conductances and the bulk cytoplasmic subspace conductances. The pacemaker unit conductances include the following conductances and fluxes: inward Ca^{2+} current (I_{Ca}), inward Na^+ current (I_{Na}), non-selective cation conductance (I_{NSCC}), plasma membrane Ca^{2+} -ATPase (I_{PM}), outward Na^+ pump (I_{NaP}), sarco-endoplasmic reticulum Ca^{2+} ATPase (J_{SERCA}), IP3 receptor Ca^{2+} flux (J_{IPR}), mitochondrial Ca^{2+} uniporter (J_{MCU}), mitochondrial Na^+/Ca^{2+} exchanger (J_{NCX}), subspace 1/subspace 2 Ca^{2+} diffusion (J_{S1S2}), subspace 2/bulk cytoplasmic Ca^{2+} diffusion (J_{S2Cy}). The bulk cytoplasmic subspace conductances and fluxes include T-Type Ca^{2+} current ($I_{Ca(T)}$), Ca^{2+} extrusion pump ($I_{Ca(Ext)}$), $K(v1.1)$ K^+ current ($I_{K(v1.1)}$), ERG K^+ current ($I_{K(ERG)}$), background K^+ current (I_{KB}), non-selective inward current (I_L), bulk cytoplasmic intracellular Ca^{2+} flux (J_{Cy}). Equations for these conductances and fluxes are provided in the Appendix.

Communication of IC and SMC

The communication between SMC and IC (Figure 1(a)) was modeled as proposed by Koenigsberger *et al.*¹⁴ for one 2D layer of SMC. Gap junctions are modeled as a single global conductance/permeability between SMC and ICC (Figure 1(a)). The electrical coupling between cells is modeled as

$$V_{coupling} = -g * (v - V_m)$$

in which the gap junctional electrical coupling coefficient g is related to the gap junctional conductance G by

$$g = G/C_m$$

where C_m is the cell membrane capacitance. The conductance G reflects the composite junctional conductance to small cytoplasmic ions.¹⁴ The term for electrical coupling was added for each cell to the equations for dv/dt .

Fig. 1.

Figure 1 Schematic representation of major ionic fluxes in the model. (a) Schematic representation of normal detrusor: IC coupled with SMC through gap junction. (b) Schematic representation of major ionic fluxes in the model of SMC. SR: sarcoplasmic reticulum. (c) Schematic representation of major ionic fluxes in the model of IC. ER: endoplasmic reticulum; M: mitochondria; PU1-5: pacemaker unit 1-5

The calcium coupling describing the intercellular calcium diffusion is modeled by

$$J_{\text{coupling}} = -p \cdot (c - C_{Cy})$$

in which the gap junctional calcium coupling coefficient p is related to the gap junctional permeability P by

$$p = P/l$$

where l represents the mean diffusion path in a cell in the order of the cell width.¹⁴ The term for calcium coupling was added for each cell to the equations for dc/dt .

Model programs

The above described models were implemented into MATLAB Package, which allows dynamic analysis of the corresponding nonlinear dynamic systems.

Results

Simulation of individual isolated SMC revealed behavior incompatible with the experimental results (e.g. Jiang *et al.*¹²): small amplitude and fast frequency (5.5 transients/min) of Ca^{2+} transients and membrane potential waves (Figure 2(a) and (b), dotted line). When SMC were coupled with IC through calcium (Figure 2(c)) and electrical (Figure 2(d)) coupling, slow spontaneous Ca^{2+} transients

and action potentials with a frequency corresponding to experimental recordings of the bladder contractile activity (Figure 2(a) and (b), full line) appeared in the SMC. The IC served as the pacemaker, as the spontaneous Ca^{2+} transients (Figure 2(e)) and action potentials (Figure 2(f)) of IC preceded those of SMC (Figure 2(a) and (b)).

Next, we tried to model the effects of several pharmacological interventions affecting Ca^{2+} handling that had previously been tested experimentally. Ryanodine selectively

Figure 2 Model of normal detrusor consisting of SMC and IC. (a) Spontaneous Ca^{2+} transients in an isolated SMC (dotted line) and normal detrusor SMC (SMC coupled with IC, full line). (b) Membrane potential in an isolated SMC (dotted line, no action potential) and normal detrusor SMC (SMC coupled with IC, full line, action potentials present). (c) Calcium coupling between SMC and IC in normal detrusor model (full line). The zero dotted line shows no coupling of the isolated SMC model. (d) Electrical coupling between SMC and IC in normal detrusor model (full line). The zero dotted line shows no coupling of the isolated SMC model. (e) Spontaneous Ca^{2+} transients in IC. (f) Membrane potential (action potentials) in IC

Table 1 Parameters of the model

Parameter	Norm.	Norm. Ryan Low	Norm. Ryan High	Norm. Caff	Norm. Nim	OAB	OAB Ryan Low	OAB. Ryan High	OAB Caff	OAB Nim
GCa	0.00175	0.00175	0.00175	0.00175	0.00001	0.00175	0.00175	0.00175	0.00175	0.00001
CC	75	52.5	4	0.2	75	25	13.5	2	0.2	25
sc	2	2.3	2.3	2	2	2	2	2	2	2
cc	0.85	0.85	0.85	0.7	0.85	0.85	0.85	0.85	0.7	0.85
λ RyR	1	1	1	1	1	0.4	0.4	0.4	0.4	0.4
Rk	12	12	12	12	12	12	12	12	12	12
Gk	0.0046	0.0046	0.0046	0.0046	0.0046	0.003	0.003	0.003	0.003	0.003
pp	0.1	0.1	0.1	0.1	0.1	0.225	0.225	0.225	0.225	0.225
gg	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11

Note: Only parameters, which were modified according to the reported pathophysiological and/or pharmacological effects, are shown in this Table.

Norm: normal detrusor (SMC+ICC); Ryan low: low-dose ryanodine; Ryan high: high-dose ryanodine; Caff: caffeine; Nim: nimodipine; OAB: overactive bladder; GCa: whole-cell conductance for VOCC (ICaL); CC: CICR rate constant; sc: half-point of the CICR Ca^{2+} efflux sigmoidal; cc: half-point of the CICR activation sigmoidal; λ RyR: CICR coefficient; Rk: maximum slope of the K_{Ca} activation sigmoidal; Gk: whole-cell conductance for K^{+} efflux; pp: gap junctional calcium coupling coefficient; gg: gap junctional electrical coupling coefficient.

affects the ryanodine receptor/ Ca^{2+} -release channels (RyR) of the sarcoplasmic reticulum and the effect is dose-dependent: at low doses it preferentially activates RyR, whereas at high doses it blocks RyR.¹⁷ These dose-dependent effects are probably reflected by the conflicting results of several studies, which reported either reduced amplitude with no change in the frequency of spontaneous bladder muscle contraction (i.e. block of Ca^{2+} -release) or increased frequency with no change in amplitude of the contraction (i.e. activation of Ca^{2+} release).^{12,18} By modifying the parameters of sarcoplasmic reticulum Ca^{2+} -release (Table 1), the model was able to reproduce both types of behavior: an increased frequency of spontaneous Ca^{2+} transients (Figure 3(a), (c), (e)) or reduced amplitude of Ca^{2+} transients (Figure 3(b), (d), (f)). The higher frequency of Ca^{2+} transients was associated with slightly higher fluxes through RyR (J_{RyR} , Figure 3(c)) and Ca^{2+} -dependent K^{+} channels (J_{KCa} , Figure 3(e)), thus reproducing the effects of a low dose of ryanodine. The effect of high-dose ryanodine was modeled by reducing the sarcoplasmic reticulum Ca^{2+} -release (Figure 3(d)), which was associated with a reduced amplitude of Ca^{2+} transients (Figure 3(b)) and diminished Ca^{2+} -dependent K^{+} -currents (Figure 3(f)).

Caffeine is known to lock RyR in open state and to mobilize intracellular Ca^{2+} stores, consequently suppressing Ca^{2+} -induced Ca^{2+} -release from the endoplasmic/sarcoplasmic reticulum via RyR, Ca^{2+} transients and contractions.⁹ It also activates the Ca^{2+} -dependent K^{+} -conductance probably due to a (local) increase in Ca^{2+} concentration.¹⁹ These effects were completely reproduced by the model: inhibition of phasic Ca^{2+} -release led to a substantial suppression of Ca^{2+} -transients (Figure 4(a)), whereas Ca^{2+} -dependent K^{+} -conductance was significantly increased (higher amplitude and longer duration, Figure 4(c)).

Nimodipine, a blocker of voltage-operated Ca^{2+} -channels (VOCC), was reported to suppress spontaneous detrusor contractions.¹² The inhibition of VOCC (Figure 4(d)) completely suppressed the spontaneous Ca^{2+} transients (Figure 4(b)), which matched the experimental data.

In the second part of the study, we tested whether the model could reproduce the OAB phenotype. In OAB, the gap junctional coupling between SMC and IC was reported to be increased,^{9,10} but the number of RyR¹⁰ and Ca^{2+} -dependent K^{+} -currents²⁰⁻²² were found to be reduced. A model with decreased RyR conductance and Ca^{2+} -dependent K^{+} -conductance and with augmented gap junctional coupling (Table 1) reproduced the OAB phenotype: spontaneous Ca^{2+} transients with variable amplitude and high frequency (Figure 5(a)) and action potentials with high frequency (Figure 5(b)). The elevated gap junctional calcium coupling coefficient (Table 1) resulted in increased gap junctional coupling fluxes (J_{coupling} , Figure 5(c)). Interestingly, a reduction of RyR conductance and Ca^{2+} -dependent K^{+} -conductance (Table 1) did not result in a reduction of the RyR Ca^{2+} flux (J_{RyR} , Figure 5(d)) or the Ca^{2+} -dependent K^{+} -current (J_{KCa} , Figure 5(e)). These paradoxical findings may be explained by higher Ca^{2+} levels in the sarcoplasmic reticulum (Figure 5(f)).

Finally, the pharmacological interventions affecting Ca^{2+} handling were tested in an OAB setting. Both the stimulatory (low dose) and inhibitory (high dose) effects of ryanodine on RyR were reproduced by the model: low-dose ryanodine (stimulatory) intervention was associated with larger RyR Ca^{2+} -release (Figure 6(e)) and preserved Ca^{2+} transients (Figure 5(a)), high-dose ryanodine (inhibitory) intervention led to suppression of both RyR Ca^{2+} -release (Figure 6(e)) and Ca^{2+} transients (Figure 6(b)). Modeling the effects of caffeine led to a suppression of Ca^{2+} transients (Figure 6(c)) and elevated Ca^{2+} -dependent K^{+} -current (J_{KCa} , Figure 6(f)). Inhibition of VOCC by nimodipine (Figure 6(g)) completely suppressed spontaneous Ca^{2+} transients (Figure 6(d)). In general, the model completely reproduced the experimental data reported earlier in the OAB setting.¹²

Discussion

The primary aim of this study was to develop a model of bladder detrusor muscle that would involve both

Figure 3 Modeling the dual effect of ryanodine in normal detrusor. (a) Spontaneous Ca^{2+} transients in the absence (control, dotted line) and the presence of low-dose ryanodine (full line) in the model of normal detrusor. (b) Spontaneous Ca^{2+} transients in the absence (control, dotted line) and the presence of high-dose ryanodine (full line) in the model of normal detrusor. (c) RyR Ca^{2+} fluxes in the absence (control, dotted line) and the presence of low-dose ryanodine (full line) in the model of normal detrusor. (d) RyR Ca^{2+} fluxes in the absence (control, dotted line) and the presence of high-dose ryanodine (full line) in the model of normal detrusor. (e) Ca^{2+} -dependent K^{+} -currents in the absence (control, dotted line) and the presence of low-dose ryanodine (full line) in the model of normal detrusor. (f) Ca^{2+} -dependent K^{+} -currents in the absence (control, dotted line) and the presence of high-dose ryanodine (full line) in the model of normal detrusor

SMC and IC, and to test whether such a model is able to reproduce previous experimental data. To the best of our knowledge, there is currently no model of bladder IC available; therefore, in this study a model of bladder IC was developed based on available models of IC in the gastrointestinal tract.^{15,16} In the gastrointestinal tract, IC generate spontaneous rhythmic electrical activity that was attributed to intracellular Ca^{2+} dynamics (for review see Sanders *et al.*²³). This pacemaker activity is supposed to be generated

within pacemaker units that include the endoplasmic/sarcoplasmic reticulum, mitochondria, and plasma membrane.²⁴ Faville *et al.*¹⁵ developed a model that described such an activity in a single pacemaker unit. This pacemaker unit model was later expanded to implement a discrete pacemaker unit population within the whole cell modeling framework,¹⁶ thus being superior to the model of Corrias and Buist²⁴ with a single bulk pacemaker unit. Based on the analysis of the unitary potential amplitude, five pacemaker

Figure 4 Modeling the effect of caffeine and nimodipine in normal detrusor. (a) Spontaneous Ca^{2+} transients in the absence (control, dotted line) and the presence of caffeine (full line) in the model of normal detrusor. (b) Spontaneous Ca^{2+} transients in the absence (control, dotted line) and the presence of nimodipine (full line) in the model of normal detrusor. (c) Ca^{2+} -dependent K^+ currents in the absence (control, dotted line) and the presence of caffeine (full line) in the model of normal detrusor. (d) VOCC currents in the absence (control, dotted line) and the presence of nimodipine (full line) in the model of normal detrusor

units were suggested as the lower limit of the number of pacemaker units, because smaller population sizes would produce unitary potentials greater than half the amplitude of full slow waves. Based on this recommendation, five pacemaker units were implemented in our IC model as a compromise between the computational expense of simulating and physiological relevance. A simpler model with a single pacemaker unit was also tested, and the differences between the models with a single pacemaker unit and five pacemaker units were negligible. Furthermore, the original Faville's model of IC pacemaker unit did not include ryanodine-sensitive Ca^{2+} -stores, only IP_3 -sensitive stores.¹⁵ Since Ca^{2+} -release from ryanodine-sensitive stores in IC has now been documented,⁶ we refined the pacemaker unit model by implementing Ca^{2+} -fluxes from ryanodine-sensitive intracellular stores (endoplasmic reticulum) to both of the cytoplasmic spaces of the model ($\text{J}_{\text{ICRER/S2}}$ and $\text{J}_{\text{ICRER/Cy}}$). It should be mentioned, however, that the theory of pacemaker unit (Sander's hypothesis¹⁶) was recently substantially challenged^{25,26} and a novel model of small intestinal IC pacemaker activity that incorporates Ca^{2+} -activated Cl^- current and may be relevant also for bladder IC, was developed.²⁷

The model of bladder SMC was derived from previous models of vascular SMCs^{13,14} and it involves all major ionic

fluxes of the plasma membrane and of the endoplasmic/sarcoplasmic reticulum. Advanced gastrointestinal smooth muscle models may represent another valuable alternative (e.g. Corrias and Buist²⁸), especially for multiscale modeling of tissue or organ function.^{29,30} Since the IC located in the bladder detrusor are closely associated with SMCs⁴ and were reported to express connexin 43,³¹ it is likely that they communicate through gap junctions. Therefore, the gap junctional coupling between IC and SMC was modeled according to Koenigsberger *et al.*¹⁴ as the electrical coupling that reflects the composite permeability to small cytoplasmic ions and the calcium coupling for the diffusion of Ca^{2+} . The coupling of IC and SMC in our model was necessary for the induction of spontaneous activity, suggesting that IC may act as the pacemaker of bladder detrusor muscle at basal conditions with no significant nervous and/or humoral stimulation present. Nevertheless, the gap junctional communication must be regarded as a hypothetical model since the gap junctional coupling between SMC and IC was never unambiguously documented. Gap junctions were shown between bladder SMCs^{32,33} as well as between IC^{34,35} but, to the best of our knowledge, never between SMC and IC.

Although the model was developed and optimized for studying the (patho)physiology of the bladder, it may

Figure 5 Modeling OAB. (a) Spontaneous Ca^{2+} transients in OAB. (b) Membrane potential (action potentials) in normal detrusor (dotted line) and in OAB (full line). (c) Gap junctional coupling between SMC and IC in normal detrusor (dotted line) and in OAB (full line). (d) RyR Ca^{2+} fluxes in normal detrusor (dotted line) and in OAB (full line). (e) Ca^{2+} -dependent K^{+} -currents in normal detrusor (dotted line) and in OAB (full line). (f) Ca^{2+} levels in the sarcoplasmic reticulum of normal detrusor (dotted line) and of OAB (full line)

be easily adapted and used for modeling other organ systems. IC have been described in a number of smooth muscle organs (e.g. gastrointestinal tract, urinary tract, reproductive tract, blood vessels), and they probably can form gap junctions with each other and with adjacent SMCs in these tissues.¹ The models of both IC and SMC used in this study are rather complex and include, to the best of our knowledge, all of the major ionic currents and transporters described so far in SMC and IC including their regulation by intracellular Ca^{2+} . By adjusting the relative contributions

of individual currents/transporters according to current experimental results, the SMC and IC of different organs may be reproduced. If a new significant transport mechanism is found, it can be easily incorporated into the existing model. Similar approach was already repeatedly employed in the field of gastrointestinal physiology on several levels of biological complexity, from (sub)cellular models to tissue and whole organ levels providing a complex in silico organ modeling framework for addressing complex clinical problems.^{29,30} The interactions between IC and SMC were

Figure 6 Modeling the effects of pharmacological interventions in OAB. (a) Spontaneous Ca^{2+} transients in the presence of low-dose ryanodine in the model of OAB. (b) Spontaneous Ca^{2+} transients in the presence of high-dose ryanodine in the model of OAB. (c) Spontaneous Ca^{2+} transients in the presence of caffeine in the model of OAB. (d) Absence of spontaneous Ca^{2+} transients in the presence of nimodipine in the model of OAB. (e) RyR Ca^{2+} fluxes in the absence of ryanodine (control OAB, dotted line), in the presence of low-dose ryanodine (full line) and in the presence of high-dose ryanodine (dash-and-dotted line) in OAB. (f) Ca^{2+} -dependent K^+ -currents in the absence (control OAB, dotted line) and in the presence of caffeine (full line) in OAB. (g) VOCC currents in the absence (control OAB, dotted line) and the presence of nimodipine (full line) in OAB

recently modeled also in uterine tissue.³⁶ In a system consisting of myocytes coupled to passive (IC-like) cells, which was extended to a simple two-dimensional lattice model of the tissue, they were able to demonstrate that an increase in inter-cellular electrical coupling strongly facilitated the appearance of spontaneous action potentials that may eventually lead to parturition.

OAB is defined as symptom complex comprising urinary urgency, with or without urge incontinence, usually with frequency and nocturia in the absence of other local or metabolic factors that would account for the symptoms (e.g.³⁷). The prevalence of OAB is estimated at between 10 and 20%, increasing with age and roughly similar in men and women.^{38,39} The pathophysiology of OAB is not clear, the main underlying mechanisms may be divided into neurogenic,⁴⁰ myogenic,⁴¹ or combined. In this study, only the myogenic theory was addressed, since the model is limited to SMC and IC. The model, however, reproduced experimental results obtained with OAB preparations, which suggests that myogenic mechanism might be dominant in some conditions. A number of alterations were described in OAB SMC that could contribute to the OAB phenotype. RyR protein expression was shown to be reduced to approximately 25% of control expression in a rat model of detrusor instability and bladder outlet obstruction.¹² A consequent reduction in Ca^{2+} -release through RyR would decrease the available Ca^{2+} -dependent K^{+} -conductance, as the large conductance Ca^{2+} -activated K^{+} (BK) channels were demonstrated to be activated by local Ca^{2+} release through RyR.⁴² Beside this Ca^{2+} -dependent mechanism, the Ca^{2+} -dependent K^{+} -conductance in OAB is also reduced as demonstrated by several studies in both experimental models^{20,21} and patients.^{20,22} Another interesting mechanism that could contribute to the development of OAB is that c-kit labelling showed significantly more IC in OABs,^{9,11,43} and that the c-kit receptor blockers had inhibitory effects on the overactive human detrusor.⁴³ Since there is a substantial body of evidence that points to a central role of IC in suburothelial sensory processing and the modulation of detrusor smooth muscle spontaneous activity, the increased population of IC is rather likely to be a significant contribution to OAB. When these mechanisms were tested in our model, the reduction in Ca^{2+} -release through RyR together with the reduction of Ca^{2+} -dependent K^{+} -conductance and increased level of gap junctional coupling indeed induced an OAB-like activity pattern.

The results of this model were similar to experimental findings when the effects of several pharmacological agents were tested. Most of the experimental results were obtained from muscle strips. Although the strips are usually peeled away from the mucosa and lamina propria, they represent, in our opinion, a mixed preparation that contains both SMC and IC.⁸ The effect of ryanodine is complicated by its dose-dependence. Low doses of ryanodine (~ 10 nM) were reported to increase the frequency of single RyR channel openings to a normal conductance level, intermediate ryanodine doses (~ 1 μM) are reported to induce very-long-duration open events with reduced conductance, and high doses of ryanodine (~ 100 μM) were reported to lock

the channel in a closed configuration.¹⁷ This dose-dependency, together with species differences, was probably responsible for the conflicting results of the experimental studies, which reported either reduced amplitude with no change in the frequency of spontaneous pig bladder muscle contraction¹⁸ or increased frequency with no change in amplitude of rat bladder muscle contraction in the presence of ryanodine.¹² By modifying sarcoplasmic reticulum Ca^{2+} -release, the model could reproduce both inhibitory (high dose) and stimulatory (low dose) types of ryanodine effects in both normal detrusor and OAB.

Caffeine, the opener of RyR,⁴⁴ was reported to deplete intracellular calcium stores and increase the intracellular Ca^{2+} concentration, consequently activating the large conductance Ca^{2+} -dependent K^{+} -channels.¹⁹ The spontaneous contractile activity of the detrusor muscle was attenuated by caffeine.¹² The reduction of phasic Ca^{2+} -release (due to depletion) in the model was associated with an increase in Ca^{2+} -dependent K^{+} -conductance and a substantial attenuation of the spontaneous activity in both normal detrusor and OAB, in a manner consistent with experimental results.

Ca^{2+} influx through VOCC is essential for the spontaneous contractions of detrusor muscle; it contributes to initiating the rise in intracellular Ca^{2+} contraction, maintaining intracellular Ca^{2+} levels, and refilling intracellular Ca^{2+} stores.^{12,45} Consequently, the application of VOCC blockers (e.g. nimodipine) suppresses spontaneous contractile events.^{12,45} In our model, the suppression of VOCC completely diminished the spontaneous activity in both normal detrusor and OAB as expected.

Study limitations

An inherent limitation of the modeling studies is the fact that a number of parameters used in the equations are set arbitrarily, and therefore it is unclear to what extent they correspond to real processes. This model is based on previous models of vascular SMC and gastrointestinal IC and it is possible (even likely) that some quantitative differences between these tissues and the bladder were neglected, and that the model requires further refinements to better correspond to bladder SMC and IC. These refinements will require a more precise information about bladder SMC and IC (e.g. relative size, volume, membrane area of cells, numbers of cells that communicate, conductances), which is not at the moment, to the best of our knowledge, available. The model was simplified by considering the ratio of 1 SMC:1 IC only. In real 3D tissue, this ratio is probably different, thus rising complex questions of summation and synchronization, which, however, are beyond the scope of this study.

The model only involves SMC and IC, ignoring various other cell types present in the bladder. Although the model reproduced the experimental results obtained in detrusor muscle strips very closely, it should be emphasized that some other structures such as the urothelium and nerve supply must be included for modeling the more complex behaviors of the bladder.

The intracellular Ca^{2+} transients are presented as the endpoint of this modeling study. Although there is clear

relationship between intracellular Ca^{2+} and contraction, and consequently Ca^{2+} transients may be extrapolated to the contractile events; in some conditions significant differences may occur. Therefore, the model must be further extended to cover the processes beyond Ca^{2+} dynamics to increase its relevance.

Conclusions

By combining models of IC and SMC, a model of normal detrusor muscle was developed that reproduced the behavior of detrusor muscle strips under various experimental conditions.

After appropriate alterations, the model of normal detrusor tissue may be easily modified to a model of OAB, which again was fully consistent with previous experimental data.

Authors' contributions: All authors participated in the design, interpretation of the studies, analysis of the data and review of the manuscript; JR developed the mathematical models of SMC and interstitial cell, MB was involved in the mathematical modeling, MS was responsible for physiological interpretation of the results, and MS and JR wrote the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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