Original Article

Analysis of Spontaneous Depolarization-Linked Hyperpolarizations in Mouse Detrusor Smooth Muscle Cells

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Abstract

Background: Urinary bladder detrusor smooth muscle cells exhibit spontaneous electrical activities comprising various signal types. **Aims and Objectives:** This article introduces and analyzes a rare category of signals observed in such activity, named spontaneous depolarization-linked hyperpolarization (sDLH). **Materials and Methods:** A mouse model was used in the study, where all the occurrences of sDLHs were pooled together from multiple intracellular recording sessions. Four features – (i) resting membrane potential (RMP) (*R*, in mV), (ii) depolarization amplitude (*D*, in mV), (iii) hyperpolarization amplitude (*H*, in mV), and (iv) time course of the hyperpolarization (*T*, in ms) – were evaluated from all sDLHs. **Results:** The analysis of results indicated that (a) the signals appear more frequently in cells with higher RMP, (b) the depolarization amplitudes seem to be distributed randomly and have no correlation with other features, (c) hyperpolarization amplitudes show two distinct clusters and exhibit strong correlation with the RMP, and (d) time course of hyperpolarization phase shows no distinct groups and is distributed in a window larger than that of any other signals seen in the intracellular recordings. With the help of the results obtained from the analysis, a hypothesis for the biophysical origin of these signals is proposed. **Conclusions:** This needs to be tested experimentally, and if proved right, would help extend the boundaries of our current understanding of the detrusor smooth muscle system.

Keywords: Detrusor, electrophysiology, mouse model, smooth muscle, spontaneous activity, urinary bladder

Introduction

Spontaneous electrical activities observed in the mouse detrusor smooth muscle cells (DSMCs) primarily contain passive signals called spontaneous excitatory junction potentials (sEJPs) and active signals called action potentials (APs).[1-4] However, these cells also exhibit signals which do not fall in either of the above two categories such as aborted APs (aAPs) and spontaneous depolarization-linked hyperpolarizations (sDLHs). The former, as the name suggests, are suprathreshold signals which initiate the active ion-channel mechanisms in the cell but fail to become a full-fledged AP, possibly due to the excessive current leakage from the recording cell. sDLHs, on the other hand, are characterized by a prominent hyperpolarization phase following a subthreshold depolarization. A comparison of typical profiles of these four signal types are shown in Figure 1.

The sEJPs and spontaneous APs (sAPs), being common components in most smooth muscle tissues, were extensively analyzed and were used to explore the membrane and syncytial properties of smooth muscle cells (SMCs) in various tissues.[1-10] However, the presence of aAPs and sDLHs in the mouse DSMC spontaneous electrical activity is still not acknowledged in the scientific literature. An analysis of one of these two hitherto-unreported signals – sDLH – is presented in this work as an attempt to partially fill this void.

What makes sDLHs interesting is the association of a subthreshold depolarization and a hyperpolarization. It is known that the neurotransmitters acting on the DSMCs – acetylcholine and adenosine triphosphate (ATP) – have an excitatory effect on the cell membrane and do not induce a hyperpolarization. This means that the hyperpolarization observed in the sDLHs

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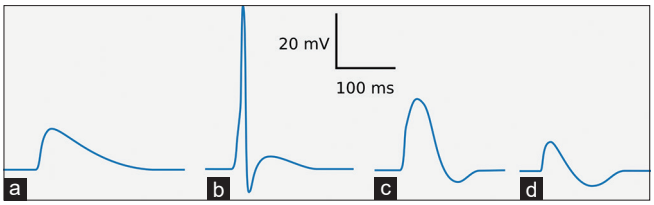

Figure 1: Typical profiles of basic signal types observed in the intracellular recordings from mouse detrusor smooth muscle cells. (a) Spontaneous excitatory junction potential, (b) spontaneous action potential, (c) aborted action potential, and (d) spontaneous depolarization-linked hyperpolarizations

is caused by some active ion channels present in the cell membrane. However, such ion channels are usually activated by a suprathreshold stimulus. In sDLHs, the depolarizations observed are subthreshold, which are intriguing. One plausible explanation for such a phenomenon is the involvement of complex calcium dynamics present in the DSMCs. The subthreshold depolarizations, most likely caused by the spontaneous neurotransmitter release, could on occasions initiate a local calcium transient at the membrane. If these transients appear near the intracellular Ca^{2+} stores of the endoplasmic reticulum (ER), they could open up the store causing a sharp rise in the intracellular Ca^{2+} concentration, thereby activating the large-conductance (BK) and small-conductance (SK) potassium channels, thereby resulting in a prominent hyperpolarization. However, such a theory is yet to be tested.

The probability of appearance of the sDLH signals is very low. For instance, in our database containing intracellular recordings from 73 cells, sDLHs appeared only in 13. Even in those 13 recordings, their frequency of appearance is very low, as evident from the fact that the total number of occurrence of sDLHs in the entire database used in the study was mere 38, whereas the number of instances of other signal types – sEJPs, sAPs, and aAPs – was in the order of thousands. An overlapped plot of all available sDLHs is shown in Figure 2. The signals are onset-aligned and direct current-shifted for ease of comparison. It can be observed that even among this small set of signals, there is a very high variability present in their depolarization and hyperpolarization phases and their overall time courses.

Through this study, we aim to investigate the properties of sDLHs and come up with hypotheses on their biophysical origin. Such hypotheses, if supported by experimental and simulation studies, could give insights on the ion-channel properties, Ca dynamics, and other mechanisms present in the mouse DSMC syncytium.

Methods

Electrophysiological data were obtained from mouse detrusor following the procedure explained in Meng *et al*.[3] Six mice (Balb/C strain) were sacrificed to obtain 13 intracellular recordings used in this study.

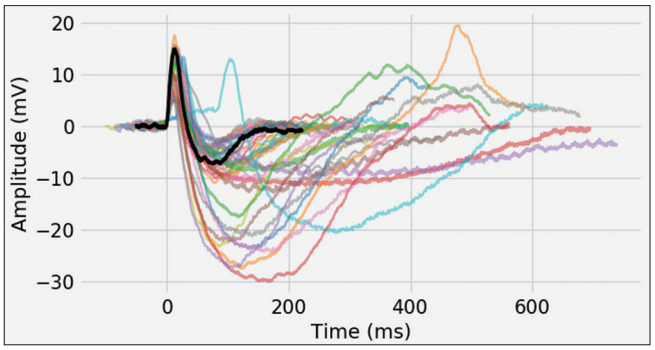

Figure 2: Cluster plot showing all spontaneous depolarization-linked hyperpolarizations used in the study. The signals were onset-aligned and DC-shifted for readability. One typical spontaneous depolarization-linked hyperpolarization, chosen at random, is highlighted to indicate the template

The profile of an sDLH was captured using three features defined as follows:

1. Depolarization amplitude (*D*): The highest amplitude attained in the depolarization phase in millivolts
2. Hyperpolarization amplitude (*H*): The highest amplitude during the hyperpolarization phase, measured as the absolute deviation from the resting membrane potential (RMP) in millivolts
3. Hyperpolarization duration (*T*): The time span of the hyperpolarization phase measured between the instant after the depolarization phase when the membrane potential falls below the RMP and the end of the signal.

Apart from the three features defined above, RMP was defined as the fourth feature (indicated as *R*) included in the analysis. A schematic figure indicating the features of sDLH is given in Figure 3. Once the feature values were evaluated for all the sDLHs, a two-step analysis was conducted. First, the histograms of individual features were studied to identify their properties. In the second step, the correlations between all pairs of features were determined to investigate the set of features that vary together. The results thus obtained and the inferences derived are given in the upcoming sections.

Results

All 38 sDLHs thus available from the database were isolated for the study. For each of those signals, the features explained in the "Methods" section were evaluated. Histograms of individual feature values thus obtained are provided in Figure 4a-d. The histogram of the RMP values [Figure 4a] indicates that majority of the sDLHs appeared when the RMP of the cell was greater than or equal to its average value, which is −44 mV.[3] The values of the depolarization amplitudes were observed to vary between 5 and 18 mV [Figure 4b]. Although the lower amplitude depolarizations (D < 10 mV) seemed to be of higher frequency compared to the high-amplitude depolarizations (D > 10 mV), the difference was not significant ($P \gg 0.1$). The hyperpolarization amplitude [Figure 4c] exhibits a much larger spread between 5 and 30 mV. As indicated in the figure, two groups can be formed: one with H <15 mV and the one where H >15 mV.

The number of sDLHs appearing in the former group was significantly higher than in the latter ($P < 0.05$). This indicates that there may be two different biophysical mechanisms operating for the production of hyperpolarizations in sDLHs. The final histogram shows the variation in the time course of the hyperpolarization phase [Figure 4] and exhibits an exponentially decaying trend, where the larger time courses had lesser frequencies of appearance. An interesting observation is that the time course of hyperpolarization can be as prolonged as 600 ms. Even in APs, where we expect the maximum participation from the active ion channels, hyperpolarization phases of duration >350 ms were not observed.

The correlation between the pairs of features was then analyzed. The values of the correlation coefficients thus obtained are tabulated in Table 1. From the table, it can be observed that all pairs gave very low ϱ values, except two pairs – (i) RMP versus hyperpolarization amplitude and (ii) hyperpolarization amplitude versus time course of hyperpolarization. The unexpected observation was that the amplitude of the depolarization phase, which is assumed to be the trigger for the signal, does not affect or get affected by any of the features in sDLH. Scatter plot of the two pairs of features exhibiting significant correlation values [highlighted in Table 1] is shown in Figure 5. The amplitude of the hyperpolarization phase was found to be larger for cells with higher RMP. It could also be noted from Figure 5a that all of the sDLHs with large hyperpolarizations (H > 15 mV) appeared in cells with RMP >−35 mV. As described in the discussion section, this might indicate that the mechanisms which cause the production of the large hyperpolarizations require channels which are more available in the depolarized

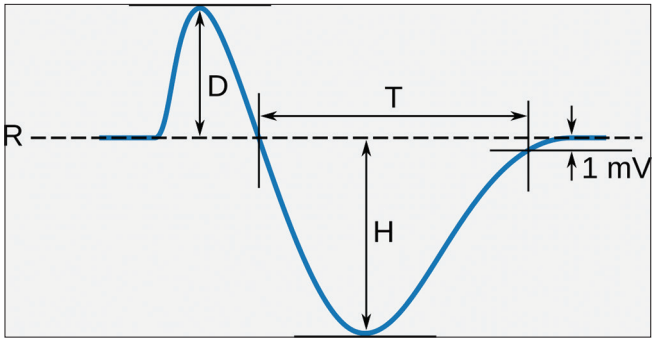

Figure 3: Schematic figure showing the features of a spontaneous depolarization-linked hyperpolarization. Abbreviations used are as follows – R: Resting membrane potential, D: Depolarization amplitude, H: Hyperpolarization amplitude, and T: Time course of hyperpolarization

Table 1: The values of correlation coefficients (ϱ) evaluated between the pairs of features observed from the spontaneous depolarization-linked hyperpolarization signals

	D	H	T
R	0.14	**0.58**	0.34
D		0.21	0.28
H			**0.64**

The pairs with significant correlation are highlighted. R: Resting membrane potential, D: Depolarization amplitude, H: Hyperpolarization amplitude, T: Time course of hyperpolarization

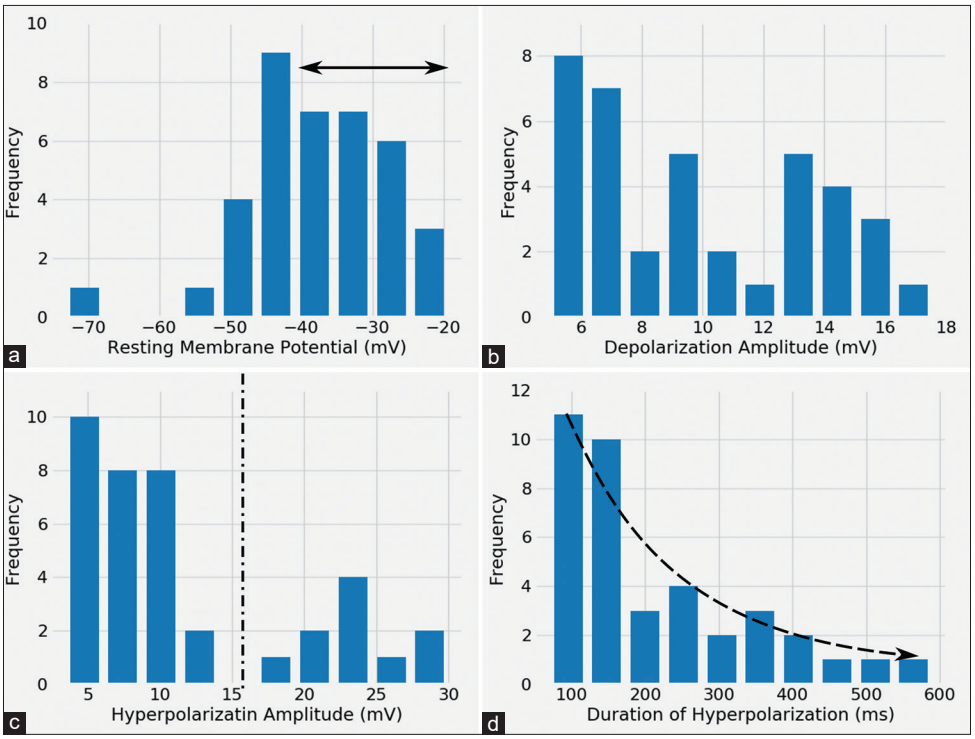

Figure 4: Histograms of the feature values evaluated from the population of spontaneous depolarization-linked hyperpolarizations. (a) Resting membrane potential, R, (b) Depolarization amplitude, D, (c) Hyperpolarization amplitude, H, and (d) Duration of hyperpolarization, T

membrane. Figure 5b indicates the correlation between the amplitude and the duration of the hyperpolarization phase in sDLHs. The correlation was expected, as it would require more time to repolarize the membrane from the larger hyperpolarized state. However, it should be noted that there were exceptions, where sDLHs with smaller hyperpolarization amplitude took a comparatively longer duration to repolarize.

Discussion

A signal type named sDLH is introduced and analyzed in this work. In mouse DSMCs, such signals make rare appearances. Its biophysical origin or their role in the detrusor smooth muscle operation is as yet unknown. Analysis of the features of sDLHs presented here is an initial step toward the exploration of these domains.

Four features which describe the nature of the sDLH signals were chosen, and their variations observed across the population of sDLHs were analyzed. The histogram of individual features provided some hints helpful in predicting the sources of these signals. For example, the fact that the sDLHs appeared more frequently in cells with a higher RMP revealed the involvement of some voltage-gated ion channels in the membrane, such as T-type calcium channels.[11,12] The depolarization phases displayed a distributed amplitude histogram. It appeared that even the low amplitude depolarizations were capable of producing the sDLHs. However, there is a possibility that the combined signal may not indicate the original amplitude of the depolarization phase. If the ion channels causing hyperpolarizing currents get activated before the completion of the depolarization phase, such currents could cancel out a smaller or large part of the depolarizing currents, thereby attenuating the amplitude of the depolarization phase.

It was observed that the amplitudes of hyperpolarization phases were clustered into two groups, one with a smaller amplitude (<15 mV) and the other with larger amplitudes (>15 mV). This observation could be correlated with the two hyperpolarizing components given by Padmakumar *et al*.[4] in the four-component model of the spontaneous APs in DSMCs. The two hyperpolarizing components were named (i) slow afterhyperpolarization (sAHP) and (ii) very sAHP (vsAHP) components. The sAHP components are small in their amplitude (up to 10 mV) and duration (up to 150 ms), whereas those in vsAHP were large up to 30 mV and 350 ms, respectively. The two groups of hyperpolarization amplitudes observed in the sDLHs could be caused by the same biophysical elements that cause the said components in the DSMC sAPs. Two major ion channels which cause hyperpolarization in DSMC are calcium-activated small-conductance (SK) and big-conductance (BK) potassium channels.[9,13,14] The amplitudes and time courses of operation of sAHP component matched that of SK channel operation, whereas the vsAHP component amplitude matched with the amplitude scales that BK channels are capable of inducing. Thus, by comparison, the same channels could be candidate channels operating to produce sDLHs as well. However, unlike the amplitude histogram, such a close similarity between the sAP components was absent in the histogram of the time course feature of sDLHs. Two distinct groups were not observed, and the window of time courses observed was much broader (100–600 ms) compared to that observed in sAPs (150–350 ms).

The two groups discernible in the histogram of *H* were also observed in the scatter plot of *H* versus *R* shown in Figure 5a. It was also observed that higher amplitude hyperpolarizations appeared only in cells with RMP >−35 mV and that there is a significant jump from *H* amplitudes above 15 mV. These observations also support our hypothesis that the hyperpolarizations in sDLHs were caused by the outward K currents mediated by BK channels. It was demonstrated by Herrera *et al*.[15] that the depolarization of the DSMC membrane potential produces a substantial effect on the BK channel activity by (i) increasing the frequency and amplitude of the Ca^{2+} sparks from the internal stores and (ii) increasing the sensitivity of BK channels to the intracellular Ca^{2+} concentration. It can be observed from 5a that the X-intercept of the regression line falls at −66 mV, close to the reversal potential of K in DSMCs, which is reported as −75 mV.[12] This indicates that the hyperpolarization observed in the sDLHs are caused by potassium ions.

The inferences obtained from the signal analysis can be connected to form a hypothesis that could explain the origin

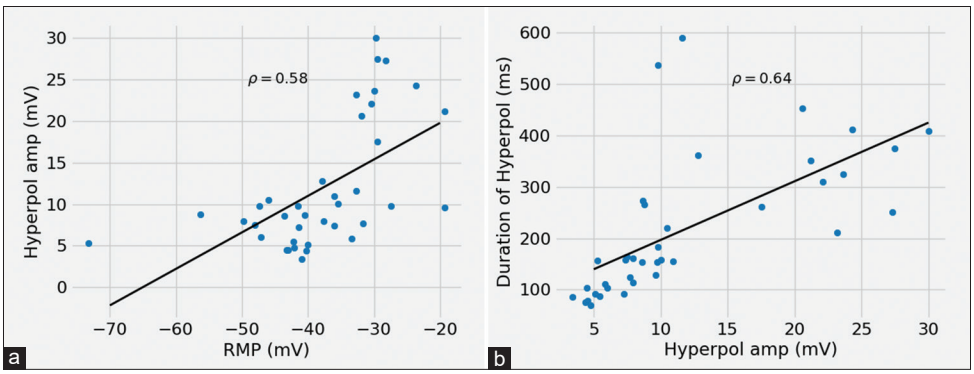

Figure 5: Scatter plots of feature pairs exhibiting significant correlation. (a) Hyperpolarization amplitude versus resting membrane potential and (b) time course of hyperpolarization versus hyperpolarization amplitude

of sDLHs. One such hypothesis is shown schematically in Figure 6. According to this, the initiation of an sDLH is caused by a neurotransmitter release event, shown as State 0 in Figure 6. The purinergic receptors get activated by ATP released as a neurotransmitter from the varicosities near the SMC. These receptors, when activated, allow influx of ions nonselectively, thereby depolarizing the cell membrane. This is State 1, seen as the onset of the signal. This depolarization, in turn, activates voltage-gated T-type calcium channels which selectively allow Ca^{2+} ions from the extracellular fluid (ECF) to enter the cell. This ion flow further depolarizes the membrane as shown in State 2. Up to this stage, the series of events appear in the same order as presented in the work of Mahapatra *et al*.[12] In that study, it is shown that the activity of T-type calcium channels depolarizes the membrane up to the threshold potential at which the voltage-gated L-type calcium channels are activated, which initiates the positive feedback loop eventually developing into an AP. In the case of sDLH, the threshold is not reached. In the framework of the proposed hypothesis, this is because of a rare event that occurs near the cell membrane [shown in Figure 6, State 3]. On occasions where the T-type calcium channels are located very close to a section of ER – an intracellular structure which acts as a store of Ca^{2+} ions – the course of events diverts into a drastically different direction. The influx of calcium by the T-type channels causes local calcium transients and local increment in Ca^{2+} ion concentration (see figure). These transients activate the receptors at the wall of ER, causing it to release Ca^{2+} ions. This comprises a chain reaction, as the increment of calcium in the ICF causes more receptors to get activated at the ER membrane, causing a calcium wave, during which a significant increment of Ca^{2+} concentration is observed throughout the cell [shown as shade of yellow in Figure 6, State 3]. Existence of such Ca^{2+} waves were studied in the past, for example, field stimulation of the mouse bladder can initiate such local calcium waves in the DSMCs.[16] This rise in calcium concentration activates large number of SK and BK channels at the plasma membrane, causing a quick outflow of potassium ions. This outflow of K ions, being stronger than the inflowing calcium current by the T-type channels, causes

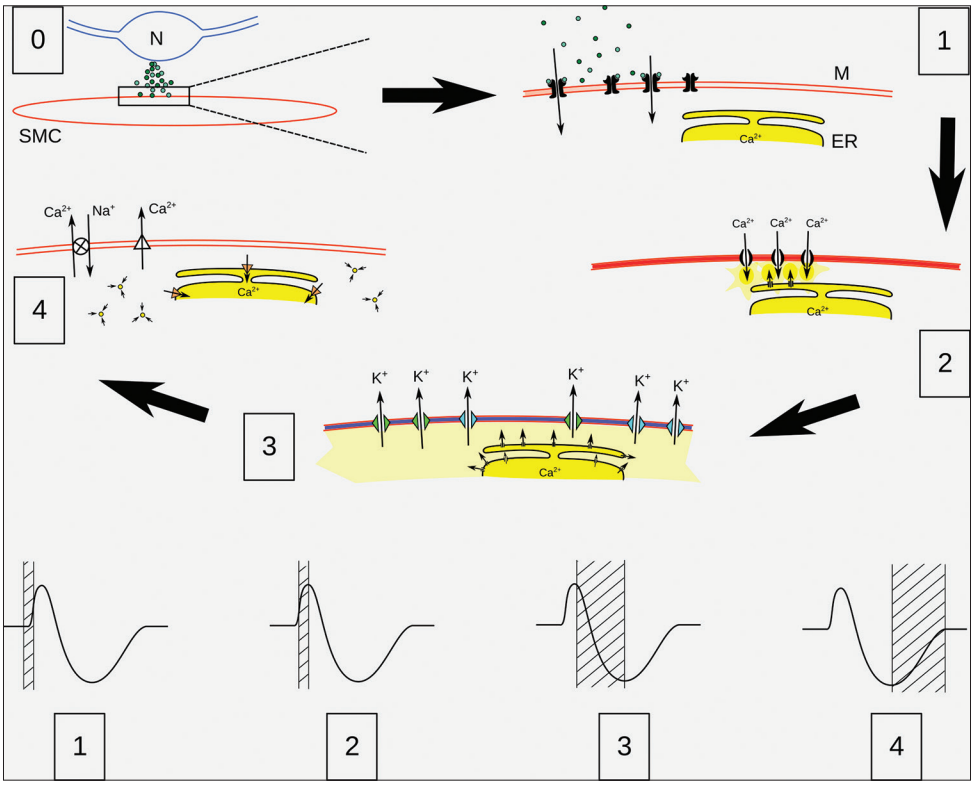

Figure 6: Schematic figure of a hypothesis explaining the origin of spontaneous depolarization-linked hyperpolarization signals. The upper panel, chronologically numbered as States 0–4, indicated a series of biophysical events occurring in the cell environment, initiated by a neurotransmitter release event near a SMC in resting state (State 0). The lower panel indicates the corresponding variations in membrane potential during each state, eventually generating typical sDLH signal template. Descriptions of individual states in brief: State 0 – Neurotransmitter release event; N-neuron varicosity, SMC. State 1 – Purinergic receptors activated by ATP, initiate inward currents causing SMC membrane to depolarize; M-SMC plasma membrane, ER. State 2 – Voltage-gated calcium channels (T-type channels) open up causing inflow of Ca. Local rise of calcium concentrations activates the receptors of ER, resulting in the release of Ca^{2+} ions from ER. The cell continues to depolarize. State 3 – The release of Ca^{2+} from ER increases the intracellular Ca^{2+} concentration. This elevated calcium level activates large-conductance (BK) and small-conductance (SK) potassium channels causing strong outward current, resulting in membrane hyperpolarization. State 4 – SK and BK channels shut down, halting further hyperpolarization of the membrane. Prolonged activity of various calcium pumps, exchangers, and buffers restores the resting state Ca^{2+} concentration in intracellular fluid (ICF), thereby repolarizing the membrane to RMP

a net outflow of current, repolarizing the membrane and thus preventing it from reaching the threshold. These potassium currents are maintained as long as there is an elevated level of ICF Ca^{2+} concentration, which is roughly on the order of a hundred milliseconds.[13] During this period, the membrane repolarizes to RMP and then continues to hyperpolarize. As the membrane hyperpolarizes, the strength of the potassium currents diminishes due to three reasons: (i) BK channels get deactivated as a depolarized membrane potential is required for sustaining their active state, (ii) the electrostatic driving force for the potassium channel declines as the membrane potential nears the reversal potential of potassium, and (iii) the free Ca^{2+} ions present in the ICF are cleared by the calcium buffers, exchangers, and various pumps in action, thereby deactivating the SK and BK channels. Finally, in State 4, the outward K + current vanishes. The excess free Ca^{2+} ions in ICF are removed by the following: (i) sarco/ER Ca ATPase (SERCA) pumps, actively transporting Ca^{2+} into the ER; (ii) plasma membrane Ca^{2+} ATPase (PMCA) pumps, actively transporting Ca^{2+} ions from ICF to ECF; (iii) sodium-calcium exchangers, transporting free Ca^{2+} ions to ECF in exchange for Na ions from ECF to ICF; and (iv) the calcium buffers which bind and inactivate free Ca^{2+} ions to form complex molecules.[11] As a result of all these operations, calcium concentration in the ICF is restored to resting levels, and the cell repolarizes to its RMP.

The key of the proposed hypothesis lies in State 3, where the ryanodine receptors at ER are activated, bypassing the L-type calcium channels. This is possible only if there is a section of ER near a cluster of T-type calcium channels. Furthermore, in order to get these T-type channel clusters to activate completely at low depolarization levels, it is required to have the neurotransmitter action that occurs very close to them so that they experience a strong local depolarization in their vicinity. Thus, according to the proposed hypothesis, an sDLH signal arises only if the neurotransmitter release event occurs in a region of DSMC membrane where there are (i) purinergic receptors, (ii) T-type calcium channel clusters, and (iii) very close access to sections of ER. Arguably, there is a very low probability for all these conditions to be simultaneously met, which we assume to be the reason for the low probability of appearance of sDLHs in the spontaneous electrical activities of DSMCs. The hypothesis is also capable of explaining the seemingly random distribution of depolarization amplitudes across sDLHs. Depending on how close the site of action of neurotransmitter lies to the sDLH hotspots – where the three necessary elements listed above are clustered together – the depolarization required to initiate an sDLH would vary.

It may be difficult at present to test the proposed hypothesis experimentally, owing to the low probability of occurrence of the sDLHs, as well as the technical difficulties inherent in monitoring the above-outlined subcellular events, which take place at a nanometer scale. However, our conjecture can be tested *in silico*. Mahapatra *et al*.[12] have developed a computational model of DSMCs in which nine active channels were included. Various elements of calcium dynamics are developed by Dave and Manchanda[11] and SK and BK channels reported by Mahapatra *et al*.,[12] and Gupta and Manchanda[13] could be used to set up a simulation framework to test the proposed hypothesis. If such simulation studies could reproduce the sDLH signals, experimental tests can be designed to reproduce the results *in vitro*. Such studies will validate our current understanding of the biophysical mechanisms in DSMC and will take us one step closer to the comprehensive understanding of the detrusor smooth muscle syncytium.

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Conflicts of interest

There are no conflicts of interest.

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