9 Mucosa-Dependent, Stretch-Sensitive Spontaneous Activity in Seminal Vesicle

Mitsue Takeya, Tokumasa Hayashi, Hikaru Hashitani, and Makoto Takano

Abstract

Seminal vesicles (SVs), a pair of male accessory glands, contract upon sympathetic nerve excitation during ejaculation while developing spontaneous phasic constrictions in the inter-ejaculatory storage phase. Recently, the fundamental role of the mucosa in generating spontaneous activity in SV of the guinea pig has been revealed. Stretching the mucosa-intact but not mucosa-denuded SV smooth muscle evokes spontaneous phasic contractions arising from action potential firing triggered by electrical slow waves and associated Ca^{2+} flashes. These spontaneous events primarily depend on sarco-endoplasmic reticulum (SR/ER) Ca^{2+} handling linked with the opening of Ca^{2+}-activated chloride channels (CaCCs) resulting in the generation of slow waves. Slow waves in mucosa-intact SV smooth muscle are abolished upon blockade of gap junctions, suggesting that seminal smooth muscle cells are driven by cells distributed in the mucosa. In the SV mucosal preparations dissected free from the smooth muscle layer, a population of cells located just beneath the epithelium develop spontaneous Ca^{2+} transients relying on SR/ER Ca^{2+} handling. In the lamina propria of the SV mucosa, vimentin-immunoreactive interstitial cells including platelet-derived growth factor receptor α (PDGFRα)-immunoreactive cells are distributed, while known pacemaker cells in other smooth muscle tissues, e.g. c-Kit-positive interstitial cells or α-smooth muscle actin-positive atypical smooth muscle cells, are absent. The spontaneously-active subepithelial cells appear to drive spontaneous activity in SV smooth muscle either by sending depolarizing signals or by releasing humoral substances. Interstitial cells in the lamina propria may act as intermediaries of signal transmission from the subepithelial cells to the smooth muscle cells.

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Seminal vesicle · Spontaneous contraction · Mucosa · Slow wave · Intracellular calcium release · Calcium-activated chloride channel · Stretch · Male reproductive glands · Seminal fluid · Male fertility

9.1 Introduction

Seminal vesicles (SVs), male accessory reproductive glands, produce the major components of the seminal fluid that is required for male fertility. Both sympathetic and parasympathetic nerves innervate the SVs and regulate their contractions and secretion [1, 2]. During emission, i.e., the early phase of ejaculation, SVs contract and expel their secretion into the urethra when noradrenaline is released from sympathetic nerve excitation and activates α_1-adrenoceptors (α_1-ARs) in SV smooth muscle [3]. Accordingly, silodosin, a selective α_{1A}-AR antagonist, used for the treatment for benign prostate hyperplasia (BPH), has a commonly seen adverse effect, namely ejaculation disorders [4] resulting from the impaired propulsion of the seminal fluid from the SVs [5, 6].

During the inter-ejaculatory storage phase, SVs do not remain quiescent; rather they generate spontaneous phasic contractions that do not rely on autonomic nervous activity. The spontaneous contractions of SVs may contribute to the maintenance of the quality of seminal fluid vital for male fertility. Despite the fact that the SV spontaneous contractions were first reported over a hundred years ago [7], the mechanisms underlying their generation are little understood. Recently, a critical role of the SV mucosa in generating spontaneous phasic contractions in SV smooth muscle of the guinea pig has been demonstrated [8]. In this chapter, properties of SV spontaneous contractions in several mammals are briefly summarized to update our knowledge of the mechanisms underlying SV spontaneous activity based on these recent findings.

9.2 Spontaneous Contractions Recorded from Rodent and Human SVs

9.2.1 Anatomy

9.2.1.1 Gross Anatomy

Human SVs form a paired saclike structure that joins the ampulla of the vas deferens to form the beginning of the ejaculatory ducts that pass through the prostate and terminate within the prostatic urethra [9]. Some rodents including guinea pig, rat, hamster, and mouse have a well-developed pair of SVs, while the domestic rabbit has a large unpaired SV [10, 11].

9.2.1.2 Microanatomy

The SV wall consists of the luminal epithelial layer, thin lamina propria, muscularis and an abluminal serosal layer [9, 12]. The muscularis of the guinea pig SVs is composed of an inner circular smooth muscle layer and an outer longitudinal layer confined to its urethral end. In the distal portion of SVs, only a layer of circularly orientated smooth muscle cells is present [2]. Electron microscopic studies of guinea pig SV revealed spindle-shaped fibroblasts that are distributed in the lamina propria just beneath the basement membrane of the epithelial cells [12, 13].

9.2.2 Spontaneous Contractions of SVs In Vivo

During inter-ejaculatory phases, SVs secrete and store their fluid contents. Spontaneous contractions during the storage phase in vivo have been reported by recording intraluminal pressure of the SVs in anesthetized rodents. In SVs of the adult rabbit, small irregular contractions are continuously generated without nerve stimulation [11]. The SVs of adult rat, which produce vigorous contractions in response to electrical nerve stimulation, also continually exhibit spontaneous phasic contractions during their inter-ejaculatory phases

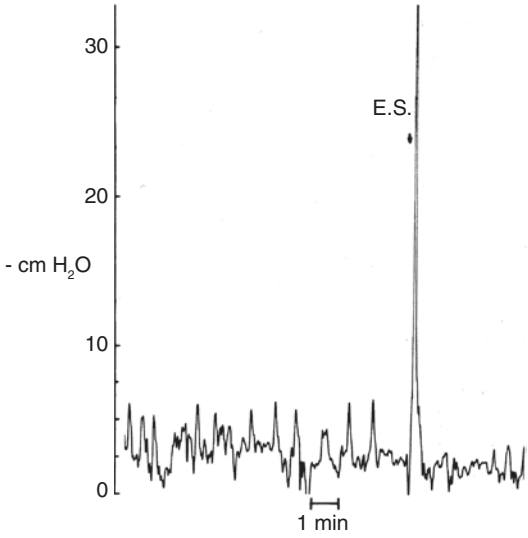

Fig. 9.1 In vivo recording of contractile activity of rat SV. Electrical nerve stimulation (E.S. *arrow*)-induced vigorous contraction and spontaneous irregular contractions (reproduced from Hib et al. [14] with permission of John Wiley & Sons). In further experiments by Hib et al. [15], the E.S.-induced contractions were confirmed to be prevented by phentolamine but not atropine

(Fig. 9.1 [14]). These spontaneous contractions are not affected by the administration of either phentolamine, a nonselective α-AR antagonist, or atropine, a muscarinic antagonist; however, these antagonists successfully prevent the facilitative effects of phenylephrine or acetylcholine (ACh), respectively, on SV contractions [15].

In 2-month-old adult rats, measurement of the luminal pressure during continuous infusion of SVs with normal saline demonstrates that the SV spontaneous contractions are more frequently seen at the nearly maximum infusion rate associated with organ distension [16].

9.2.3 Spontaneous Contractions in Excised Whole SVs

A recent study reported the contractile behavior of the excised guinea pig whole SVs during perfusion with physiological salt solution (PSS) by measuring their intraluminal pressure [6]. These "isolated" whole SVs develop irregular spontaneous phasic contractions independently of neuronal activity, as they are not affected by tetrodotoxin (TTX) that blocks nerve-evoked contractions (Video 9.1). The motility patterns of the spontaneous contractions in the whole SV preparations vary with time, and thus both retrograde and antegrade peristaltic contractions are randomly generated, indicating that any region of SVs can drive the contractions. Both frequency and amplitude of the spontaneous contractions are enhanced by increasing the internal hydrostatic pressure as the SVs are distended (Fig. 9.2). Nifedipine, a blocker of L-type voltage-dependent Ca^{2+} channels (LVDCCs), abolishes the spontaneous contractions [6], suggesting that the contractions primarily rely on the Ca^{2+} influx through LVDCCs.

9.2.4 Spontaneous Contractions of SVs In Vitro

"Spontaneous" rhythmic contractions in excised rat and guinea pig SV tissues were first recorded by Waddell in 1916 [7]. Later, spontaneous TTX-resistant, twitch-like contractions were recorded from the circular smooth muscle in the guinea pig SV [8, 17, 18]. Isometric tension recordings of human SV strips also demonstrate spontaneous contractions [19, 20].

The smooth muscle cells in circumferential muscle SV strips also develop TTX-insensitive spontaneous pacemaker-like depolarizations which trigger action potentials that are associated with the phasic contractions [21]. The generation of the spontaneous contractions is not inhibited upon blockade of α-ARs [18], nicotinic or muscarinic ACh receptors [17]. The periodical spontaneous contractions were generated in ring segments (4 mm width) obtained from either the proximal or distal guinea-pig SVs with a total length of 6–8 cm (M. Takeya and M. Takano, unpublished data). Thus, the site of origin of the SV periodical spontaneous contractions appears not to be localized but distributed along the whole organ.

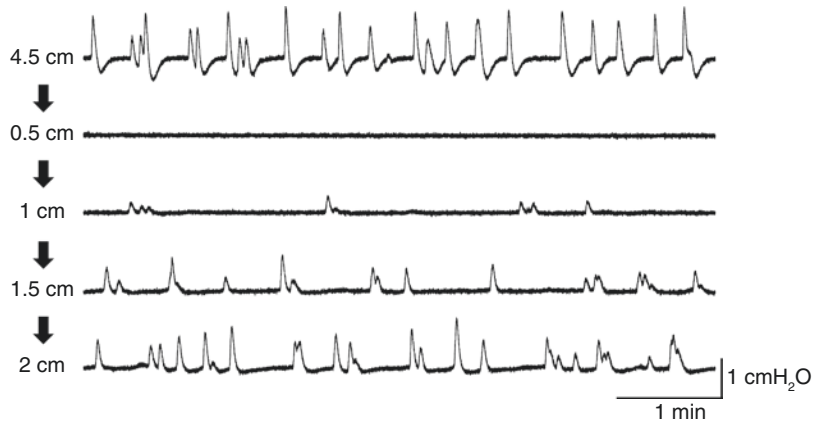

Fig. 9.2 TTX-insensitive phasic contractions in an "isolated" whole-guinea pig SV when the height of the liquid surface of the irrigator was set at 0.5–4.5 cm higher than the preparation. Application of the higher internal hydrostatic pressure by elevating the irrigator results in increasing the frequency of the SV contractions. Modified from Hayashi et al. [6]

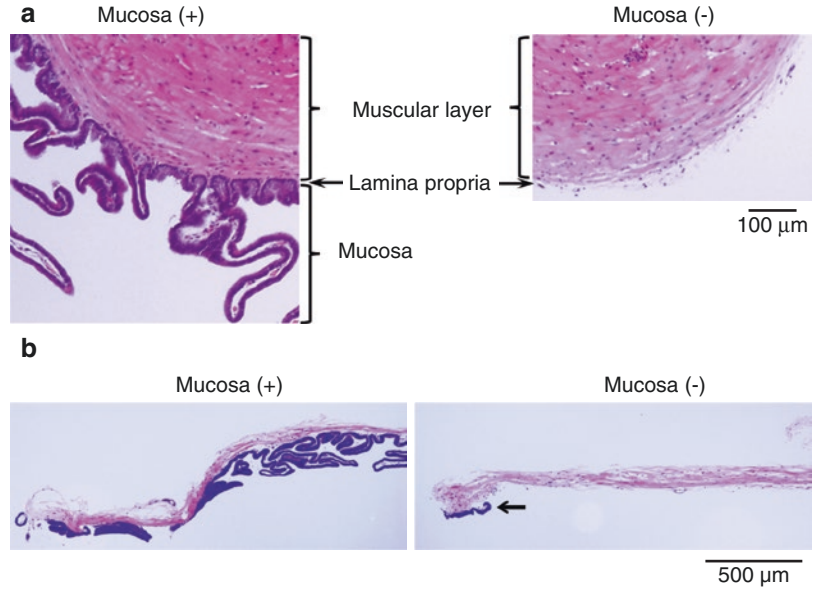

Fig. 9.3 Hematoxylin and eosin-stained coronal sections of mucosa-intact [*Mucosa* (+)] and -denuded [*Mucosa* (−)] guinea pig SV smooth muscle preparations. Reversed ring preparations which contained the whole muscular layer (**a**) were used for tension recordings. Membrane potentials or intracellular Ca^{2+} dynamics were recorded from inner smooth muscle preparations in which the bulk of muscular layers have been removed (**b**). In the right photograph in **b**, some mucosa was left attached to indicate the mucosal side (*arrow*). Adapted from Takeya et al. [8]

9.3 Role of Mucosa in Generating Spontaneous Activity of SV

Recently, the mechanisms underlying the spontaneous activity in adult guinea pig SV circular smooth muscle were investigated using mucosa-intact and mucosa-denuded smooth muscle preparations to explore the role of the mucosa [8].

9.3.1 Mucosa-Dependent Periodical Spontaneous Activity in SV Smooth Muscle

In mucosa-intact SV ring preparations containing the whole muscular layer (Fig. 9.3a), stretch of the wall induces periodical spontaneous contractions (at 36 °C) at a frequency ranging between 3 and 7 min^{-1} (Fig. 9.4a, Mucosa (+)). Mucosa-

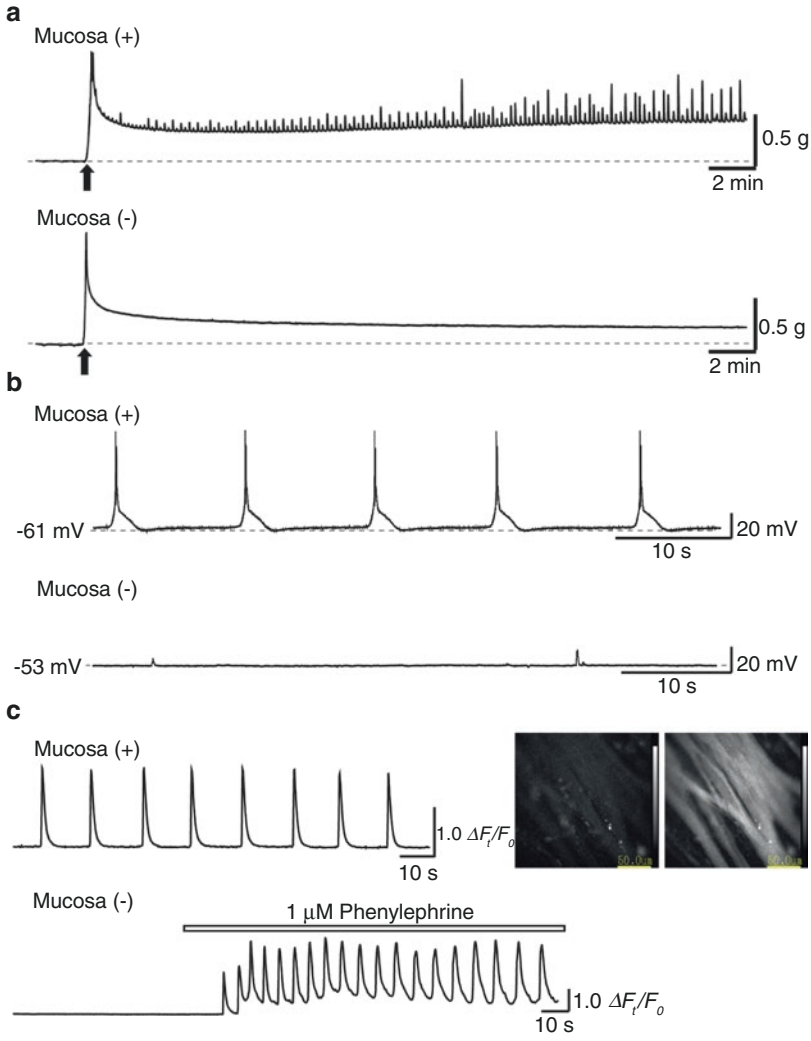

Fig. 9.4 Mucosa dependence of contractile, electrical, and Ca^{2+} activity in guinea pig SV smooth muscle. In mucosa-intact preparations [*Mucosa* (+)], stretched SV smooth muscle develops spontaneous phasic contractions (**a**), slow waves with superimposed action potentials (**b**), and synchronous Ca^{2+} flashes (**c**). *Arrows* indicate the timing of initial stretching to 1 g. Photographs in (**c**) are sequential Cal-520 fluorescence images (frame interval; 548 ms) of the mucosa-intact SV smooth muscle in normal PSS, showing that the spontaneous Ca^{2+} flashes were generated almost synchronously across SV smooth muscle cells. In contrast, mucosa-denuded SV smooth muscle [*Mucosa* (−)] fails to generate periodical spontaneous activity (**a**–**c**). Phenylephrine (1 μM) evoked oscillatory Ca^{2+} transients associated with phasic contractions in the quiescent mucosa-denuded SV smooth muscle (**c**). Adapted from Takeya et al. [8]

intact "trimmed" inner smooth muscle strips in which the bulk of muscular layers had been removed (Fig. 9.3b) also generate spontaneous contractions at a similar frequency. These spontaneous contractions appear to result from action potentials triggered by slow waves and associated Ca^{2+} flashes (Fig. 9.4b, c, Mucosa (+)). In contrast, mucosa-denuded SV smooth muscle preparations, in either a ring or strip configuration, invariably fail to generate spontaneous phasic contractions and periodical electrical or Ca^{2+} activity (Fig. 9.4a–c, Mucosa (−)). Despite the absence of spontaneous activity, the quiescent mucosa-denuded SV smooth muscle cells are

capable of generating action potentials in response to depolarizing current injection [8], as well as developing oscillatory Ca^{2+} transients and contractions upon α_1-AR stimulation (Fig. 9.4c). In addition, nerve-evoked contractions in the mucosa-denuded SV smooth muscle preparations are also well preserved, indicating that the lack of spontaneous activity is not due to impaired excitability or contractility of the SV upon removal of the mucosa. These observations are consistent with a previous report in which mucosa-denuded circular muscle strips of the guinea pig SVs do not exhibit spontaneous electrical activity and associated contractions, whereas electrical and Ca^{2+} activities are evoked by nerve excitation or α_1-AR stimulation [22].

9.3.2 Role of L-Type Voltage-Dependent Ca^{2+} Channels (LVDCCs) in Generating Spontaneous Activity in SV Smooth Muscle

Blockade of LVDCCs by 3 μM nifedipine abolishes the spontaneous phasic contractions in the mucosa-intact guinea pig SV smooth muscle (Fig. 9.5a). At a lower concentration of 1 μM,

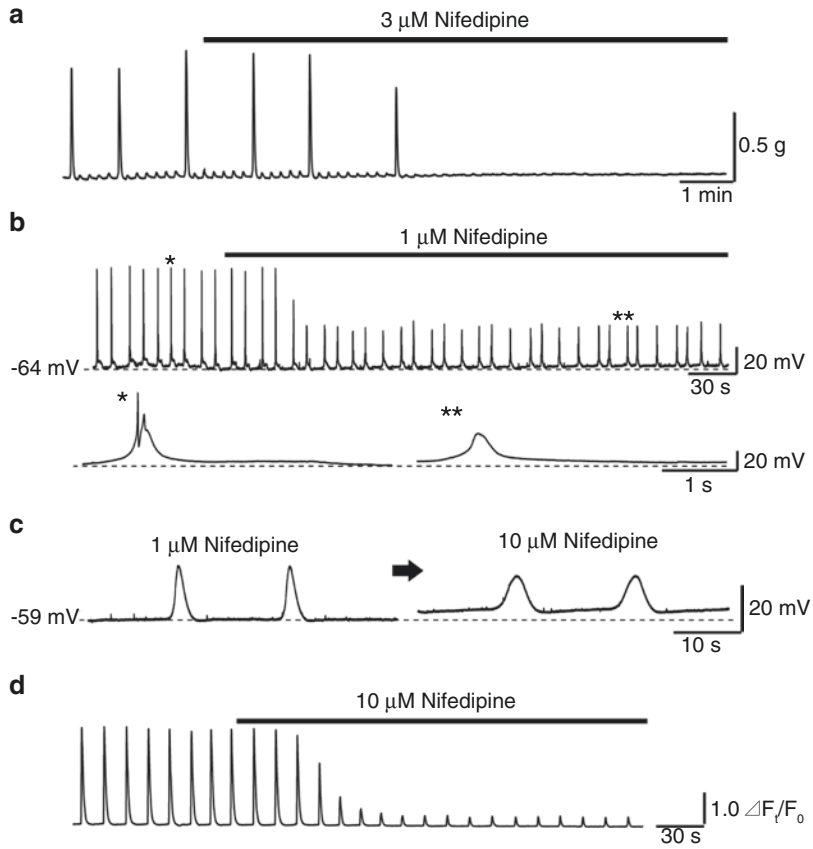

Fig. 9.5 Effects of nifedipine on spontaneous activity in mucosa-intact guinea pig SV smooth muscle. (**a**) Nifedipine (3 μM) abolished spontaneous phasic contractions in the mucosa-intact SV preparation. (**b**) Nifedipine (1 μM) abolished superimposed action potentials leaving slow waves. Lower traces show slow waves in control (*) and nifedipine (**) with an expanded time scale. Traces * and ** were obtained at the timings indicated by the corresponding marks in the upper trace. (**c**) In another mucosa-intact SV smooth muscle preparation, 10 μM nifedipine reduced the slow-wave amplitude, prolonged the slow-wave duration, and depolarized the membrane in the SV smooth muscle of control (in 1 μM nifedipine). (**d**) Spontaneous oscillatory Ca^{2+} flashes were strongly suppressed by 10 μM nifedipine. Adapted from Takeya et al. [8]

nifedipine greatly suppresses the spontaneous contractions and abolishes the superimposed action potentials without preventing the generation of slow waves (Fig. 9.5b). 10 μM Nifedipine largely suppresses spontaneous Ca^{2+} flashes (Fig. 9.5d). These results indicate that the spontaneous phasic contractions largely depend on Ca^{2+} influx via LVDCCs during action potential firing. Since the SV ring preparations consisting of the whole muscular layer develop both large and small phasic contractions (Figs. 9.4a and 9.5a), the electrical activity initiated presumably at the most inner layer of SV muscle facing to the mucosa may not consistently propagate across to the outer circular muscular layer. Similarly, the irregular spontaneous constrictions in the guinea pig-excised whole SVs [6] may result from inconsistent spread of electrical activity in both the transverse and axial directions within the SV wall. Thus, only the occasionally occurring, coordinated contractions of whole SV are strong enough to increase the intraluminal pressure, which may add further mechanical stimuli to the wall in other regions of the SV.

A higher concentration of 10 μM nifedipine reduces the slow-wave amplitude of the SV smooth muscle (Fig. 9.5c), suggesting that activation of LVDCCs partially contributes to the configuration of the slow wave. Thus, the contribution of LVDCCs to slow-wave configuration in the SV smooth muscle differs from that in the smooth muscle of guinea pig prostate [23], urethra of rabbit [24], or guinea pig [25], where LVDCC activation is involved in the plateau phase of the slow wave but not their initial upstroke.

10 μM Nifedipine depolarizes the membrane and also prolongs the slow-wave duration (Fig. 9.5c). Since TEA-sensitive, Ca^{2+}-dependent K^+ currents are recorded in the isolated smooth muscle cells of the guinea pig SV [26], it is likely that large-conductance Ca^{2+}-activated K^+ (BK) channels are activated upon Ca^{2+} entry through LVDCCs during the slow-wave generation. Thus, the blockade of LVDCCs by nifedipine may reduce the activation of BK channels in the SV smooth muscle, which causes membrane depolarization as well as prolongation of the slow-wave duration.

The frequency of the residual slow waves and spontaneous Ca^{2+} transients of SV smooth muscle are not largely affected by 10 μM nifedipine (Fig. 9.5c, d), suggesting that the intrinsic periodicity of spontaneous activity in SV smooth muscle does not depend on the activation of LVDCCs. Thus, the mucosa of guinea pig SV is fundamental in generating LVDCC-independent electrical activity in SV smooth muscle associated with Ca^{2+} transients. Mucosa-dependent slow waves trigger the activation of LVDCCs to amplify themselves resulting in action potential firing that appears to play a major role in generating contractions in SV smooth muscle. Synchrony of the periodical spontaneous Ca^{2+} transients in the SV smooth muscle cells is preserved during the blockade of LVDCCs by 10 μM nifedipine (Fig. 9.6b). This is in marked contrast to the spontaneous Ca^{2+} transients in atypical smooth muscle cells of the renal pelvis [27] or suburothelial venular smooth muscle cells [28, 29] in which LVDCCs play a critical role in maintaining the synchrony amongst spontaneously active cells.

9.3.3 Role of Intracellular Ca^{2+} Stores in Spontaneous Activity of SV Smooth Muscle

Blockade of SR/ER Ca^{2+}-ATPase (SERCA) by 10 μM cyclopiazonic acid (CPA) abolishes the slow waves and synchronous spontaneous Ca^{2+} transients in the 10 μM nifedipine-pretreated mucosa-intact SVs (Fig. 9.6), suggesting that both events depend on Ca^{2+} handling by the SR/ER. Thus, in common with most visceral smooth muscle organs, spontaneous phasic contractions in SV circular smooth muscle appear to be primarily driven by the cycle of Ca^{2+} uptake and release by the SR/ER. Since mucosa-denuded smooth muscle of guinea pig SVs develops LVDCC-independent, CPA-sensitive increases in intracellular Ca^{2+} concentration upon the activation of α_1-ARs that are known to couple with Gq proteins [22], inositol 1,4,5-trisphosphate (IP_3) receptors may well be involved in Ca^{2+} release

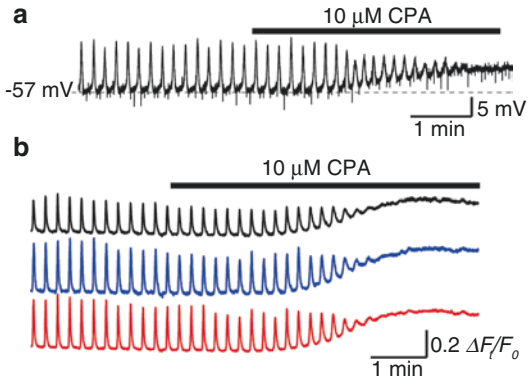

Fig. 9.6 Blockade of SERCA abolished nifedipine-resistant slow waves and spontaneous Ca^{2+} transients in mucosa-intact guinea pig SV smooth muscle. (**a**) In a 10 μM nifedipine-pretreated SV smooth muscle preparation, CPA depolarized the membrane by about 5 mV and prevented the generation of the slow waves. (**b**) Intracellular Ca^{2+} dynamics recorded from three regions of interest in another 10 μM nifedipine-pretreated SV smooth muscle preparation. Note that the synchrony of the spontaneous Ca^{2+} transients amongst smooth muscle cells was maintained in the presence of 10 μM nifedipine. CPA increased the basal Ca^{2+} level and abolished synchronous spontaneous Ca^{2+} transients. Reproduced from Takeya et al. [8]

from SR in SV smooth muscle. However, the relative contribution of IP_3- and ryanodine-receptor Ca^{2+} release channels to the generation of mucosa-dependent activity in SVs has not yet been established.

9.3.4 Role of Ca^{2+}-Activated Cl^- Channel in Spontaneous Activity of SV Smooth Muscle

The LVDCC-independent slow waves in the mucosa-intact guinea pig SV smooth muscle are abolished by lowering extracellular Cl^- concentration from 130 to 13 mM [8]. Nonselective Ca^{2+}-activated Cl^- channel (CaCC) blockers such as DIDS (300 μM) and niflumic acid (100 μM) also abolish or greatly reduce the generation of slow waves in 10 μM nifedipine-pretreated SV smooth muscle preparations [8]. Thus, generation of the slow waves in SV smooth muscle may depend on Cl^- efflux via CaCCs triggered by spontaneous Ca^{2+} release from the SR/ER. Similar mechanisms underlying the slow-wave generation have been confirmed in interstitial cells of Cajal (ICCs) in gastrointestinal (GI) tract [30] and urethra [31].

Anoctamin1 (ANO1) has been established to function as CaCCs that underlie slow waves in ICCs of the GI tract [30]. In guinea pig SV smooth muscle, 3 μM $T16A_{inh}$-A01, a selective ANO1 inhibitor, does not affect the SV slow-wave generation, suggesting the contribution of CaCCs other than ANO1 channels [8]. Since ANO1 immunoreactivity in the guinea pig SVs is restricted to the apical surface of the secretory columnar epithelium (Fig. 9.7), ANO1 channels appear to be involved in Cl^- secretion into seminal fluid [32–34] but not the generation of the SV slow waves.

9.4 How Does the Mucosa Drive Spontaneous Activity in SV Smooth Muscle?

9.4.1 Spontaneously Active Cells in SV Mucosa

Since an intact mucosa is required for generating synchronous spontaneous Ca^{2+} transients and corresponding CaCC-dependent slow waves in guinea pig SV smooth muscle, both events may be driven by spontaneously active cells within the mucosa.

In the basal surface of the mucosal preparations dissected from the muscular layer of guinea pig SV (Fig. 9.8a), a population of cells distributed just beneath the columnar epithelial cells develop asynchronous, irregularly occurred Ca^{2+} transients lasting for more than several seconds in 10 μM nifedipine (Fig. 9.8b, c). The spontaneous Ca^{2+} transients in the subepithelial cells are abolished by 10 μM CPA (Fig. 9.8c), suggesting that these cells generate spontaneous Ca^{2+} activity relying on Ca^{2+} handling by the SR/ER.

Application of 100 μM ATP evokes almost synchronous, robust Ca^{2+} transients in the spontaneously active subepithelial cells (Fig. 9.8b, Video 9.2). The morphology of the subepithelial cells, having irregular-shaped cell bodies with

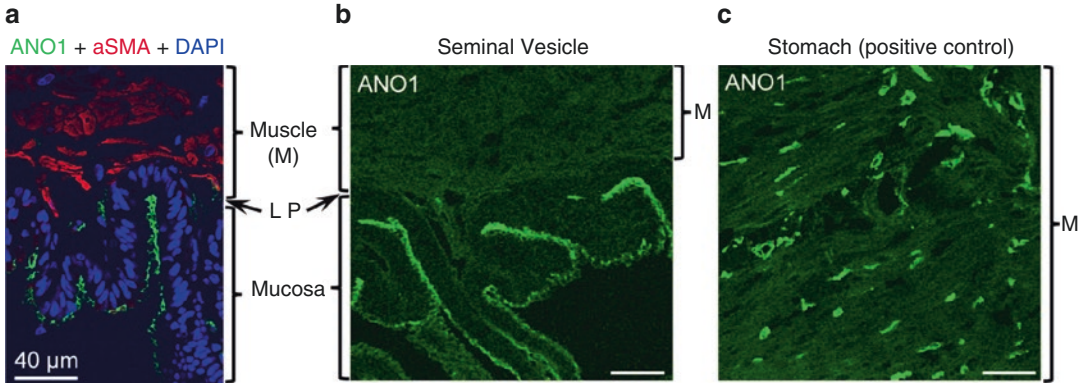

Fig. 9.7 Distribution of ANO1 in guinea pig SV and stomach. (**a**) In the coronal section of guinea pig SV, the apical side of the mucosa, but not lamina propria (*LP*) or muscular layer (*M*), was immunopositive for ANO1. The sections of another guinea pig SV (**b**) and gastric antrum (**c**) were immunolabelled with anti-ANO1 antibody using the same protocol. In the SV, ANO1 immunoreactivity localized in the apical side of the mucosa (**b**), while ANO1-immunoreactive cells were detected in the muscular layer of the stomach. αSMA, α-smooth muscle actin. All scale bar = 40 μm. Modified from Takeya et al. [8]

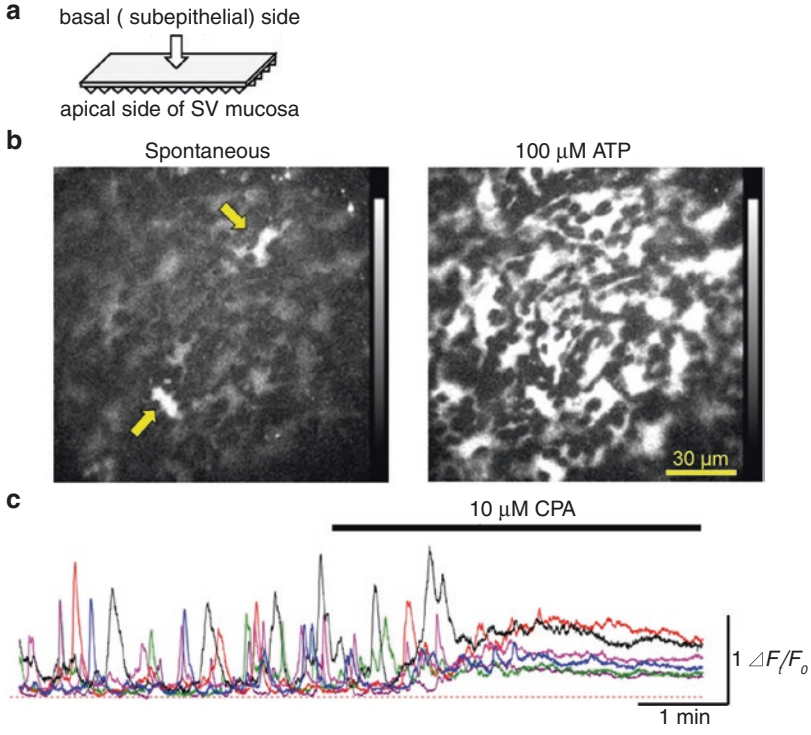

Fig. 9.8 Spontaneous and ATP-induced Ca^{2+} transients in SV "isolated" mucosal preparations. (**a**) An illustration of dissected SV mucosa from the muscular layer used for the Cal-520 fluorescence imaging and for the whole-mount preparation in immunohistochemistry. Functional and morphological properties were examined from basal (subepithelial) side of the preparations. (**b**) In a mucosal preparation that had been pretreated with 10 μM nifedipine, a population of cells located just beneath the columnar epithelium generated the spontaneous Ca^{2+} transients (left image, *arrows*). Subsequent 100 μM ATP evoked a massive increase in the intracellular Ca^{2+} in the spontaneously active subepithelial cells (right image). (**c**) Asynchronous spontaneous Ca^{2+} transients recorded from six subepithelial cells in another 10 μM nifedipine-pretreated preparation. Individual subepithelial cells generated "irregularly occurring" Ca^{2+} transients independently of each other. CPA (10 μM) increased the basal Ca^{2+} level and abolished the Ca^{2+} transients. Adapted from Takeya et al. [8]

several short processes, could clearly be visualized during the ATP-induced Ca^{2+} transients. The density of the ATP-sensitive subepithelial cells is about 30–40 cells per (100 μm)^2 and their cell bodies have a length of 15–20 μm and a width of 6–10 μm [8].

9.4.2 Cellular Composition of SV Mucosa

In the guinea pig SV mucosal preparations (Fig. 9.8a), vimentin-immunoreactive (IR) interstitial cells in lamina propria are situated beneath the pancytokeratin-IR epithelial cells (Fig. 9.9a). In the lamina propria, platelet-derived growth factor receptor α (PDGFRα)-positive interstitial cells are a subpopulation of the vimentin-IR interstitial cells, as evident by the intermediate filaments of these PDGFRα-positive interstitial cells being also stained by a vimentin antibody (Fig. 9.9b, c). In contrast, other vimentin-IR interstitial cells do not express PDGFRα.

The distribution of c-Kit-IR interstitial cells has not been demonstrated in the guinea pig SV mucosa or muscular layer [8]. Furthermore, α-smooth muscle actin (αSMA)-IR cells are observed only in vascular smooth muscle cells in guinea pig SV mucosa [8]. Thus, the origin of spontaneous activity in guinea pig SV mucosa appears to be different from well-known pacemakers such as c-Kit-positive ICCs in GI tract or atypical smooth muscle cells expressing αSMA in renal pelvis.

The morphological characteristics of the subepithelial cells firing spontaneous Ca^{2+} transients (Fig. 9.8b) appear to be different from PDGFRα-IR interstitial cells with their slenderer cell shape (>20 μm in cell length) (Fig. 9.9) in terms of their cell shape or size. PDGFRα-IR and/or vimentin-IR interstitial cells in the lamina propria that ***have*** a longer cell length than the spontaneously active subepithelial cells are also distributed in the mucosal surface of mucosa-denuded SV smooth muscle preparations (Fig. 9.10a, T. Hayashi and M. Takeya, unpublished observation). These morphological examinations reveal that interstitial cells are located between the subepithelial cells firing spontaneous Ca^{2+} transients and the smooth muscle cells (Fig. 9.10b). Since the spontaneous periodical activity is not generated in mucosa-denuded SV

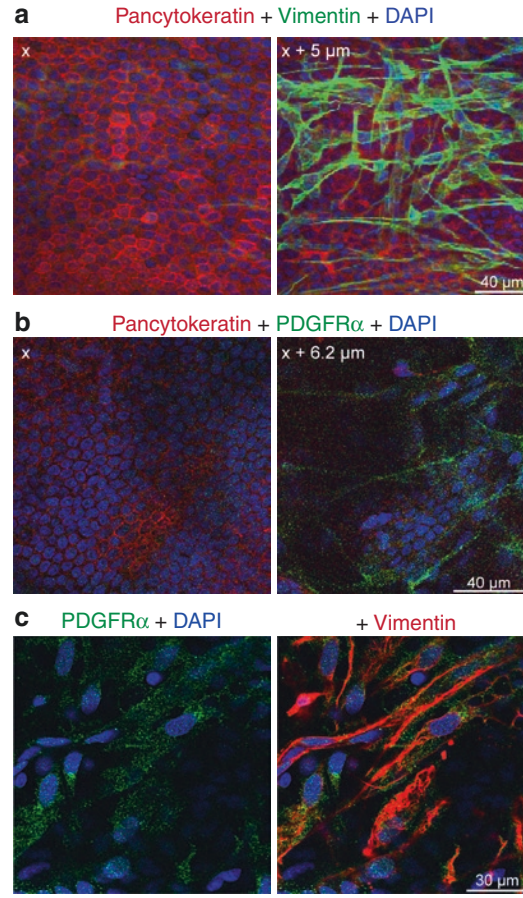

Fig. 9.9 Epithelium and interstitial cells in the lamina propria distributing on the basal surface of the "isolated" SV mucosal preparations. Whole-mount preparations of dissected guinea pig SV mucosa from the muscular layer (Fig. 9.8a) were observed using confocal microscopy. (**a**) Serial images were obtained from the basal (subepithelial) side of the mucosa preparations immunolabelled with anti-pancytokeratin (red: a marker of epithelial cell) and vimentin (green: a marker of interstitial cell). "*x + 5 μm*" in the right image indicates that the image was obtained 5 μm from the basal side of "*x*." Vimentin-immunoreactive (IR) cells located beneath the epithelial layer. (**b**) Serial images of another SV mucosal preparations. PDGFRα-IR (green) cells are found beneath the epithelial layer (*x + 6.2 μm*). (**c**) In other SV mucosal preparation, PDGFRα (green)-IR-positive cells in the lamina propria were immunoreactive for vimentin (red). Adapted from Takeya et al. [8]

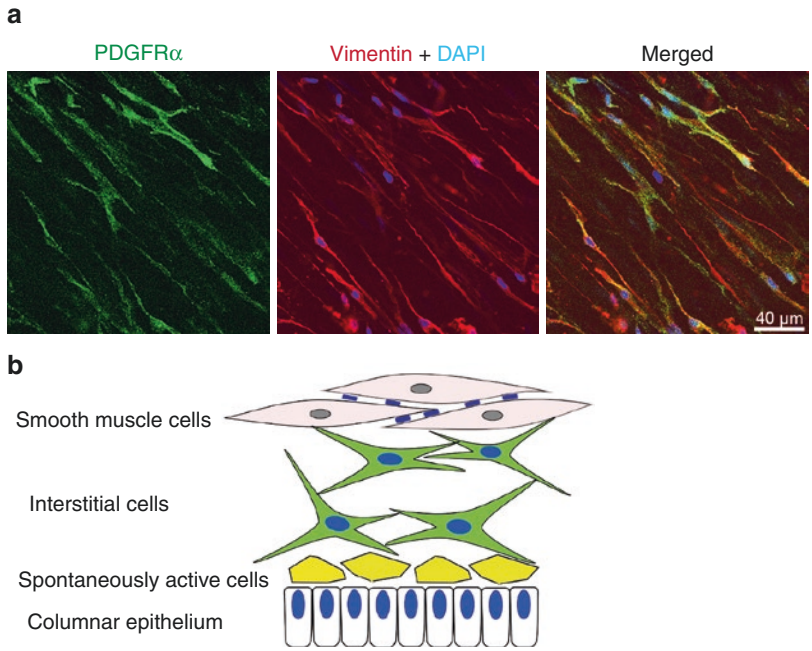

Fig. 9.10 Interstitial cells in the lamina propria also exist on the mucosal surface of SV smooth muscle preparation after removing the mucosa. (**a**) PDGFRα (green)-IR and/or vimentin-positive (red) interstitial cells distributing on the mucosal surface of the mucosa-denuded guinea pig SV smooth muscle. (**b**) A schematic drawing of the guinea pig SV wall based on distribution of vimentin-IR interstitial cells in the striped surface of both dissected mucosal and muscular preparations. There may be interstitial cells between the subepithelial cells firing asynchronous spontaneous Ca^{2+} transients and the smooth muscle cells

smooth muscle, the interstitial cells attached to mucosa-denuded smooth muscle are not capable of driving the auto-rhythmicity in smooth muscle cells.

9.4.3 Communication Between Mucosal and Muscular Cells

Synchronous spontaneous electrical and Ca^{2+} activity generated in SV smooth muscle cells could be driven by either depolarizing signals or humoral substances originated from unidentified mucosal "pacemaker" cells, presumably the subepithelial cells.

9.4.3.1 Role of Humoral Substances Released from Mucosal Cells

Spontaneous Ca^{2+} transients in the subepithelial cells may well trigger the Ca^{2+}-dependent synthesis and/or release of humoral substances. The mucosa-derived factors, if any, may enhance subthreshold "auto-rhythmicity" in the syncytium of SV smooth muscle cells by stimulating IP_3 production to facilitate "cytosolic Ca^{2+} oscillators," i.e., the cycle of Ca^{2+} uptake by SERCA and Ca^{2+} release [35]. This notion is supported by the finding that the "quiescent" mucosa-denuded guinea pig SV smooth muscle invariably develops oscillatory contractions, depolarizations, and Ca^{2+} transients upon the activation of α_1-ARs that couple with Gq signaling pathways (Fig. 9.4c) [22]. However, these mucosa-derived factors triggering SV spontaneous contractions are neither noradrenaline nor ACh, as inhibition of α-ARs or muscarinic receptors does not prevent these spontaneous phasic contractions of the guinea pig SV strips [17, 18].

Since application of ATP evokes robust Ca^{2+} transients in the spontaneously active subepithelial cells (Fig. 9.8), endogenous ATP could be involved in the generation and/or amplification of the spontaneous Ca^{2+} transients in the SV subepithelial cells. In guinea pig and human bladder mucosal preparations, UTP, a P2Y agonist, evokes ATP

release, suggesting that ATP autocrine/paracrine signaling may enhance further ATP release from the urothelium via activation of P2Y receptors [36]. Stretch-induced ATP release from guinea pig bladder mucosa has also been demonstrated [36]. Since SV smooth muscle develops mucosa-dependent periodical activity when stretched, mechanosensitive ATP release from epithelium or the spontaneously active subepithelial cells may be involved in the SV spontaneous contractions.

Prostaglandins (PGs), whose contribution to mucosa-dependent contractions in SV remains to be determined, are well known to be produced as constituents of seminal fluid by SV epithelium [1, 37]. Endogenous PGs play a fundamental role in generating pyeloureteric contraction of several species [38]. In the rabbit corpus cavernosum, cyclooxygenase-2 (COX-2)-dependent PGs may be released by intramuscular interstitial cells to develop the spontaneous phasic activity [39]. In murine gastric antral muscle, production of COX-2-dependent PGs in intramuscular ICC (ICC-IM) is involved in stretch-mediated chronotropic activity [30, 40].

9.4.3.2 Role of Gap Junction in Generating the Mucosa-Dependent Activity in SV Smooth Muscle

In mucosa-intact SV smooth muscle, the CaCC-dependent slow waves are abolished by 100 μM carbenoxolone, a gap junction blocker (Fig. 9.11). The simplest interpretation is that CaCC-dependent slow waves originate in the mucosal "pacemaker" cells and spread into SV musculature via gap junctions. In this case, a functional syncytium appears to be formed by at least three cell populations: spontaneously active subepithelial cells that act as pacemaker cells, interstitial cells in lamina propria that may act as an electrical conducting pathway, and "driven" smooth muscle cells. Since spontaneous Ca^{2+} transients in the SV subepithelial cells are generated independently of each other (Fig. 9.8), the individual subepithelial cells may irregularly generate spontaneous transient depolarizations (STDs) due to opening of CaCCs. Such irregular, asynchronous spontaneous depolarizing signals from the subepithelial cells may act as "point sources" of excitation, and the periodicity of slow waves and Ca^{2+} transients in SV smooth muscle may be determined by the refractory period of the interstitial cells in the lamina propria and/or smooth muscle cells, e.g., reduction in SR/ER Ca^{2+} contents or inactivation/deactivation of ion channels.

Even in cases where mucosa-derived humoral factors diffuse into the smooth muscle layer to activate their subthreshold "auto-rhythmicity," gap junction-mediated coupling may play a fundamental role in the subepithelial cells or intestinal cells in lamina propria. Gap junction coupling amongst the subepithelial "pacemaker" cells allows the cells to trigger a synchronous "surge" release of the humoral factors. Alternatively, mucosa-derived humoral substances released

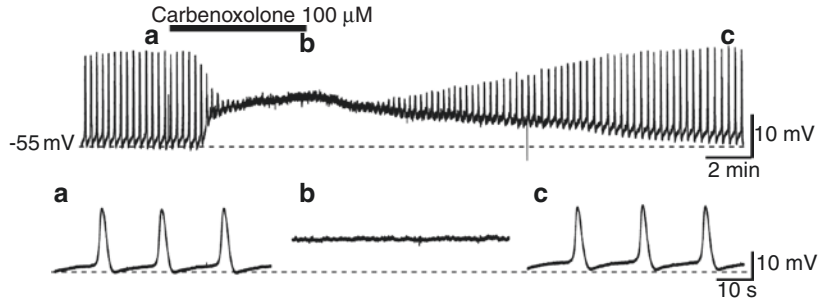

Fig. 9.11 Blockade of gap junctions abolishes CaCC-dependent slow waves in mucosa-intact SV smooth muscle. In a 10 μM nifedipine-pretreated preparation, 100 μM carbenoxolone depolarized the membrane and prevented the generation of slow waves. Lower traces were displayed in control (**a**), carbenoxolone (**b**), and after recovery (**c**) with an expanded time scale. Traces **a**–**c** were obtained at the timings indicated by the corresponding characters in the upper trace. Reproduced from Takeya et al. [8]

from the subepithelial cells may activate interstitial cells in the lamina propria that function as a "mediator" to induce the auto-rhythmicity in SV smooth muscles via gap junctions. In our preliminary observation, placing dissected mucosal preparations close to the quiescent mucosa-denuded SV smooth muscle preparations failed to restore the spontaneous phasic contractions (M. Takeya and M. Takano, unpublished data). These results could be explained by a disruption of gap junction connections between the interstitial cells in the lamina propria and muscular layers. Of course, the results could simply result from insufficient diffusion of the humoral substances from the subepithelial cells to smooth muscle layer due to their degradation.

Since dissecting the mucosa away from the SV smooth muscle layer mechanically breaks their intercellular communications, fundamental mechanisms in generating the mucosal spontaneous activity and/or transmitting the mucosal signals to the muscular layer may have been lost in these "isolated" mucosal preparations. In particular, interstitial cells in the lamina propria that may function as intermediaries or integrators of mucosal signaling will be divided into the mucosal and muscular sides (Figs. 9.9 and 9.10a). The spontaneously active subepithelial cells that generate asynchronous spontaneous electrical and Ca^{2+} activity may well develop "synchronous" oscillatory activity by coupling with the underlying SV smooth muscle syncytium via interstitial cells in lamina propria.

9.5 Perspective: Role of SV Spontaneous Contractions in Male Fertility

SVs secrete the major components of seminal fluid including fructose, prostaglandins, antioxidant agents, as well as a variety of other bioactive proteins such as cytokines [1, 37]. Although seminal fluid is often regarded simply as a vehicle to transport sperm to fertilize the oocyte, novel roles of seminal fluid in reproductive medicine are becoming evident in recent years. In most animals, even though viable pregnancies can be initiated using epididymal or washed ejaculated sperm during in vitro fertilization followed by embryo transfer, their success rate, i.e., normal pregnancy and live birth, is low. It has been demonstrated that the addition of seminal plasma to assisted reproductive procedures can significantly improve pregnancy success in several animals as well as humans [41–43]. Growing evidence indicates that signaling agents in seminal fluid drive multifactorial changes within the maternal uterus to establish an environment conducive to optimal embryo implantation and pregnancy outcome [42, 44]. It is envisaged that spontaneous constrictions of SVs during inter-ejaculatory phase have a "mixing function" to improve the fluidity of the viscous contents and quality of the bioactive substances that are vital for male fertility.

9.6 Conclusions

SVs develop spontaneous phasic contractions during the inter-ejaculatory phase. These events may be produced or enhanced upon muscle wall stretch. Besides the secretion of seminal fluid that are required for male fertility, the mucosa of guinea pig SV plays a crucial role in generating spontaneous contractions. Mucosal signals may well be transmitted to SV smooth muscle via gap junction coupling to drive CaCC-dependent SWs triggering the opening of LVDCCs to contract the SV muscularis. A candidate for the SV pacemaker, is the subepithelial cell that exists just beneath the columnar epithelium and fires spontaneous Ca^{2+} transients which relyies on SR/ER Ca^{2+} cycling; although the identification of the pacemaker cell remains to be determined. There are interstitial cells in lamina propria that may act as an intermediator between the spontaneously active subepithelial cells and the SV smooth muscle cells. It should also be explored whether mucosa-derived humoral substances drive the spontaneous activity in SV smooth muscle. Thus, the pathway of signal transmission from the SV mucosa to muscularis remains to be of great interest.

References

1. Aumüller G, Riva A. Morphology and functions of the human seminal vesicle. Andrologia. 1992;24:183–96. https://doi.org/10.1111/j.1439-0272.1992.tb02636.x.
2. Al-Zuhair A, Gosling JA, Dixon JS. Observation on the structure and autonomic innervation of the guinea-pig seminal vesicle and ductus deferens. J Anat. 1975;120:81–93.
3. Clement P, Giuliano F. Physiology and pharmacology of ejaculation. Basic Clin Pharmacol Toxicol. 2016;119:18–25. https://doi.org/10.1111/bcpt.12546.
4. Capogrosso P, Serino A, Ventimiglia E, Boeri L, Dehò F, Damiano R, et al. Effects of silodosin on sexual function—realistic picture from the everyday clinical practice. Andrology. 2015;3:1076–81. https://doi.org/10.1111/andr.12095.
5. Hisasue S, Furuya R, Itoh N, Kobayashi K, Furuya S, Tsukamoto T. Ejaculatory disorder caused by alpha-1 adrenoceptor antagonists is not retrograde ejaculation but a loss of seminal emission. Int J Urol. 2006;13:1311–6. https://doi.org/10.1111/j.1442-2042.2006.01535.x.
6. Hayashi T, Takeya M, Nakamura K, Matsuoka K. Effects of silodosin and tamsulosin on the seminal vesicle contractile response. LUTS. 2016;8:55–61. https://doi.org/10.1111/luts.12072.
7. Waddell JA. The pharmacology of the seminal vesicles. J Pharmacol Exp Ther. 1916;9:113–20.
8. Takeya M, Hashitani H, Hayashi T, Higashi R, Nakamura KI, Takano M. Role of mucosa in generating spontaneous activity in the guinea pig seminal vesicle. J Physiol. 2017;595:4803–21. https://doi.org/10.1113/JP273872.
9. Gonzales GF. Functional structure and ultrastructure of seminal vesicles. Arch Androl. 1989;22:1–13.
10. Price D, William-Ashman HG. The accessory reproductive glands of mammals. In: Young WC, editor. Sex and internal secretions, vol. 1. 3rd ed. Baltimore, MD: The Williams and Wilkins Co.; 1961. p. 366–448.
11. Melin P. In vivo recording of contractile activity of male accessory genital organs in rabbits. Acta Physiol Scand. 1970;79:109–13. https://doi.org/10.1111/j.1748-1716.1970.tb04706.x.
12. Veneziale CM, Brown AL, Prendergast FG. Histology and fine structure of guinea pig seminal vesicle. Mayo Clin Proc. 1974;49:309–13.
13. Tse MKW, Wong YC. Structural study of the involution of the seminal vesicles of the guinea pig following orchiectomy. Acta Anat. 1980;108:68–78. https://doi.org/10.1159/000145283.
14. Hib J, Ponzio R, Vilar O. In vivo recording of contractile activity of pelvic urethra and seminal vesicle in rats. Effects of electrical stimulations and neurohypophysial hormones. Andrologia. 1983;15:480–5. https://doi.org/10.1111/j.1439-0272.1983.tb00173.x.
15. Hib J, Ponzio R, Vilar O. Effects of autonomic drugs on contractions of rat seminal vesicles *in vivo*. J Reprod Fertil. 1984;70:197–202. https://doi.org/10.1530/jrf.0.0700197.
16. Turek PJ, Aslam K, Younes AK, Nguyen HT. Observation of seminal vesicle dynamics in an in vivo rat model. J Urol. 1998;159:1731–4. https://doi.org/10.1097/00005392-199805000-00102.
17. Ohkawa H. Further evidences for the cholinergic and adrenergic mechanisms in the isolated hypogastric nerve-seminal vesicle preparation of the guinea-pig. Bull Yamaguchi Med Sch. 1973;20:73–84.
18. Ohkawa H. Evidence for adrenergic transmission in the circular smooth muscle of the guinea-pig seminal vesicle. Tohoku J Exp Med. 1981;134:141–58. https://doi.org/10.1620/tjem.134.141.
19. Birowo P, Ückert S, Kedia GT, Sonnenberg JE, Thon WF, Rahardjo D, et al. Characterization of the effects of various drugs likely to affect smooth muscle tension on isolated human seminal vesicle tissue. Urology. 2010;75:974–8. https://doi.org/10.1016/j.urology.2009.09.034.
20. Narita H. The study of sperm transport through the human genital tract—pharmacological responses in vitro of the human genital tract. Jpn J Urol. 1983;74:1734–48. https://doi.org/10.5980/jpnjurol1928.74.10_1734.
21. Ohkawa H. Excitatory junction potentials recorded from the circular smooth muscles of the guinea-pig seminal vesicle. Tohoku J Exp Med. 1982;136:89–102. https://doi.org/10.1620/tjem.136.89.
22. Kubota Y, Hashitani H, Fukuta H, Sasaki S, Kohri K, Suzuki H. Mechanisms of excitatory transmission in circular smooth muscles of the guinea pig seminal vesicle. J Urol. 2003;169:390–5. https://doi.org/10.1016/S0022-5347(05)64134-1.
23. Shigemasa Y, Lam M, Mitsui R, Hashitani H. Voltage dependence of slow wave frequency in the guinea pig prostate. J Urol. 2014;192:1286–92. https://doi.org/10.1016/j.juro.2014.03.034.
24. Hashitani H, van Helden DF, Suzuki H. Properties of spontaneous depolarizations in circular smooth muscle cells of rabbit urethra. Br J Pharmacol. 1996;118:1627–32. https://doi.org/10.1111/j.1476-5381.1996.tb15584.x.
25. Hashitani H, Edwards FR. Spontaneous and neurally activated depolarizations in smooth muscle cells of the guinea-pig urethra. J Physiol. 1999;514:459–70. https://doi.org/10.1111/j.1469-7793.1999.459ae.x.
26. Sadraei H, Beech DJ. Ionic currents and inhibitory effects of glibenclamide in seminal vesicle smooth muscle cells. Br J Pharmacol. 1995;115:1447–54. https://doi.org/10.1111/j.1476-5381.1995.tb16636.x.
27. Lang RJ, Hashitani H, Tonta MA, Parkington HC, Suzuki H. Spontaneous electrical and Ca^{2+} signals in typical and atypical smooth muscle cells and interstitial cell of Cajal-like cells of mouse renal pelvis. J Physiol. 2007;583:1049–68. https://doi.org/10.1113/jphysiol.2007.137034.
28. Hashitani H, Takano H, Fujita K, Mitsui R, Suzuki H. Functional properties of suburothelial microvessels in the rat bladder. J Urol. 2011;185:2382–91. https://doi.org/10.1016/j.juro.2011.02.046.
29. Hashitani H, Mitsui R, Shimizu Y, Higashi R, Nakamura K. Functional and morphological properties of pericytes in suburothelial venules of the mouse

bladder. Br J Pharmacol. 2012;167:1723–36. https://doi.org/10.1111/j.1476-5381.2012.02125.x.
30. Sanders KM, Ward SM, Koh SD. Interstitial cells: regulators of smooth muscle function. Physiol Rev. 2014;94:859–907. https://doi.org/10.1152/physrev.00037.2013.
31. Sergeant GP, Hollywood MA, McCloskey KD, Thornbury KD, McHale NG. Specialised pacemaking cells in the rabbit urethra. J Physiol. 2000;526:359–66. https://doi.org/10.1111/j.1469-7793.2000.t01-2-00359.x.
32. Levine N, Rinaldo JE, Schultz SG. Active chloride secretion by *in vitro* guinea-pig seminal vesicle and its possible relation to vesicular function *in vivo*. J Physiol. 1975;246:197–211. https://doi.org/10.1113/jphysiol.1975.sp010886.
33. Liao SB, Cheung KH, O WS, Tang F. Adrenomedullin increases the short-circuit current in the mouse seminal vesicle: actions on chloride secretion. Biol Reprod. 2014;91:31, 1–6. https://doi.org/10.1095/biolreprod.113.116848.
34. Jang Y, Oh U. Anoctamin 1 in secretory epithelia. Cell Calcium. 2014;55:355–61. https://doi.org/10.1016/j.ceca.2014.02.006.
35. van Helden DF, Imtiaz MS. Ca^{2+} phase waves: a basis for cellular pacemaking and long-range synchronicity in the guinea-pig gastric pylorus. J Physiol. 2003;548:271–96. https://doi.org/10.1111/j.1469-7793.2003.00271.x.
36. Sui G, Fry CH, Montgomery B, Roberts M, Wu R, Wu C. Purinergic and muscarinic modulation of ATP release from the urothelium and its paracrine actions. Am J Physiol Ren Physiol. 2014;306:F286–98. https://doi.org/10.1152/ajprenal.00291.2013.
37. Gonzales GF. Function of seminal vesicles and their role on male fertility. Asian J Androl. 2001;3:251–8.
38. Lang RJ, Davidson ME, Exintaris B. Pyeloureteral motility and ureteral peristalsis: essential role of sensory nerves and endogenous prostaglandins. Exp Physiol. 2002;87:129–46. https://doi.org/10.1113/eph8702290.
39. Hashitani H, Yanai Y, Shirasawa N, Soji T, Tomita A, Kohri K, Suzuki H. Interaction between spontaneous and neutrally mediated regulation of smooth muscle tone in the rabbit corpus cavernosum. J Physiol. 2005;569:723–35. https://doi.org/10.1113/jphysiol.2005.099309.
40. Won KJ, Sanders KM, Ward SM. Interstitial cells of Cajal mediate mechanosensitive responses in the stomach. Proc Natl Acad Sci U S A. 2005;102:14913–8. https://doi.org/10.1073/pnas.0503628102.
41. Robertson SA. Seminal plasma and male factor signalling in the female reproductive tract. Cell Tissue Res. 2005;322:43–52. https://doi.org/10.1007/s00441-005-1127-3.
42. Bromfield JJ. Seminal fluid and reproduction: much more than previously thought. J Assist Reprod Genet. 2014;31:627–36. https://doi.org/10.1007/s10815-014-0243-y.
43. Crawford G, Ray A, Gudi A, Shah A, Homburg R. The role of seminal plasma for improved outcomes during *in vitro* fertilization treatment: review of the literature and meta-analysis. Hum Reprod Update. 2015;21:275–84. https://doi.org/10.1093/humupd/dmu052.
44. Robertson SA, Sharkey DJ. Seminal fluid and fertility in women. Fertil Steril. 2016;106:511–9. https://doi.org/10.1016/j.fertnstert.2016.07.1101.