Many types of smooth muscle contract spontaneously, with each contraction being accompanied by a slow rhythmic oscillation of the membrane potential (slow wave), a discharge of spike potentials or both [1]. The spontaneous generation of a single spike or bursts of spikes is detected in smooth muscles of the urinary bladder, the uterus (longitudinal muscle layer), the prostate, the isolated longitudinal muscle of the intestine or the portal vein. Slow waves are observed in smooth muscle of the stomach, the circular layer of the intestine and uterus of many species, the renal pelvis, the urethra and the ureter. In several of these muscles, spike potentials are followed. These include the renal pelvis, ureter, uterus (circular muscle), lymphatic vessels and the urethra [2]. As many enteric nerves generate bursts of activity, the transmitters released from these nerves can modulate the activity of smooth muscles [3], and there is a possibility that smooth muscle activity could result indirectly as a consequence of neuronal activity. However, this is rarely the cause of muscle activity since many smooth muscles remain active after neuronal activity has been inhibited.

Key words: gastrointestinal muscle, slow waves, myogenic activity, interstitial cells, Ca release.

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blocks by applying the neurotoxin tetrodotoxin [2, 4]. Similarly, the inhibition of the postjunctional receptors activated by the chemical transmitter substances released by autonomic nerves, for example, acetylcholine, noradrenaline, nitric oxide, substance P or histamine [3], does not inhibit spontaneous activity in most smooth muscles [1]. These results support the idea that the spontaneous activity originates in smooth muscles, i.e. it is a “myogenic” activity [1, 2].

The best understood example of myogenic activity is that of the heart, where the sequential activation of sets of ion channels results in the rhythmic oscillation of the membrane potential in the sino-atrial node [5]. However, in smooth muscle, the membrane events responsible for the generation of spontaneous activity remain unclear. In the heart, activity in autonomic nerves innervating the pacemaker region alters the frequency of heart beat by altering the slope of diastolic phase of the pacemaker potential through modulation of the activity of specific ion channels [5]. In smooth muscles, on the other hand, the main effect of autonomic nerve activation is to modulate the intensity of contractile force with only minor effects on the frequency of activity [1]. These differences might suggest that the ionic mechanisms responsible for the pacemaker activity differ between cardiac and smooth muscles.

Although much attention has been paid to the myogenic activity of smooth muscles, an understanding of the cellular mechanisms underlying spontaneous activity has not been forthcoming. A major problem arises because activity is most readily detected in a preparation of tissue containing hundreds of cells, rather than in single smooth muscle cells which have been dispersed after enzyme treatment. It is reasonable to speculate that the activity originates in a group of special cells analogous to sino-atrial node cells of the heart, and the population of such cells is too small to detect after applying conventional techniques for isolating single cells. As examples, specialized “pacemaker cells” appear to be scattered within smooth muscle tissues of the renal pelvis [6]. A thin layer of specialized smooth muscle cells located in the submucosal layer of the colon is required to generate spontaneous activity in this tissue [7–9]. In intestinal smooth muscles, on the other hand, spontaneous activity has been considered to originate in non-muscle cells, the so-called “interstitial cells of Cajal” (ICC), distributed at the myenteric region [10, 11]. ICC differ from smooth muscle cells in that they lack a contractile apparatus, they contain vimentin filaments and display immuno-reactivity to anti-c-Kit antibody [12–14]. However they share some ultrastructural similarities with smooth muscle cells, having caveolae in the plasma membrane and a basal lamina around their outer membranes [15]. Indeed ICC are the source of activity, gastrointestinal activity may not be generated by smooth muscle cells, and a re-consideration for the criteria of “myogenic” activity may be required.

Here, attempts will be made to overview our present knowledge of the cellular mechanisms underlying spontaneous activity in gastric smooth muscle cells. There are many reports relating to the spontaneous activity of gastrointestinal smooth muscle cells; these have been reviewed by several authors [1, 10, 11, 13, 16, 17]. Descriptions of the membrane events underlying the spontaneous activity of intestinal smooth muscle are well documented. Consequently, this review will start with a brief overview of such activity in intestinal smooth muscles. It is hoped that this will provide a basis for understanding the properties of gastric smooth muscle cells.

**Spontaneous Activity of Intestinal Smooth Muscles**

The intestinal wall consists of two muscle layers, a longitudinal layer on the serosal side and a circular muscle layer on the mucosal side. The myenteric plexus lies between these two muscle layers, and each muscle layer receives one or more projections from this plexus [3]. In addition, many types of ICC are distributed within the intestinal wall. Those which are thought to be required for spontaneous activity form a single-layer network in the myenteric region [13, 14]. Individual ICC are spindle-shaped with long processes that contact nearby ICC via gap junctions to form a network. ICC also form contacts with two layers of smooth muscle cells. Within each layer, smooth muscle cells are electrically coupled to neighboring cells. Thus, the three types of cells form an array of interconnected networks, suggesting that electrical signals generated in one cell can propagate to surrounding cells electrotonically in a three-dimensional manner unless rectification occurs at individual gap junctions.

Experiments using isolated preparations of circular and longitudinal muscles of the cat intestine indicated that only the latter was spontaneously active, and therefore the pacemaker was considered to be located in the longitudinal muscle layer [18]. Recording the electrical activity of smooth muscle cells from different levels of the wall of mammalian intestine (cat, dog, opossum, rabbit, human) showed that activity could be detected from many cell layers [19]. The amplitude of the activity was largest at the border of the myenteric layer, and decreased with distance from the
Dissection experiments using the intestine of dog also suggested that the activity originates at the border of the circular and longitudinal muscle layers as removal of the myenteric layer abolished activity [19]. These data suggest that the pacemaker cells initiating intestinal motility locate in the myenteric region. In a series of anatomical studies, Thuneberg [12] found that ICC in the myenteric layer form gap junctional connections with nearby smooth muscle cells and are rich in mitochondria. He hypothesized that ICC function as pacemaker cells in the intestine, that energy production by mitochondria is essential for the maintenance of rhythmic activity and that gap junctions enable signals to propagate to other cells.

As pointed out, ICC are widely distributed in the intestinal wall. They can be visualized by staining with methylene blue [12]. When methylene blue-stained cells are exposed to strong light, the cells are destroyed due to damage to the mitochondria. In canine colonic smooth muscles, the removal of functional ICC using this method resulted in the inhibition of spontaneous activity with associated depolarization of the membrane [20, 21]. Damage to mitochondria by the incubation of tissues with rhodamin 123 also induced disorder in intestinal muscle activity [22]. Electron microscopic observation indicated that rhodamin 123 severely damages the ICC distributed in the myenteric regions [22]. These results therefore support the idea that intestinal activity originates in ICC [10, 11].

The discovery of abnormal motility patterns in the intestines of mice in which the development of ICC had been impaired with an antibody to protooncogene c-kit [23] opened new pages for understanding the mechanisms of intestinal activity. c-kit encodes a membrane receptor tyrosine kinase, and its structure and function are similar to the platelet-derived growth factor [24]. When baby mice are injected with antibodies to the c-Kit receptor domain, the expression of c-kit immunoreactivity disappears from ICC in the intestine along with an associated abolishment of spontaneous activity in smooth muscle cells [25, 26]. There are several types of ICC in the intestinal wall. The majority of c-kit-positive cells are found distributed in the myenteric region, but others are distributed through the adjacent muscle layers [14]. In mice, the white-spotting (W) locus is allelic with c-kit, and mutation of the W locus occurs spontaneously. Although homozygotes (WW) are fetal, there are different levels of point mutations, such as W^s, in which tyrosine kinase activity is not completely abolished. In these mutant mice, rhythmic activity of the membrane in intestinal smooth muscle cells is absent with associated reduction in the number of ICC in the myenteric layer [25–27] (Fig. 2). The stem cell factor (SCF) gene is syntenic with the Steel (Sl) locus, and a nonlethal level of the steel mutation in mice also abolishes both membrane electrical activity and normal development.
of ICC in intestinal smooth muscles [28]. These results are in good agreement with the observation that patients with several types of digestive disorders often associate with impaired ICC in the intestine [11]. Thus, ICC are likely the pacemaker cells for the spontaneous activity of intestinal smooth muscles. This may also explain the reason why enzymatically dispersed intestinal smooth muscle cells often fail to produce rhythmic activity.

ICC distributed in the myenteric layer of the canine colon are spontaneously active with irregular generation of square-type depolarizing responses [29]. In ICC isolated from the canine colon, voltage clamp experiments reveal that there are two different populations of voltage-sensitive Ca-channels with low and high thresholds; the former have properties similar to T-type Ca-channels, while the latter resemble L-type Ca-channels [30]. As colonic smooth muscle cell has only the high-threshold Ca-channels which are sensitive to organic Ca-antagonists [31], the pacemaker potential of the ICC is assumed to be produced by a sustained inward current carried through the low-threshold Ca-channels, analogous to pacemaker potentials in cardiac muscle. However, cultured ICC of the mouse intestine produce spontaneous membrane potential changes through opening Ca-activated Cl-channels [32]. The processes for the generation of spontaneous activity are suggested to be as follows: Ca$^{2+}$ released from internal stores activates Cl-channels to depolarize the membrane and, when the potential exceeds the threshold level, opens L-type Ca-channels to form a large depolarization. These mechanisms observed in the cultured ICC resemble those reported in the lymphatic [33] and urethral [34, 35] smooth muscle cells. In isolated ICC from the mouse small intestine, on the other hand, the membrane generates rhythmic depolarizing responses, and the potentials are produced by inward currents that are insensitive to the inhibitors of L-type Ca-channels [36] (Fig. 3). The reversal potential of the depolarizing response is $+10$ mV, suggesting that the inward current involved is produced by a mixed cation current through voltage-insensitive channels [36]. It remains unclear whether these differing results relate to species differences or the different experimental protocols used.

Close apposition of the processes of ICC distributing in muscle layers with axon varicosities of autonomic nerves suggests that some of the ICC may take place as an intermediary in enteric neurotransmission in the gizzard muscles of the love bird [37] and the esophagus of opossum [38]. In the dog colon, nerves innervating ICC are thought to be inhibitory since they contain vasoactive intestinal polypeptide (VIP), a candidate inhibitory transmitter substance of enteric nerves [39, 40]. In the mouse stomach, transmural nerve stimulation (TNS) evokes a non-adrenergic non-cholinergic inhibitory junction potential (NANC i.j.p.) which is produced by nitric oxide (NO) and a second unidentified substance. However the i.j.p.s evoked in muscles from the stomach of W/W$^v$ mutant mice are found lack a nitregic component [41]. As the hyperpolarization produced by sodium nitroprusside (SNP), an NO donor, is somewhat diminished in the mutant mice, intact ICC are thought to be essential for the generation of the NO-mediated responses in the smooth muscle layers. These phenomena were explained by assuming either that the receptors for neurotransmitters distribute mainly in ICC or the dominant target of innervation are ICC. Thus, NO released from nerves or applied exogenously produce responses mainly in ICC, and then they are transmitted to smooth muscle cells through gap junctions [41]. ICC contain the appropriate type of NO synthase [42], and surprisingly NO triggers an increase in the intracellular concentration of Ca$^{2+}$ ([Ca$^{2+}$]), the opposite response to that of smooth muscle cells [43]. Thus, stimulation of ICC with NO enhances the production of NO in ICC, inducing a strong inhibition of smooth muscle cells. In this way ICC appear to be not only the intermediary of inhibitory transmission but also an amplifier of NO signals [44].

**Properties of Spontaneous Activity in Gastric Smooth Muscles**

The electrical properties of gastric smooth muscles differ from region to region of the stomach. These “myogenic” differences between regions have been described in the dog [45, 46], the guinea-pig [47] and the rat [48]. In each species, smooth muscles of the fundus region are quiescent (Fig. 4), and in the guinea-pig, spike potentials are generated only when sustained depolarization is given to the membrane [49]. This property of the fundus muscle may be responsible for the initiation of smooth muscle contraction when extremely strong stretch is applied to the stomach following ingestion of a large amount of food.
Bundles of muscle isolated from the corpus regions produce simple slow waves while those isolated from pyloric regions generate groups of spike potentials on top of each slow wave. Antrum muscles generate slow waves with an initial spike-like component. Because of the rhythmical changes in membrane potential, it is often hard to determine the resting membrane potential in gastric smooth muscle cells. Taking the most negative level as the resting membrane potential, it is around \(-250\) mV in the fundus region and around \(-265\) mV in the pylorus region. The value becomes successively more negative along the gastric wall from fundus to pylorus, but the ionic mechanisms responsible for these changes remain unclear [47, 48].

The localization of points of initiation of slow waves has been investigated in the intact stomach of dog using extracellular electrodes; the observations indicated that the “pacemaker site” shifted at times [51]. Later, it is reported that the rhythmic activity first appeared in the corpus and then propagated successively to pyloric regions across the antrum [52]. However, re-examination of the points of initiation of myogenic activity in the dog stomach revealed that activity started from variable regions and occasionally propagated in an “antidromic” direction [53]. This indicates that, unlike the heart, the pacemaker site is not fixed but can change its location within the stomach. As segments of tissues isolated from any region of the stomach show intrinsic rhythmic activities, it is reasonable to understand that each segment possesses intrinsic pacemaker cells or pacemaker functions. In general, segments isolated from the corpus region contract much faster than those from the pylorus region [10]. By analogy with the heart [5], it seems reasonable to speculate that, in \(in vivo\), the activity appearing with the highest frequency (possibly in the corpus) dominates the activity from regions with lower frequency rhythms.

In isolated preparations of gastric muscle, the frequency of slow waves is reduced by cooling and stopped by metabolic poisons or anoxia, suggesting that they are linked to the metabolic activity of some cells lying in the stomach wall [1]. In isolated gastric muscles of the guinea-pig, blocking oxidative phosphorylation with cyanide reduces the frequency and amplitude of spontaneous activity [54], while the inhibition of glycolysis with iodoacetic acid (IAA) abolishes it [55]. As the inhibition by these metabolic inhibitors of muscle activity does not parallel the reduction of ATP contents in the cell, slow waves do not appear to be directly related to the hydrolysis of ATP to ADP [55]. The sites of ATP consumption/production remain unclear, but the importance in ICC appears likely since these cells are rich in mitochondria [12].

Although there is variation between regions in the stomach and between species, gastric slow waves usually occur some 2–8 times/min. Characteristically, their frequency is barely modulated by the changes in the membrane potential [1]. For example, in the antrum smooth muscle of the guinea-pig stomach, the frequency of slow wave changes by 10–20% with 20 mV membrane depolarizations or hyperpolarizations [56]. These values are much smaller compared to the 50% increase in the frequency of slow waves produced by a 5 mV depolarization in the cat colon [57]. Depolarization of the membrane will increase [Ca\(^{2+}\)] in smooth muscle cells, suggesting that the activation of pacing mechanisms of gastric muscles does not require an increase in [Ca\(^{2+}\)]. However, this does not seem to be the case since the frequency of slow wave decreases in low Ca\(^{2+}\) solutions and increases in high Ca\(^{2+}\) solutions [1]. The metabolic activity in smooth muscle is carried out mainly in mitochondria whose Ca\(^{2+}\) homeostasis is coupled to that of cytosolic [Ca\(^{2+}\)] [58]. Therefore, it is reasonable for slow
waves to occur at higher frequencies in depolarized conditions. It remains unclear why the frequency of slow waves is less sensitive to depolarization in the stomach muscles.

The frequency of slow wave is also not significantly modulated by autonomic nerve stimulation. In the antrum smooth muscles, the amplitude of slow waves is increased by cholinergic nerve stimulation and is decreased by NANC inhibitory effects, but in either case, only a minor change in the frequency of slow waves is detected [59, 60] (Fig. 5). These properties of slow waves are in contrast with the neural regulation of cardiac pacemaker cells; in the heart, autonomic nerve stimulation alters the frequency of pacemaker activity by changing the slope of the pacemaker potential [5]. In the pylorus muscles of the guinea-pig stomach, the voltage dependency of the frequency of slow wave appears after removal of the longitudinal muscle layers and, possibly, ICC distributed in the myenteric layer [61]. In this case, pacemaker mechanisms occurring in ICC may be largely independent from the membrane potential since ICC couple to smooth muscle cells electrically through gap junctions. Alternatively, changes in the membrane potential of smooth muscle cells may not directly couple to those of ICC, perhaps because there may be rectification at the gap junctions connecting these two cell types.

Fig. 5. Effects of transmural nerve stimulation on slow waves recorded from circular muscle of the guinea-pig stomach. A train of stimuli (A, 0.5 Hz; B, 1 Hz) was applied in the presence of physostigmine (A) or atropine (B). During nerve stimulation, the amplitude of slow waves is increased (A) or decreased (B) with no marked alteration in the frequency.

Cellular Mechanisms of Spontaneous Activity in Gastric Smooth Muscle

The ionic mechanisms responsible for the generation of slow waves in gastric smooth muscle cells have been assessed in experiments where the membrane potential has been changed or by applying solutions containing different ionic compositions or selective inhibitors of specific ion channels. The effects of organic Ca-antagonists, such as verapamil, diltiazem, nifedipine or nicardipine, on spontaneous electrical responses of gastric smooth muscles have been tested in several species. In the dog antrum muscles, diltiazem and nicardipine reduce the amplitude and duration of plateau potentials with no marked alteration of the initial spike component [46, 62], suggesting that the plateau component is produced by an influx of Ca$^{2+}$ through L-type Ca-channels. However in the guinea-pig stomach, verapamil [63], diltiazem [64] and nifedipine [65] inhibit spike potentials but not slow waves (Fig. 6); i.e., L-type Ca-channels contribute only to generate spike potentials, whereas slow waves are produced by ionic mechanisms different from the spike potentials. Thus, although the effects of Ca-antagonists on gastric muscles differ between dog and guinea-pig, both smooth muscle tissues produce spontaneous activity by mechanisms which do not involve Ca-currents carried through L-type Ca-channels.

Several types of agents which specifically block a range of different ion channels have been tested on slow waves, and these results are summarized elsewhere [1, 17]. Initially, the involvement of the Na-K pump in the generation of slow waves was considered since their frequency showed a high-temperature coef-
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By E.M. Dickens, G.D.S. Hirst, and H. Suzuki, unpublished observation). As slow waves are produced by inward currents [56], the ions involved should have equilibrium potentials positive to the resting membrane potential. The amplitude of slow wave decreases with depolarization and disappears around −30 mV, i.e., the level may be the reversal potential for slow waves. These observations suggest the involvement of chloride ions in the generation of slow waves since the value is similar to the equilibrium potential for these ions [71]. However, low Cl− solutions do not prevent generation nor dramatically alter the configuration of slow waves [1]. Niflumic acid and DIDS, inhibitors of Ca-activated Cl-channels, are also ineffective in inhibiting or modulating slow waves in the guinea-pig stomach [60, 72]. It is reasonable to speculate that Ca-activated K-channels contribute to the falling phase of slow waves since slow waves are associated with the elevation of [Ca2+]i, even in the presence of nifedipine [72]. However, the configuration of slow waves is not markedly altered in the presence of apamin or chalibudotoxin, inhibitors of Ca-activated K-channels [73]. Thus, at the moment, the well-known ion channel inhibitors are not effective in modulating slow waves generated in the guinea-pig gastric antrum.

The amplitude of slow waves is a function of membrane potential, and in the guinea-pig antrum muscles, hyperpolarization increases while depolarization reduces the amplitude [56]. Marked membrane hyperpolarizations reduce the amplitudes of slow waves to a size which becomes insensitive to further polarization. Thus, slow waves can be divided into two components, voltage-insensitive and sensitive components [1]. The voltage-insensitive component has a plateau-type potential, and depolarization of the membrane to a certain level (possibly threshold potential) triggers the secondary voltage-sensitive component (Fig. 7). The voltage-insensitive component of the slow waves is sensitive to temperature, with a Q10 value of about 2.7, suggesting that this is linked to the metabolic activity of the cells [56]. Voltage clamp experiments using double sucrose-gap methods indicate that currents flow inwardly during the generation of either the voltage-sensitive or the voltage-insensitive components of slow waves [56]. The ionic conductance of the membrane estimated from the amplitudes of electrotonic potential increases during the generation of slow waves [1, 69], suggesting that the second component of the slow waves is produced by ions whose equilibrium potentials distribute more positive than the resting membrane potential.

Isolated segments of gastric muscles contain three kinds of cells, circular and longitudinal smooth muscle cells and ICC. Attempts have been made to identify the different cell types by loading them with neurobiotin, each cell type being characterized by its pattern of spontaneous activity [65]. Circular muscles produce slow waves having triangular in shape with 20–30 mV amplitudes, longitudinal muscles produce square-shaped potentials with 20–30 mV amplitudes and ICC produce square-shaped potentials with 40–50 mV amplitudes (Fig. 8). The rate of rise of the potential is the fastest for ICC (0.4 V/s) and the slowest for circular muscle (0.08 V/s). Responses of these cells are well synchronized and insensitive to nifedipine. Furthermore, the excitation of ICC always proceeds that of circular and longitudinal muscle cells.
These properties may indicate that ICC are the pacemaker cells in gastric muscles, as in the case of intestinal muscles. The suggested pathway is as follows. A large driving potential, generated in ICC is transmitted electrotonically to circular and longitudinal muscle cells through gap junctions, and this potential triggers active responses (i.e., a voltage-sensitive component of slow wave) in the circular muscles while it produces only an electrotonic potential in longitudinal muscle cells. A low concentration of caffeine inhibits the voltage-sensitive component of the slow wave generated in circular muscles but does not alter the responses generated in longitudinal muscle cells and ICC [65]. These results also allow speculation that the gap junctional connection between ICC and circular muscles has a rectifying property since the frequency of voltage-insensitive component is hardly modulated by the polarization of circular smooth muscles [56].

**Properties of Regenerative Potentials Generated by Bundles of Circular Smooth Muscle**

As has been pointed out, many types of interstitial cells are distributed through the wall of the gastrointestinal tract, although they differ somewhat from the interstitial cells originally described by Cajal some 100 years ago (see [14]). Again, as pointed out, ICC may function as pacemaker cells in the small intestine [10, 11]. In this review, interstitial cells contributing to the spontaneous activity of gastrointestinal smooth muscles will all be categorized simply as ICC, as has been done in other reviews [10, 11]. The gastric wall has a complex distribution of interstitial cells, a network of ICC lies in the myenteric region and many spindle-shaped ICC are distributed through the circular muscle layer [74]. In cultured gastric muscles of mice where the development of ICC was inhibited by binding an antibody to c-Kit receptors, slow waves were absent [75]. This suggests that ICC may also be essential for the generation of slow waves in stomach. If this is the case, isolated circular muscles which are devoid of myenteric ICC should be quiescent, as is the case for isolated circular muscle of the small intestine [10]. Then, it would be expected that, in isolated circular muscles, electrical or chemical stimuli would evoke specific active responses. Experiments to test this possibility [76] indicated that three types of electrical responses can be recorded in small segments of circular muscles isolated from the guinea-pig antrum. The first type of response was the irregular generation of unitary depolarizations with amplitudes up to 15 mV. The second type of response was the periodic generation (every 10–20 s) of slow oscillatory potentials (duration 5–10 s) with large amplitudes (20–30 mV) termed regenerative potentials. A third type of response, a spike potential, was often triggered at the peak of the regenerative potentials. Nifedipine rapidly abolished the spike potentials but had no effect on either the unitary or regenerative potentials. A spectral analysis of the regenerative potential indicated that it was produced by summing together many of the unitary potentials [77]. Regenerative potentials resembled slow waves in form, but were significantly different in their sensitivity to caffeine; 1 mM caffeine abolished the regenerative potential but spared a part in the slow wave (Fig. 9). The difference between these two preparations appears to be the presence or

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**Fig. 8. Spontaneous electrical responses recorded from circular (A) and longitudinal muscles (C) and ICC (B) of the guinea-pig gastric antrum in the presence of 10^{-6} M nifedipine.** Modified from Dickens et al. [65].

**Fig. 9. Effects of caffeine on spontaneous activities recorded from circular and longitudinal muscles of the guinea-pig stomach antrum.** Regenerative potentials with spikes generated in circular muscle tissues (A) were absent in the presence of 1 mM caffeine (B). In longitudinal muscle tissues, spontaneously generated slow potentials with spikes (C) were changed to potentials with slow component alone in the presence of 1 mM caffeine (D).
absence of longitudinal muscle layer (and probably ICC) attached to the circular muscle layer. Caffeine inhibits part of the electrical responses generated by circular muscles but not those generated by ICC and longitudinal muscles [65]. Thus, regenerative potentials are the responses generated solely by bundles of circular muscle, whereas slow waves represent the combined activity of ICC and the circular layer, with the pacemaker potential generated by ICC passively depolarizing the circular layer to trigger the regenerative component. These results indicate that caffeine is a useful tool to identify contamination by ICC in isolated segments of circular muscles. Although we know that this methyl xanthine inhibits phosphodiesterase activity and inositol trisphosphate receptors [78], nothing is known about its detailed mechanism of action on gastric smooth muscle membrane.

In addition to caffeine, cyclopiazonic acid (CPA) and BAPTA also inhibit regenerative potentials. CPA inhibits Ca-ATPase at the membrane of the sarcoplasmic reticulum (SR), thus depleting Ca$^{2+}$ from internal stores [79]. BAPTA chelates Ca$^{2+}$, thereby reducing [Ca$^{2+}$], [80]. As pointed out, caffeine has complex actions on smooth muscles and it also depletes Ca$^{2+}$ from internal stores [81]. It is possible that this is how it abolishes regenerative potentials. These properties of regenerative potentials suggest that the generation of regenerative potential is associated with the release of Ca$^{2+}$ from internal stores. Inhibition of the functions of the Ca-store reduces the frequency of unitary depolarization, suggesting that this potential is also related to Ca$^{2+}$ release from internal stores. Electrical responses similar to unitary depolarization are also observed in lymphatic [33] and urethral smooth muscles [34]; in these tissues, they are produced by the activation of Ca-sensitive Cl-channels. However, niflumic acid, DIDS and 9-anthracenecarboxylic acid (9-AC), known inhibitors of Cl-channels, do not inhibit unitary depolarizations, regenerative potentials or slow waves [60], suggesting that ionic mechanisms underlying these signals differ.

In small segments of circular muscles of the antrum, membrane potential changes recorded simultaneously from two different cells are synchronized. This indicates that the cells are electrically coupled, possibly through gap junctions; this is proved by the production of electrotonic potentials during the injection of current into another cell [76]. Depolarization of the membrane by current injection evoked regenerative potentials in circular muscles isolated from the guinea-pig gastric antrum; they occurred after a minimum delay of about 1 s (Fig. 10). This delay is similar to the time required for the production of InsP$_3$ after the activation of receptors [82], which suggests that an unidentified second messenger may be formed in the interval between depolarization and activation of a regenerative potential. In canine intestine, the frequency of spontaneous activity is reduced in the presence of neomycin, an agent known to prevent the production of InsP$_3$ through the inhibition of phospholipase C [83]. In further support of the idea that InsP$_3$ is involved in triggering a regenerative potential is the inhibitory action of caffeine on the InsP$_3$ receptors [78]. Indeed, an alteration of receptor-activated production of InsP$_3$ by membrane potential changes has also been described [84, 85].

InsP$_3$ is almost invariably involved as a second messenger in the release of Ca$^{2+}$ from internal stores [78, 82]. There are three subtypes of InsP$_3$ receptor, and they distribute heterogeneously in different cells [78]. Each subtype of InsP$_3$ receptor seems to have a specific action on Ca$^{2+}$ stores. In cultured chicken B
lymphoma cells stimulated with ATP, expression of subtype 2 receptor results in long lasting and regular Ca^{2+} oscillations. Expression of subtype 3 causes the generation of monophasic Ca^{2+} transients, while that of subtype 1 mediates less regular Ca^{2+} oscillations [86]. In mice which genetically lack the expression of InsP3 type-1 receptor proteins [87], gastric smooth muscle fails to produce slow waves while it keeps the ability to generate spike potentials [88, 89] (Fig. 11). These observations are very similar to those made on the intestinal smooth muscle of W mutant mice lacking c-kit [90]. Thus, it seems likely that InsP3 plays a key role in the generation of spontaneous activity in gastric muscles.

Regenerative potentials, unlike action potentials, have very long refractory periods. In the small segment of circular muscles, regenerative potentials of reproducible amplitude are only evoked when two stimuli are applied at intervals of longer than 20–30 s [76]. Regenerative potentials are observed in the presence of nifedipine, indicating that L-type Ca-channels do not contribute to the generation of these potentials. Membrane conductance is increased during a regenerative potential [76], indicating that the potential is produced as a result of activation of a set of unidentified ion channels whose equilibrium potentials must be distributed positive to the resting membrane potential. The amplitudes of regenerative potentials are reduced when the membrane is depolarized by increasing [K^{+}]_o, and finally disappears at around −30 mV (K. Nose and H. Suzuki, unpublished observation), a value similar to that for slow waves [1]. The equilibrium potential for chloride ions distributes around −20 mV in smooth muscles [71], suggesting that the regenerative potentials are produced by an increase in chloride conductance. However, as pointed out, several types of chloride channel inhibitors (niflumic acid, DIDS) do not abolish regenerative potentials [60]. Thus, the ionic mechanisms underlying the generation of these potentials remain unclear.

**Closing Remarks**

Myogenic activity is detected in some smooth muscles like lymphatic vessels, the urethra or renal pelvis where activity undoubtedly arises as a result of the properties of muscle cells themselves. Our current knowledge suggests that ICC may take a central role in the generation of spontaneous activity of gastrointestinal smooth muscles. If this is the case, the word “myogenic” may be inadequate to describe this behaviour in gastrointestinal muscles since ICC are not smooth muscles. Thus, at the moment, we have no adequate term to express the spontaneous activity of gastrointestinal muscles. However, the cellular mechanisms for the generation of activity appear to be similar between different tissues, and in all cases, the elevation of [Ca^{2+}], initiates the activity. In gastrointestinal muscles, InsP3 may be involved in the release of Ca^{2+} from an internal store. However, we know nothing about the mechanisms of initiation of Ca release from internal stores and what causes the spontaneous elevation of InsP3 concentration. Alternatively, InsP3 may be involved in a train of processes which trigger Ca^{2+} release, and it’s possible that the initiation mechanisms of the spontaneous activity are separated. In smooth muscles, the spontaneous release of Ca^{2+} from internal stores is recognized as a “Ca-spark,” and “packets” of Ca^{2+} activate ion channels at the membrane to induce potential changes [91]. The Ca-spark involves ryanodine receptors on the SR membrane [91], but their relationship to InsP3 receptors remains unclear.

In addition, we must consider the properties of interstitial cells further. ICC were found by S.R. Cajal (1893) using methylene blue staining, and the history of the discovery of ICC has been reviewed by Thuneberg [12, 13]. Many types of interstitial cells are distributed in the wall of the gastrointestinal tract [74]. However, in considering their functional properties, not all interstitial cells appear to function as pacemaker cells, and it remains unclear whether all c-kit–positive or vimentine-stained cells are identical [14]. A functional classification of the different types of interstitial cells found in the gastrointestinal tract will make it clear whether the name ICC is appropriate to describe the pacemaker cells of gastrointestinal muscles.
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REFERENCES

33. Van Helden D: Pacemaker potentials in lymphatic...


64. Maginbuch T, Ohbu T, Sakamoto Y, and Yamamoto Y:
Some electrical properties of the slow potential changes recorded from the guinea-pig stomach in relation to drug actions. Jpn J Physiol 22: 333–352, 1972


83. Liu LWC, Thuneberg L, and Huizinga JD: Cyclopiazonic acid, inhibiting the endoplasmic reticulum calcium pump, reduces the canine colon pacemaker frequency. J Pharmacol Exp Ther 275: 1058–1068, 1995


85. Ganitkevich VYa and Isenberg G: Membrane potential modulates inositol 1,4,5-trisphosphate-mediated Ca\(^{2+}\) transients in guinea-pig coronary artery. J Physiol (Lond) 470: 35–44, 1993


