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Properties of Spontaneous Activity in Gastric Smooth Muscle

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Mammalian gastric smooth muscles generate spontaneous rhythmic contractions which are associated with slow oscillatory potentials (slow waves) and spike potentials. Spike potentials are blocked by organic Ca^{2+}-antagonists, indicating that these result from the activation of L-type Ca^{2+}-channel. However, the cellular mechanisms underlying the generation of slow wave remain unclear. Slow waves are insensitive to Ca^{2+}-antagonists but are blocked by metabolic inhibitors or low temperature. Recently it has been suggested that Interstitial Cells of Cajal (ICC) serve as pacemaker cells and a slow wave reflects the coordinated behavior of both ICC and smooth muscle cells. Small segments of circular smooth muscle isolated from antrum of the guinea-pig stomach generated two types of electrical events; irregular small amplitude (1 to 7 mV) of transient depolarization and larger amplitude (20 to 30 mV) of slow depolarization (regenerative potential). Transient depolarization occurred irregularly and membrane depolarization increased their frequency. Regenerative potentials were generated rhythmically and appeared to result from summed transient depolarizations. Spike potentials, sensitive to nifedipine, were generated on the peaks of regenerative potentials. Depolarization of the membrane evoked regenerative potentials with long latencies (1 to 2 s). These potentials had long partial refractory periods (15 to 20 s). They were inhibited by low concentrations of caffeine, perhaps reflecting either depletion of Ca^{2+} from SR or inhibition of InsP3 receptors, by buffering Ca^{2+} to low levels with BAPTA or by depleting Ca^{2+} from SR with CPA. They persisted in the presence of Ca^{2+}-sensitive Cl^--channel blockers, niflumic acid and DIDS or Co^{2+}, a non selective Ca^{2+}-channel blocker. These results suggest that spontaneous activity of gastric smooth muscle results from Ca^{2+} release from SR, followed by activation of Ca^{2+}-dependent ion channels other than Cl^- channels, with the release of Ca^{2+} from SR being triggered by membrane depolarization.

Key Words: Spontaneous electrical activity, Gastric smooth muscle, Intracellular Ca^{2+}, Voltage-sensitive Ca^{2+}-channel

INTRODUCTION

Gastrointestinal smooth muscles of many species show myogenic activity. The modulation of this activity by enteric and autonomic nerves facilitates digestive movements. Myogenic activity is accompanied by electrical events in the membranes of the smooth muscle cells, a slow long lasting membrane depolarization (slow wave). Slow waves can be divided into three components, a first component which is not associated with a conductance change, a second component which is voltage dependent and associated with a conductance change and a spike component (Ohba et al, 1975, Ohba et al, 1977; Tomita, 1981). Spike potentials are inhibited by Ca^{2+}-antagonists such as diltiazem (Ishikawa et al, 1984) or nifedipine (Dickens et al, 1999), indicating that they result from the activation of L-type Ca^{2+}-channels. Slow waves are inhibited by metabolic poisons and their generation is slowed at low temperatures, suggesting that they are linked to cellular metabolic activity (Tomita, 1981). However, which cellular metabolic pathways are involved in the generation of slow waves remains

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unclear.

In the colon or small intestine of dog, removal of a layer of intestinal wall containing the Interstitial Cells of Cajal (ICC) abolishes myogenic activities (Sanders, 1996). Freshly isolated (Langtiton et al, 1989) or cultured ICC obtained from dog colon (Huizinger et al, 1996) or mouse (Tokutomi et al, 1997) are spontaneously active. Furthermore, functional disorders in gastro-intestinal system are accompanied by structural abnormalities in ICC (Huizinger et al, 1997). All these observations suggest that ICC are involved in the generation of slow waves. In the guinea-pig stomach, simultaneous recording of membrane activities from ICC of the myenteric region and circular muscle indicate that potentials generated by ICC precede those recorded from smooth muscle cells of the circular muscle layer (Dickens et al, 1999). Although there are several types of immunologically distinct ICC in the guinea-pig stomach (Burns et al, 1997), ICC of the myenteric region may have a special role either to initiate a slow wave or to conduct local excitation of smooth muscle cells to a wide area of the stomach.

The ionic conductance changes occurring during slow waves remain unclear. Slow waves are accompanied by periodical increases in inward current (Ohba et al, 1977), suggesting that they are generated by ions with equilibrium potentials positive of the resting potential. Slow waves are insensitive to tetrodotoxin (Tomita, 1981) or organic Ca^{2+}-antagonists (Ishikawa et al, 1984; Dickens et al, 1999), indicating that classical voltage-sensitive Na^{+}-channels and L-type Ca^{2+}-channels may not be involved in their generation. Removal of Na^{+} or Ca^{2+} from the extracellular fluid inhibits the generation of slow waves, but these changes are associated with membrane depolarization (Tomita, 1981), indicating that modifying the ionic composition of physiological saline induces complex responses to smooth muscles.

Properties of spontaneous activity in the guinea-pig stomach

The pattern of spontaneous electrical activity recorded from the guinea-pig stomach differs from region to region. Tissues from the antrum and pylorus generate slow waves with spike potentials but those from the fundus region are quiescent (Fig. 1). The circular smooth muscle layer thickens in the pylorus region as it approaches the duodenum; here a dominant plateau follows the spike component. The amplitudes of slow waves successively decreases with bundles of muscle taken closer and closer to the fundus. Finally spontaneous activity is not detected in muscle bundles taken from the fundus region.

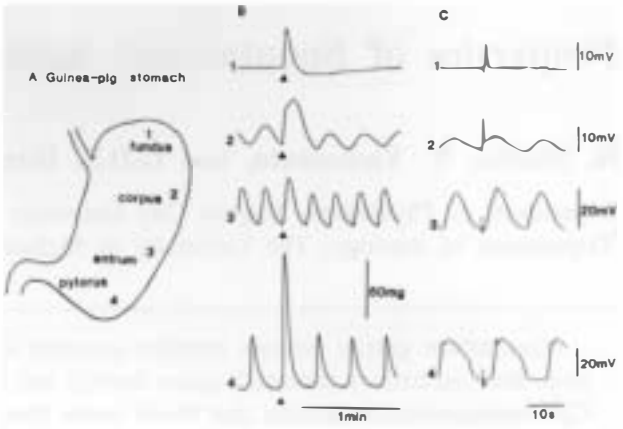

Fig. 1. Mechanical and electrical responses produced by TNS in stomach. A, Schematic drawing of the guinea-pig stomach. Mechanical (B) and electrical responses (C) produced by TNS in muscles isolated from regions shown in A (1, fundus; 2, corpus; 3, antrum; 4, pylorus). From Komori & Suzuki (Comp Biochem Physiol 91 C, 311-319, 1988).

The pattern of electrical activity recorded varies across gastric wall. The gastric wall consists of three layers, longitudinal and circular smooth muscles and ICC in the myenteric regions. In the antrum, these cells, each identified using histological techniques, generate different patterns of rhythmic membrane potential changes (Fig. 2). Muscle cells of the circular layer produce classical slow waves with their characteristic first, second and spike components (Fig. 2A). ICC generate more square-shaped potentials with rapid rates of rise (Fig. 2B). Longitudinal muscle cells generate square-shaped potentials with small amplitudes than those of ICC (Fig. 2C).

The properties of slow wave in the guinea-pig stomach have been well documented (Tomita, 1981). The amplitude of slow wave is a function of membrane potential, within a range of potentials hyperpolarization increases and depolarization decreases their amplitudes. However, the frequency of slow waves is little affected when the membrane potential is varying; substantial, depolarizations and hyperpolarizations, increase and decrease their frequency by some 10 to 20% (Ohba et al, 1975). More recently,

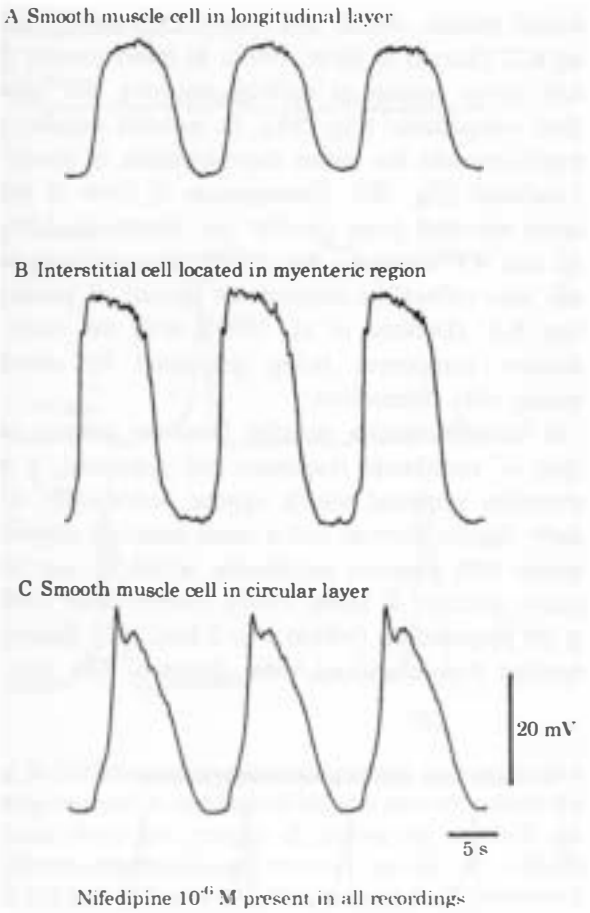

Fig. 2. Spontaneous activity of cells in the gastric wall. Comparison between spontaneous activity recorded from longitudinal (A) and circular smooth muscle cells (C) with that recorded from an ICC located in the myenteric region (B). All recordings were made from the same preparation of antral muscle.

the frequency of rhythmical potential changes recorded from bundles of circular muscles, free from the longitudinal layer, has been shown to change when the membrane potential has been changed by increasing $[K^+]_o$ or by activating intestinal K^+ channels (Huang et al, 1999). Since these preparations are probably devoid of the ICC located between the circular and longitudinal layers (myenteric region) of the stomach (Burns et al, 1997; Suzuki & Hirst, 1999), the events controlling the frequency of these regenerative potentials may differ from those controlled by ICC.

Neural modulation of spontaneous activity in the guinea-pig stomach

Transmural nerve stimulation (TNS) evokes an excitatory junction potential (e.j.p.) in muscles from the fundus region and an inhibitory junction potential (i.j.p.) in muscles from the antrum region (Komori & Suzuki, 1986). E.j.p.s are enhanced in amplitude when choline-esterases are inhibited with neostigmine and are abolished by atropine, indicating that they result from the activation of muscarinic receptors by acetylcholine. In atropinized muscles, TNS evokes an i.j.p. in many regions of the stomach. I.j.p.s have two components, a fast phase, which persists for up to 1 s, followed by a slow phase that reaches its peak some 2 to 3 s after the stimulus and decays slowly over the next 3 to 10 s (Fig. 3A). The fast component is blocked by apamin (Fig. 3B), an inhibitor of a class of the Ca^{2+}-sensitive K^+-channels (Banks et al, 1979) which are activated by ATP, PACAP or VIP (Ohno et al, 1996). However, it remains unclear which transmitter substance produces the fast i.j.p. recorded from guinea-pig stomach. In contrast the slow component is resistant to apamin. It is reduced in amplitude by nitroarginine (Fig. 3C). The block by nitroarginine is partly reversed by L-arginine (Fig. 3D), indicating that this component resulted from the release of nitric oxide (NO).

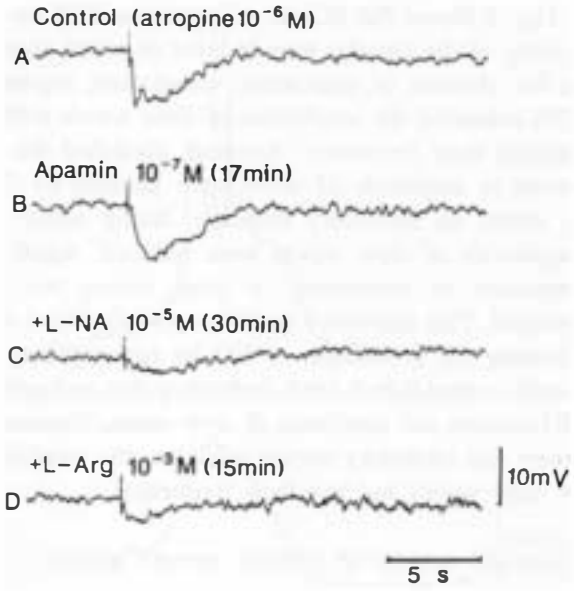

Fig. 3. Two components of the i.j.p. recorded from the fundus muscle. The i.j.p. was recorded from fundus smooth muscle before (A) and after cumulative application of 10^{-7} M apamin for 17 min (B), 10^{-5} M nitroarginine for 30 min (C) and 10^{-3} M L-arginine (D) for 15 min. All recordings were from the same cell. Atropine (10^{-6} M) was present throughout.

Fig. 4 shows the effects of repetitive TNS on the activity of the circular muscle layer of antral muscle. In the absence of muscarinic antagonists, repetitive TNS enhanced the amplitudes of slow waves without altering their frequency. Atropine abolished the increase in amplitude of slow wave induced by TNS to reveal an inhibitory response during which the amplitudes of slow waves were reduced. Again the frequency of occurrence of slow waves was unchanged. This inhibitory response was abolished after blocking the production of NO by nitroarginine (H. Suzuki, unpublished data), indicating that endogenous NO reduces the amplitude of slow wave. Thus, excitatory and inhibitory nerves modulate the amplitudes of slow waves but not their frequency.

Electrical activity of circular smooth muscle

The properties of slow waves recorded from intact tissues, which contain longitudinal muscle cells, ICC and circular muscle cells, differ from the rhythmical depolarizations recorded from isolated bundles of circular muscle, which lack longitudinal muscle cells and ICC (Suzuki & Hirst, 1999). In intact tissues, the slow waves consist of caffeine-sensitive and insensitive components (Fig. 5A). In isolated bundles of circular muscle the entire depolarization is sensitive to caffeine (Fig. 5B). Examination of form of slow waves recorded from circular and longitudinal muscles and ICC indicates the caffeine-insensitive potentials may reflect the electrotonic spread of potential from ICC (Dickens et al, 1999), with the caffeine sensitive component being generated by circular muscle cells themselves.

In isolated circular muscles from the antrum, two types of membrane responses are generated, a regenerative potential which appear periodically with nearly regular interval and a small transient depolarizations with variable amplitudes which appear irregularly (Suzuki & Hirst, 1999). Shortly after setting up the preparations (within 1 to 2 hrs), only the small transient depolarizations were detected. The rate of

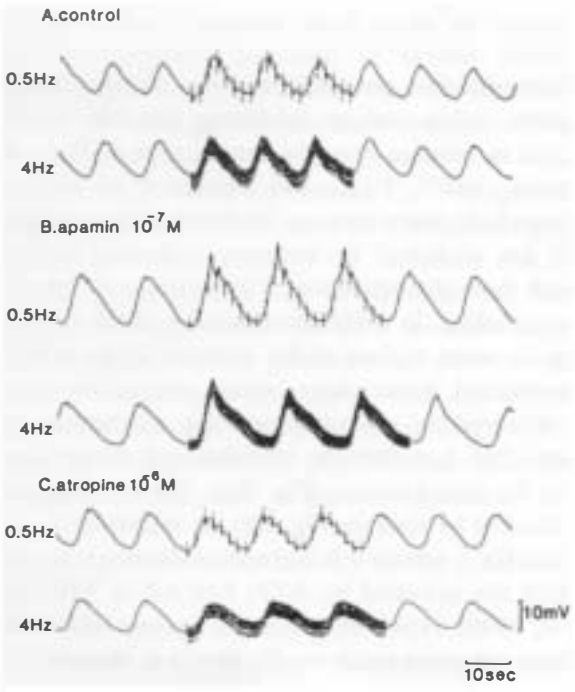

Fig. 4. Modulation of slow waves by nerve stimulation. TNS (frequency of stimulation 0.5 and 4 Hz) was applied for 30 s in the absence (A) and presence of 10^{-7} M apamin (B) and 10^{-6} M atropine (C). Antral circular muscle bundle with longitudinal cells and ICC attached. All responses were recorded from the same cell.

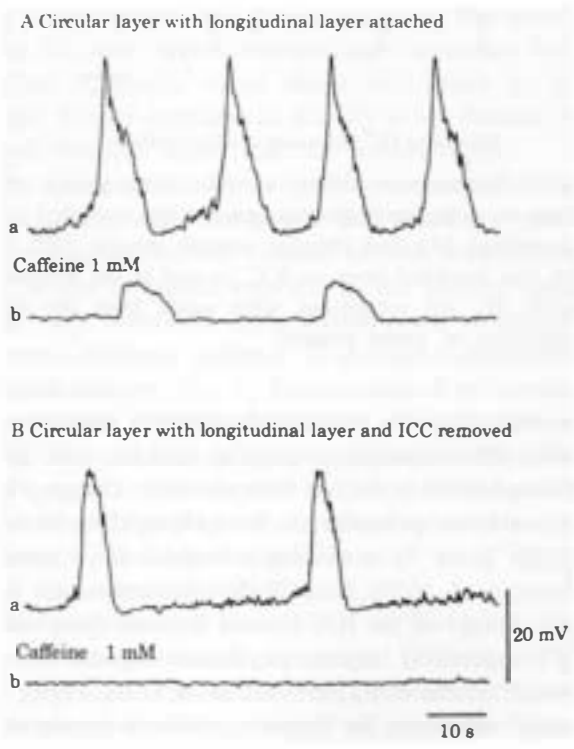

Fig. 5. Effects of caffeine on slow wave. Membrane potential changes recorded from circular smooth muscle cells of the guinea-pig antrum with longitudinal layer attached (A) and without longitudinal layer (B). In each pair of traces, a and b show responses in the absence and presence of 1 mM caffeine, respectively.

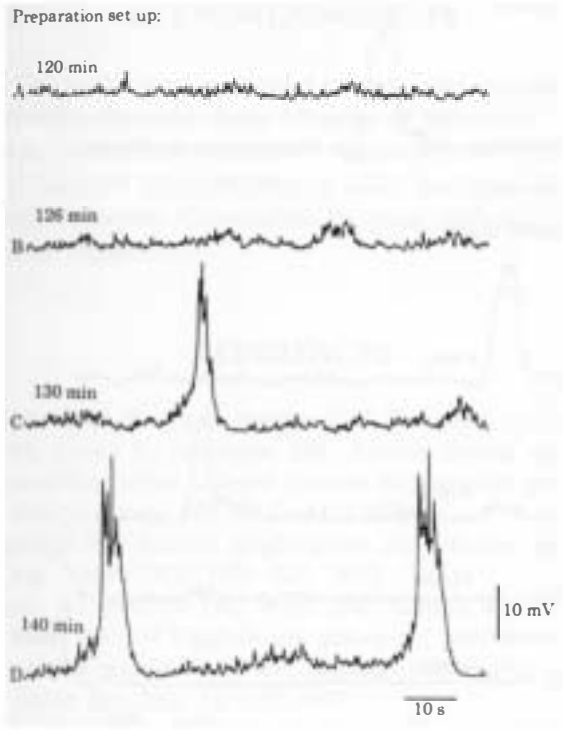

Fig. 6. Initiation of spontaneous activity in a segment of antrum muscle. A segment of circular smooth muscle was isolated from the antrum of guinea-pig stomach, and membrane potentials were recorded at 120 (A), 126 (B), 130 (C) and 140 min (D) after starting the incubation of tissue. All recordings were obtained from the same cell.

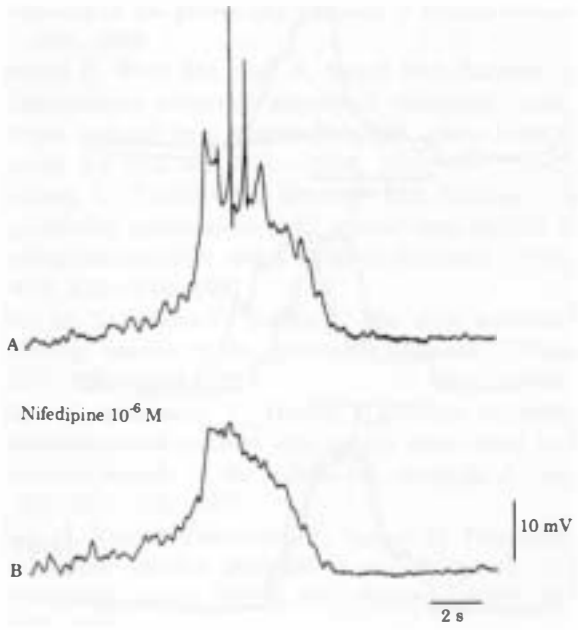

Fig. 7. Inhibition of spike potential by nifedipine in antrum muscle. Spontaneous activities recorded from antrum muscles before (A) and after application of 1 μM nifedipine (B). Both traces were recorded from the same cell.

generation of these potentials gradually increased and eventually they summed to give rise to larger depolarizations that triggered regenerative potentials (Fig. 6). Regenerative potentials in turn trigger spike potentials (Fig. 7A). Nifedipine abolished the spike potentials without altering the discharge of transient depolarizations and regenerative potentials (Fig. 7B). These experiments indicate that nifedipine is effective at blocking L-type Ca^{2+}-channels in this tissue. Clearly small transient depolarizations and regenerative potentials result from the activation of ion channels other than L-type Ca^{2+}-channels.

In these preparations of circular muscle, depolarization of the membrane by current injection from a second electrode produced an electrotonic potential in each cell. The depolarization accelerated the generation of the small transient depolarization and facilitated the generation of the regenerative potentials. The latency for generation of the regenerative potentials reduced as the intensity of injecting current was increased to reach to a stable minimum latency of about 1 s (Fig. 8). Application of brief (duration 200 to 500 ms) stronger than threshold current pulses also induced regenerative potentials, again with about 1 s delay. Thus even a brief depolarization activates a process which in turn leads to the generation of regenerative potential. As the delay cannot be explained on electrical grounds it must represent some rate-limiting step in activation process of regenerative potentials. The minimum interval required for generation of regenerative potentials of reproducible amplitude was determined by applying two stimuli at varying separations. The amplitude of the second regenerative potential was smaller than the first when the interval of stimuli was shorter than 20 s. The results indicate that the ion channels, or the pathway, involved in the generation of regenerative potentials are inactivated by excitation and require about 20 s for complete recovery from this inactivation.

The spontaneous depolarization found in lymphatic smooth muscle (Van Helden, 1992) or urethra (Hashitani et al, 1995) can be inhibited by niflumic acid or DIDS, inhibitors of the Ca^{2+}-activated Cl^--channels. However, these Cl^--channel inhibitors did not inhibit the spontaneous electrical activity recorded from gas-

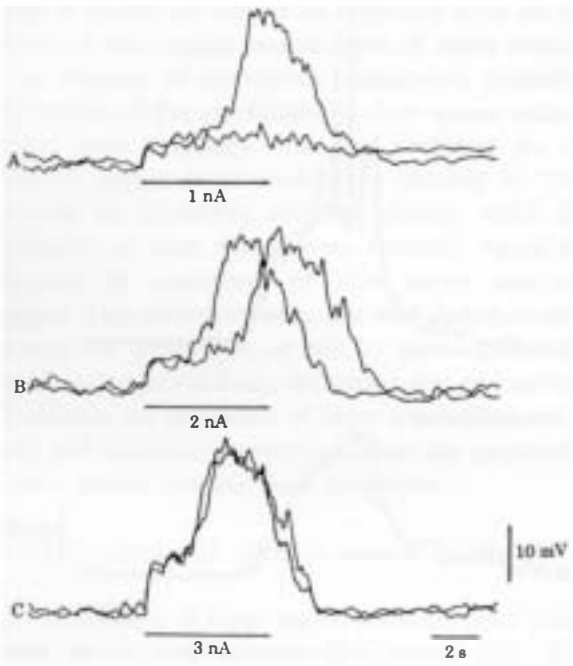

Fig. 8. Regenerative responses evoked by depolarizing pulses. Two electrodes were penetrated to different cells (separation about 100 μm) in isolated circular smooth muscle of the guinea-pig antrum, and rectangular current stimulation (A, 1 nA; B, 2 nA; C, 3 nA; 5 s duration) was applied to one electrode for two times and electrotonic potentials were recorded from the second electrode. All responses were recorded from the same cell.

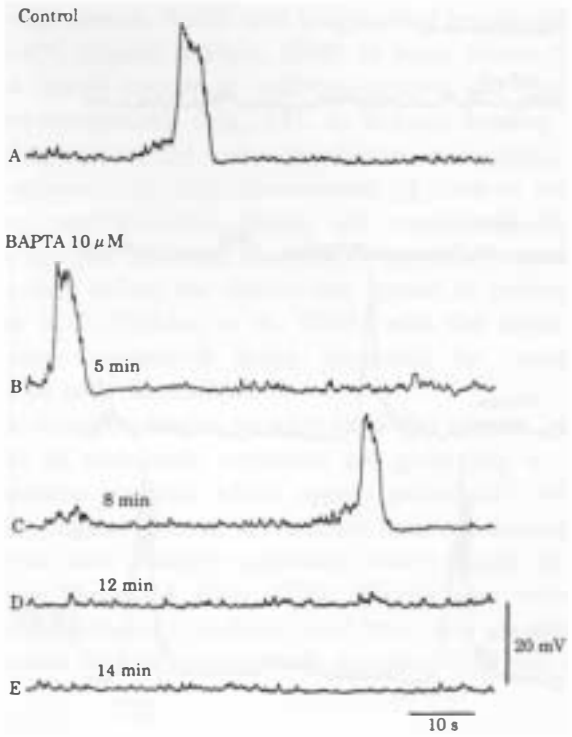

Fig. 9. Effects of BAPTA on spontaneous activity in antral circular muscle. In circular smooth muscle isolated from the antrum, membrane responses were recorded before (A) and during application of 10 μM BAPTA-AM (B, 5 min; C, 8 min; D, 12 min; E, 14 min). All recordings were obtained from the same cell.

tric circular muscles. On the other hand CPA reversibly abolished the discharge of transient depolarizations and regenerative potentials, indicating that they each require a functioning internal Ca^{2+} store. When preparations were loaded with BAPTA, to buffer $[Ca^{2+}]_i$ to a low level, the frequency of occurrence of small transient depolarizations fell and regenerative potentials were abolished (Fig. 9). Caffeine, in addition to releasing Ca^{2+} from internal store (Endo, 1977), blocks some inositol trisphosphate (InsP3) receptors (Parker et al, 1991; Missiaen et al, 1992; Berridge, 1993). In many tissues, InsP3 triggers the release of Ca^{2+} from intracellular stores. Production of InsP3 is usually accelerated by the activation of chemo-sensitive receptors on the membranes of excitable cells (Berridge, 1993). When this occurs there is invariably a delay before a response occurs. With these things in common, we speculate that the generation of small transient depolarization and regenerative potential are coupled to the release of Ca^{2+} from internal store following the activation of InsP3 receptors. Our observations suggest that in this tissue the production of InsP3 is voltage-sensitive and that depolarization increases the production of InsP3.

SUMMARY

Smooth muscles of the stomach generate slow waves and spike potentials but only the latter potential is inhibited by nifedipine or Co^{2+}, indicating that the generation of slow wave does not involve activation of voltage-sensitive Ca^{2+}-channels. Drugs that deplete Ca^{2+} from the internal store or chelate intracellular Ca^{2+} inhibit a component of the slow wave. This suggesting that this component is triggered by the release of Ca^{2+} from internal store. Depolarization of the membrane activates this component after a long delay, suggesting that depolarization-activated production of unidentified second messenger may be involved.

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