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RHYTHMIC ELECTRICAL ACTIVITY IN STOMACH AND INTESTINE OF TOAD

BY ALLEN MANGEL AND C. LADD PROSSER

Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801, *U.S.A.*

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SUMMARY

The intact stomach of the toad initiates rhythmic slow-spikes of 5–15 s duration and frequency of 3–5 min^{-1}. The spontaneous electrical waves originate in the longitudinal muscle layer; isolated circular muscle is quiescent. Aboral conduction velocity is 0·12–0·9 mm s^{-1}. Reduction of external sodium concentration from 89·5 to 15 mM produced no effect on slow spikes, although further reduction to 1·5 mM increased frequency and decreased amplitude. Slow-spikes were unaffected by ouabain or by incubation in potassium-free solution. When calcium in the medium was reduced, slow-spike amplitude and frequency decreased. Slow-spikes exhibited a change in amplitude of 16 mV per decade change in Ca_o^{2+}; slow-spikes were eliminated at 10^{-8} M Ca_0^{2+} and by blockers of calcium conductance channels.

Intact intestine of toad demonstrated slow-waves which resembled those of mammalian intestine. These were sensitive to changes in external sodium and were eliminated by 1×10^{-4} M ouabain.

It is suggested that rhythmic slow-spikes of longitudinal smooth muscle of amphibian stomach may result from periodic changes in Ca conductance whereas endogenous electrical waves of intestine may result from rhythmic extrusion of sodium.

INTRODUCTION

Myogenic rhythmicity is essential for normal digestive motility of vertebrate stomach and intestine. Several cellular mechanisms have been identified as responsible for rhythmic electrogenesis in mammals (Prosser, 1978). In the longitudinal muscle of cat small intestine several kinds of evidence point to a rhythmic electrogenic sodium pump (Connor, Prosser & Weems, 1974), but the ultimate reactions which pace this rhythm remain unknown (Connor, Kreulen & Prosser, 1976). In isolated circular muscle of mammalian small intestine spontaneous pacemaker potentials and spikes may occur arrhythmically and are apparently caused by voltage-dependent changes in calcium conductance (Liu, Prosser & Job, 1969; Weigel, 1979). Under physiological conditions spikes in the circular muscle are driven by rhythmic slow-waves in the longitudinal muscle (Connor *et al.* 1977). In stomach of dog and cat, rhythmic waves consist of an initial fast depolarization followed by a plateau, with or without spikes

(Daniel & Irwin, 1968; Papasova, Nagai & Prosser, 1968). In cat the initial wave is ouabain-sensitive (like the intestinal 'slow-waves') whereas the plateau and spikes are calcium-sensitive (Papasova *et al.* 1968). In circular muscle of guinea pig stomach, an initial electrical wave is voltage-independent and appears to be controlled by some metabolic process, other than a sodium pump, which is calcium sensitive. A second fast wave may represent a change in calcium conductance (Ohba, Sakamoto & Tomita, 1977).

Rhythmicity has been observed in stomach and intestine of a number of non-mammalian vertebrates but the ionic mechanisms have not been elucidated. The rhythmic electrical waves in the stomach of the frog (van Harn, 1968), toad (Sato, 1960) and skate (Prosser, Weems & Connor, 1976) consist of 'slow-spikes' that are clearly different from the 2-component waves of cat and dog stomach and from 'slow-waves' of cat longitudinal intestinal muscle or from the 'spikes' of cat circular intestinal muscle. In frog stomach, slow-spikes were found to originate in the longitudinal layer, to be conducted away from the cardiac end of the stomach, and to be eliminated in calcium-deficient medium (van Harn, 1968). Study of the nature of endogenous rhythmicity in amphibian stomach and intestine may provide a partial explanation of evolutionary relationships and of the generality of ionic mechanisms among vertebrates.

MATERIALS AND METHODS

Toads, *Bufo marinus*, were killed by a blow to the head; the stomach and small intestine were removed to Ringer solution of the following composition (mM): NaCl 89·5, KCl 2·97, $MgSO_4$ 1, $NaHCO_3$ 30, Na_2HPO_4 2·3, glucose 3·0, and $CaCl_2.2H_2O$ 2·52 mM. Solutions were aerated with 95%–5% (O_2–CO_2) gas mixture which, with the bicarbonate and phosphate buffering system, maintained a pH of 7·4; experiments were at room temperature (20–22 °C). In experiments with reduced sodium or with the calcium-channel blocker cobalt, MOPS buffer solution was used in place of the bicarbonate buffer.

Initial measurements were from intact stomach; in most experiments, however, the stomach was inverted and the mucosa carefully removed. The stomach was then reinverted with the serosa outside and secured to a plexiglass rod or pinned out on a sylgard based chamber. The intestine was secured on a rod or pinned out with the mucosa intact.

Electrical recordings were made with pressure electrodes (Bortoff, 1961), tube electrodes (Connor, Mangel & Nelson, 1979) and intracellular microelectrodes. Recordings were displayed on a Grass polygraph. For the intracellular recordings, 3 M-KCl-filled microelectrodes of 40–90 MΩ resistance were used and the signals were fed to a high-impedance capacity-compensated preamplifier and displayed on the polygraph.

Mechanical recordings were with a Grass transducer and were displayed on a Grass polygraph.

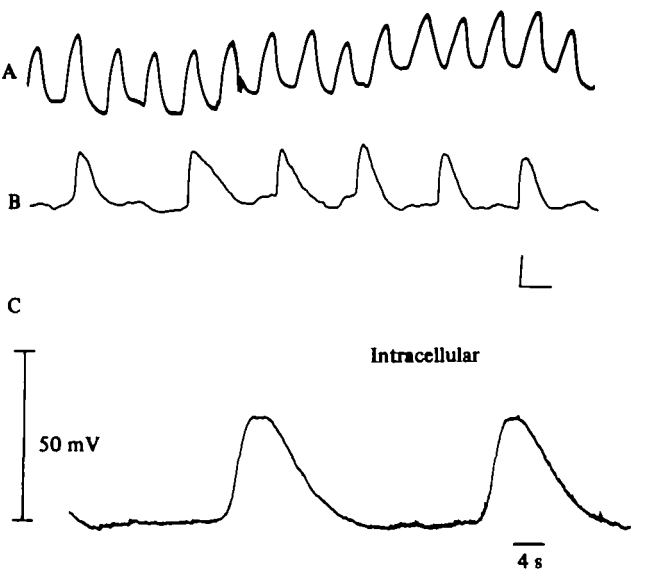

Fig. 1. Spontaneous electrical activity recorded from toad small intestine and stomach. (A) and (B) were obtained with pressure electrodes. (A) Recordings from toad intestine. (B) Recordings from toad stomach (cal. bar for (A) and (B), 0·2 mV, 10 s). (C) Intracellular recording of slow-spikes from toad stomach.

RESULTS

General properties; stomach and intestine

Electrical recordings from the external layer of the toad stomach (Fig. 1 B, C) showed spontaneous rhythmic 'slow-spikes' of 4–5 s rise-time and 12–16 s repolarization time. Each slow-spike triggered a contraction of the stomach (Fig. 2). Between slow-spikes the stomach muscle was electrically quiescent for periods of 8–20 s. Normal frequency was usually 3–5 min^{-1}. Recordings from the intestine showed near-sinusoidal 'slow-waves' of frequency 2–4 times faster (8–15 min^{-1}) than the slow-spikes of stomach, and no prolonged delay between waves (Fig. 1A). Intracellular recordings from longitudinal muscle of stomach (Fig. 1C) showed slow-spikes of similar shape and duration to those recorded with pressure electrodes. Intracellularly recorded slow-spikes were 40–50 mV in amplitude when recorded from cells with 50 mV resting potentials. The muscle fibres of the toad stomach are small and surrounded by much connective tissue, hence penetration was more difficult than in mammalian smooth muscle preparations. Since the wave-forms of recordings made with pressure electrodes were essentially the same as with microelectrodes, most recordings were made with pressure electrodes (approximately 100 μm in diameter).

Pressure electrodes give quasi-intracellular recordings; cells directly under them are depolarized and, because of the syncytial nature of smooth muscle, electrical events represent potential changes in surrounding cells. Absolute amplitudes of electrical events cannot be obtained but relative changes in amplitude can be measured and wave-form is accurately recorded.

In toad stomach, strips of circular muscle (isolated from the longitudinal layer) and

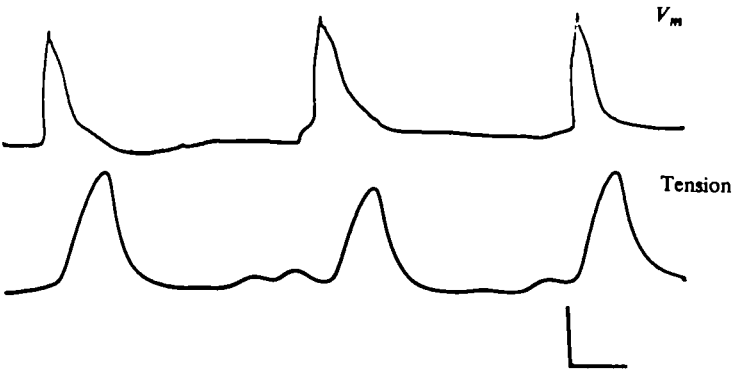

Fig. 2. Spontaneous electrical (upper trace) and mechanical (lower trace) activity recorded from toad stomach. Electrical activity was recorded with a tube electrode (cal. bar, 0·5 mV, 0·1 g, 7 s).

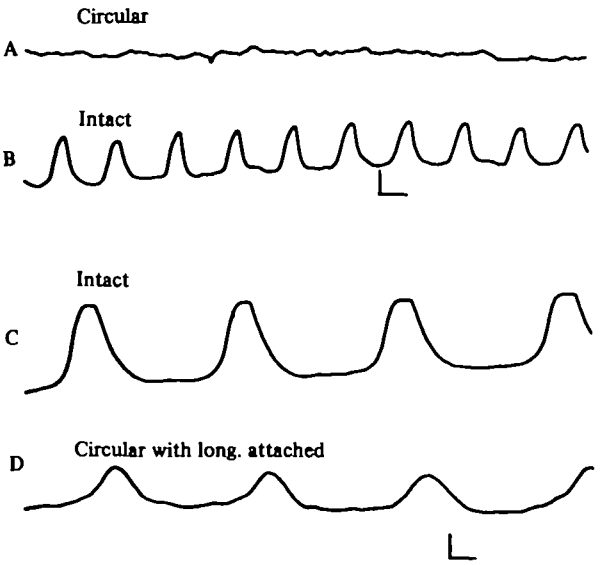

Fig. 3. Pressure electrode recordings from isolated circular and intact muscle of the toad stomach. (A) Recordings from isolated circular muscle. (B) Recordings from intact region of same preparation as in A (cal. bar for (A) and (B) 0·5 mV, 10 s). (C) Recordings from intact musculature. (D) Same preparation as (C), recording from exposed circular muscle with longitudinal attached (cal. bar (C) and (D), 0·5 mV, 6 s).

regions of stomach (from which the longitudinal layer had been removed), were electrically quiet (Fig. 3A). Histological examination showed a clear boundary between the circular and longitudinal muscle layers and confirmed the isolation of the layers from one another. However, the layers are connected by strands of connective tissue and it is probable that cells making nexal connections between the muscle layers span the interlayer region as has been observed in mammalian intestine (Taylor, Kreulen & Prosser, 1977).

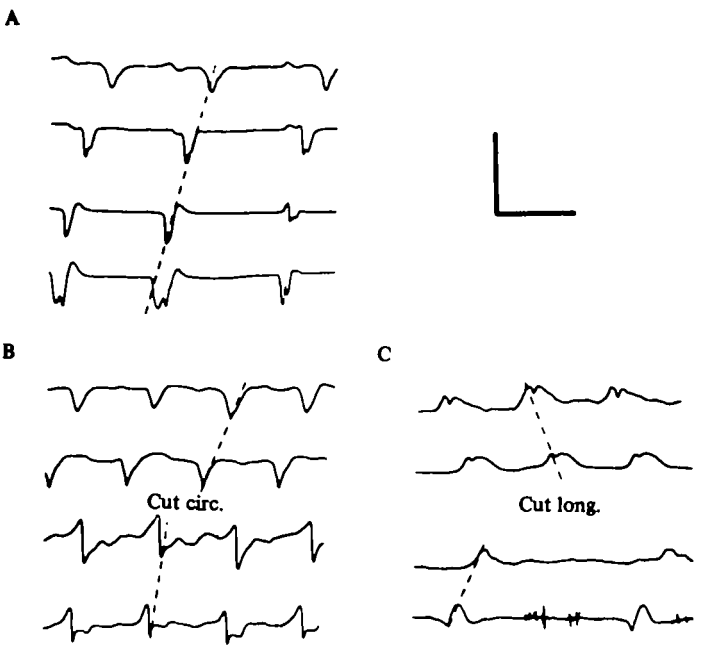

Fig. 4. Pressure electrode recordings from toad stomach to demonstrate conduction. (A) Slow-spikes originate at the oesophageal end of the stomach. The upper trace was recorded by a pressure electrode close to the pyloric region of the stomach while the ones below it are progressively closer (2–3 mm separation) to the oesophagus. (B) The effects of removing a 4–5 mm strip of circular muscle between electrodes recording traces 2–3. (C) The effects of longitudinal cut between electrodes recording traces 2 and 3 (cal. bar: traces 1 and 4, 0·8 mV; trace 2 (A and B), 0·8 mV; 0·2 mV (C); trace 3, 0·2 mV; 24 s).

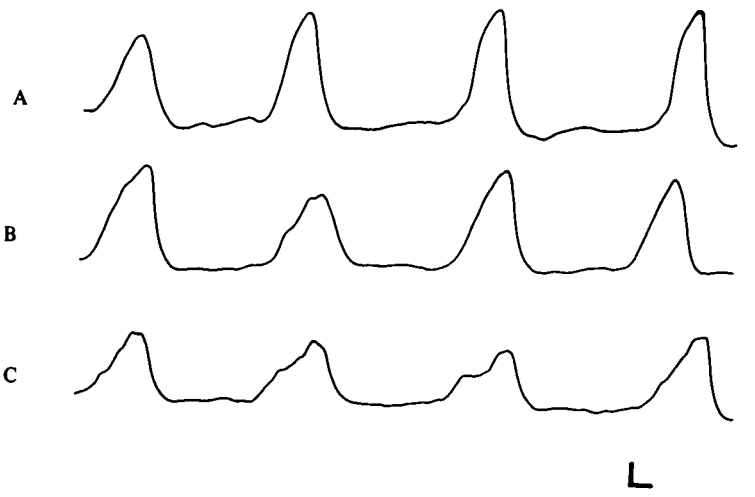

Fig. 5. Effects of low sodium solution (15 mM) on toad slow-spikes as recorded by pressure electrodes. (A) Control trace; (B) 30 min in 15 mM-NaCl (LiCl replacement); (C) return to control (cal. bar, 0·1 mV, 6 s).

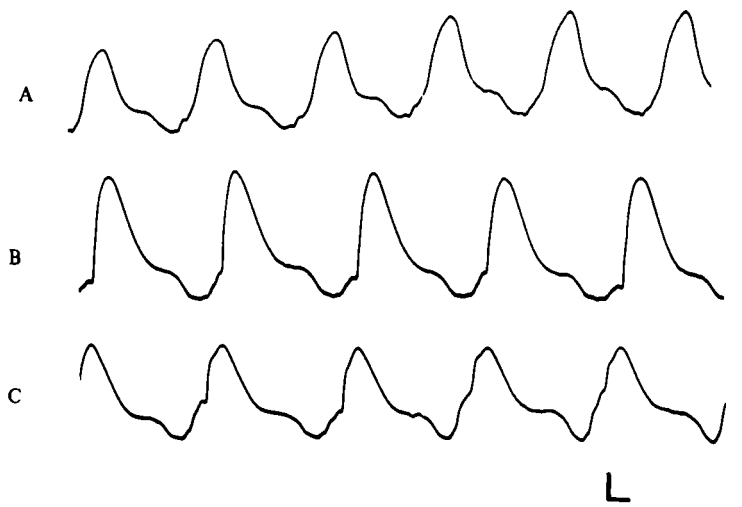

Fig. 6. Effects of 10^{-4} M ouabain on toad slow-spikes as recorded by pressure electrodes. (A) Control; (B) 30 min in 10^{-4} M ouabain; (C) return to control (cal. bar, 0·1 mV, 6 s).

There was no evidence for spontaneous spiking in the circular muscle layer such as occurs in mammalian intestine. However, when a small slit was made through the longitudinal layer and an electrode placed on circular muscle adjacent to intact regions, slow-spikes were recorded (Fig. 3C, D). Slow-spikes were recorded from inverted segments when electrodes were placed on the mucosal side of the circular muscle layer. Apparently the slow-spikes originate in longitudinal muscle and spread from the longitudinal to the circular muscle layer as do slow-waves of mammalian intestine.

Recordings by a row of pressure electrodes (Fig. 4A) showed that the slow-spikes originate normally at the esophageal end of the toad stomach and propagate in the aboral direction with a conduction velocity of 0·12–0·9 mm s^{-1}. A cut through the longitudinal layer around the stomach (Fig. 4C) or removal of a 4–6 mm wide strip of circular muscle (Fig. 4B) blocked conduction. Rhythmic slow-spikes occurred on each side of the cut but they were out of synchrony and not conducted across the cut. In these cut preparations, conduction in the lower (pyloric) region was sometimes oral and sometimes aboral (Fig. 4C). These results indicate that normal aboral conduction in the stomach may involve a cycling between the two layers much as in mammalian intestine (Connor *et al.* 1977).

Ionic mechanisms of slow-spikes in stomach

Slow-spike activity was insensitive to replacement of 90% of external sodium (all but 15 mM) by lithium (n = 5) (Fig. 5) or by sucrose (n = 3). Replacement of all but 1·5 mM sodium by lithium decreased slow-spike amplitude and increased slow-spike frequency. Reduction of sodium and calcium in the medium in proportions, such that the ratio of $\sqrt{(Ca^{2+})}/Na^{+}$ was a constant, did not result in constant slow-spike amplitude but, rather, potentiated the effects of decreased calcium or sodium

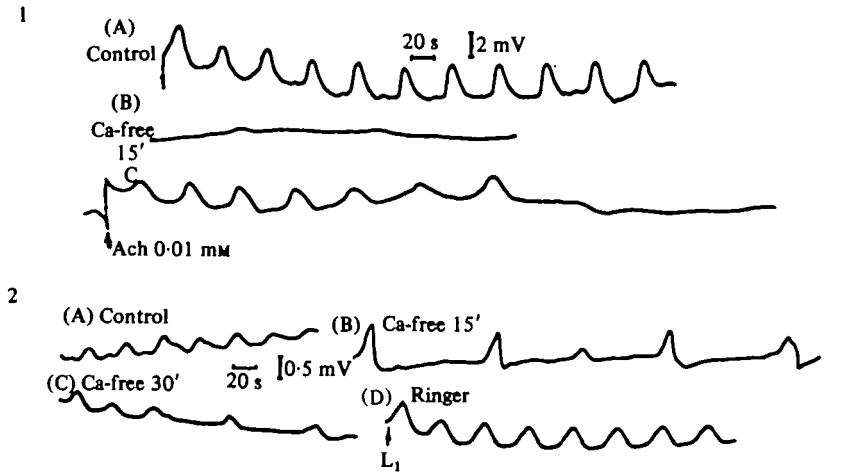

Fig. 7. Effect of calcium-free solution on slow-spikes as recorded by pressure electrodes. (1) A: Control, B: 15 min in calcium-free solution; C: Ach added in calcium-free solution. (2) A: Control in sodium Ringers solution; B: 15 min in calcium free solution; C: 30 min in calcium-free solution; D: return to lithium Ringers solution containing 2·5 mM calcium.

with respect to slow-spike amplitude. Replacement of external chloride by isethionate had no effect on slow-spike activity. Ouabain at concentrations as high as 10^{-4} M (Fig. 6) ($n = 6$), as well as incubation in potassium free medium ($n = 3$) did not alter slow-spikes. These observations indicate that slow-spike generation is not due to a rhythmic sodium pump.

In contrast to the insensitivity to sodium, reduction of calcium in toad stomach reduced both the amplitude and frequency of slow-spikes (Fig. 7 (1, 2). These effects were fully reversible in either Na^+ or Li^+ Ringer containing normal calcium. After slow-spike activity had been eliminated by incubation in calcium-deficient ($< 10^{-6}$ M-Ca) solution, application of acetylcholine could induce transient oscillations in membrane potential (Fig. 7 (1)). External calcium levels were titrated to controlled low concentrations by means of the Ca-EGTA/EGTA buffering system (Jewell & Ruegg, 1966). The effects of various concentrations of calcium on slow-spike frequency and amplitude are plotted in Fig. 8. Slow-spikes were eliminated at 10^{-8} M-Ca^{2+}. Frequency varied linearly with log Ca_0 over the range from 10^{-7} to 10^{-4} M (Fig. 8B) and was constant at concentrations above this range. Slow-spike amplitude showed a similar relation to calcium concentration (Fig. 8A) and the curve yielded a slope of 16 mV for decade change in Ca_0 in its linear region. If the active membrane were permeable only to Ca^{2+} a slope of 29 mV would be expected. However, the membrane is presumably permeable to additional ions, as evidenced by the fact that the peak voltages are so far from the calcium equilibrium potential.

Application of D600 (1 μM), verapamil (10 μM) and cobalt (5 mM) (calcium channel blockers) eliminated slow-spike activity. Contractile activity was more sensitive to reduction of external calcium than slow spikes. Contractions were eliminated at approximately 10^{-6} M-Ca^{2+}.

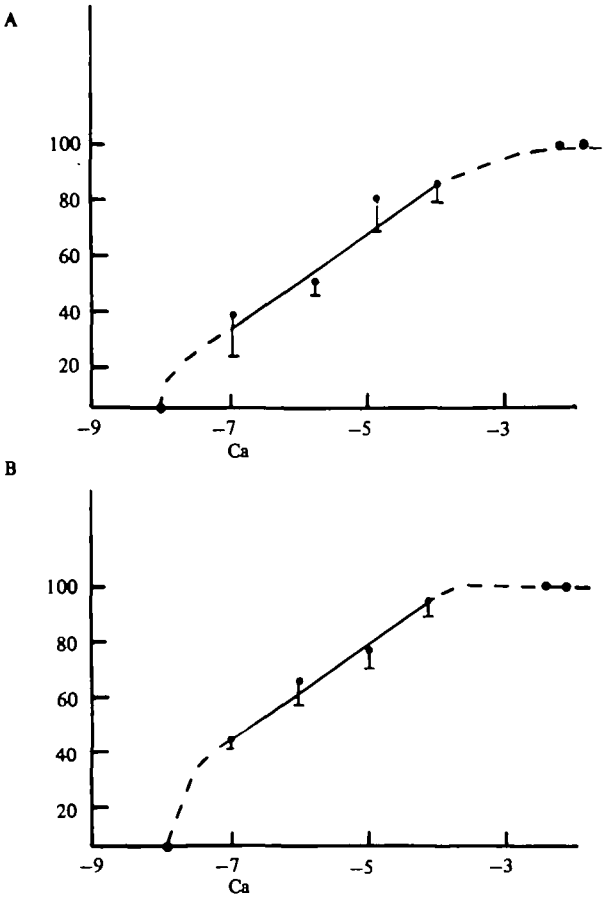

Fig. 8. Effects of various calcium levels on slow-spike frequency and amplitude as recorded with pressure electrodes. Ordinate plot is percentage of control amplitude and frequency. Solid lines were fitted by regression calculation. (A) Plot of log calcium versus slow-spike amplitude; amplitudes are relative, not absolute, because it proved impossible to perform this sequence with a single microelectrode penetration; r is 0·97. B: Plot of log calcium versus slow-spike frequency, r is 0·99. Data compiled from 20 experiments (n = 4 for each point).

Effects of ions on electrical activity in small intestine

Intestinal slow-waves differed from stomach slow-spikes in sensitivity to changes in ionic environments.

Substitution of lithium (except for 30 mM) for sodium in the Ringer-solution bathing toad intestine reversibly eliminated slow-wave activity within 20 min (n = 4) (Fig. 9). Addition of ouabain at 1×10^{-5} M had no effect (Fig. 10A) on intestinal slow-waves but at 6×10^{-5} M the amplitude was considerably reduced (Fig. 10B) and at 10^{-4} M all slow-waves were eliminated (Fig. 10C) (n = 3). Incubation in a potassium-free medium also eliminated slow-wave activity. The effects of reduced sodium concentration, ouabain and K-free medium indicate a similarity to slow-waves of mammalian intestine but a marked difference from toad stomach.

In contrast to the marked effect on slow-spikes of toad stomach, incubation of toad

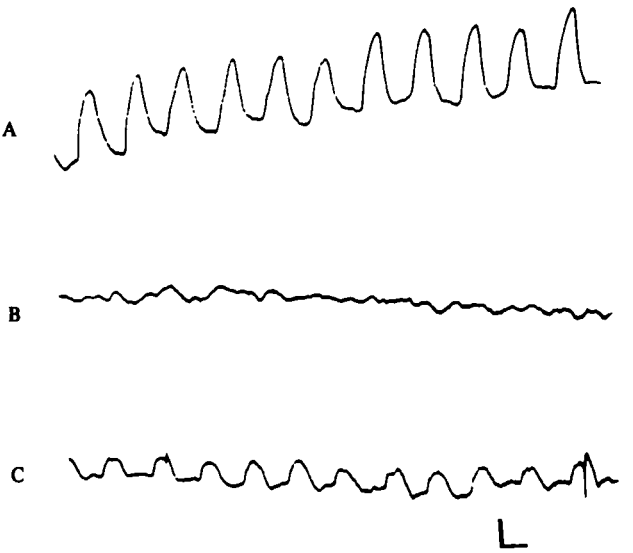

Fig. 9. Effects of low sodium solution on toad intestinal slow-waves as recorded with pressure electrodes. (A) Control; (B) incubation in 30 mM-NaCl for 20 min (LiCl replacement); (C) return to control (cal. bar, 0·1 mV, 6 s).

intestine in Ca-deficient ($< 10^{-6}$ M) Ringer had little effect on amplitude of intestinal electrical waves, and slowed frequency only slightly ($n = 4$) (Fig. 11). Substitution of chloride by isethionate did not alter the intestinal slow-waves.

DISCUSSION

Rhythmic electrical slow-spikes, as described here for toad stomach, are similar to those reported previously for frog (van Harn, 1968), toad (Sato, 1960), skate and lizard (Prosser *et al.* 1976). The slow-spikes consist of monotonic depolarizations and repolarizations; successive ones are separated by periods of electrical quiescence. Their shape is in contrast to the more sinusoidal waves of cat intestinal longitudinal muscle and also the fast spikes, often preceded by pacemaker potentials, of cat intestinal circular muscle (Liu *et al.* 1969). Slow-spikes of toad stomach originate in longitudinal muscle, as has been reported for frog (van Harn, 1968). Isolated circular muscle from the stomach shows no spontaneous spiking but slow-spikes are conducted into it from the longitudinal layer, as are slow-waves in cat intestine (Connor *et al.* 1977). Slow-spikes may spread from longitudinal to circular muscle where they initiate contractions.

The slow-spikes of toad, frog and skate are single electrical waves, unlike the stomach potentials of cat and dog which consist of initial fast events followed by plateaus with or without spikes (Daniel & Irwin, 1968; Papasova, Nagai & Prosser, 1968). They differ from those of guinea pig, which consist of an initial voltage-independent wave followed by a fast depolarization and plateau which are voltage-sensitive (Ohba *et al.* 1977).

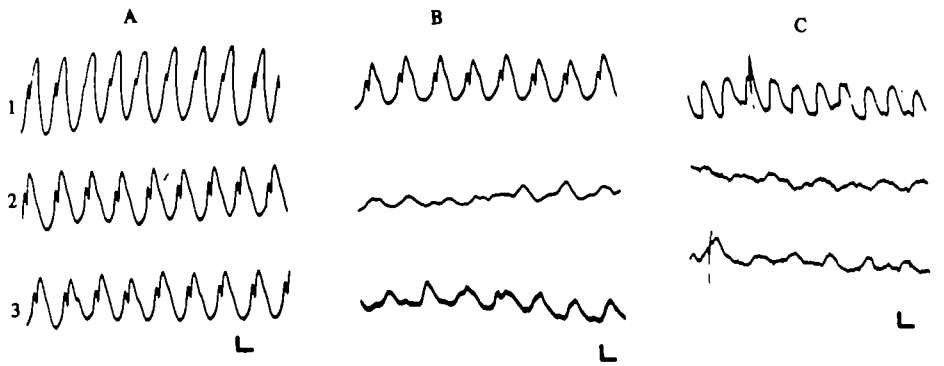

Fig. 10. Effects of ouabain on toad intestinal slow-waves as recorded with pressure electrodes. A: (1) normal solution; (2) 15 min in 10^{-5} ouabain; (3) return to control (cal. bar, 0·1 mV, 6 s). B: (1) normal solution; (2) 15 min in 6×10^{-5} ouabain; (3) return to control (cal. bar (1) 0·1 mV, 6 s; (2) and (3) 0·5 mV, 6 s). C: (1) normal solution; (2) 5 min in 10^{-4} ouabain; (3) return to control (cal. bar, 0·1 mV, 6 s).

The slow-spikes of toad stomach are sensitive in both amplitude and frequency to the concentration of calcium in the medium. In this respect, they resemble the arrhythmic spikes of circular muscle of mammalian intestine (Weigel, Connor & Prosser, 1979), as well as the slow-spikes of skate stomach (Prosser *et al.* 1976). Further resemblance is that the toad slow spikes trigger contractions. It is probable that the slow-spikes result from rhythmic changes in calcium conductance. Slopes of 17 mV for decade change in Ca^{2+} concentration for spikes in cat longitudinal muscle and 8 mV in taenia coli (Tamai & Prosser, 1966) compare well with that (16 mV) found in the toad. The slow-spikes of toad stomach resemble the plateau and spikes of mammalian stomach in their sensitivity to external calcium. Toad slow-spikes are insensitive to Na_o (15–119 mM) and to ouabain, as are slow spikes of skate stomach (Prosser *et al.* 1976), but unlike the initial fast depolarization of stomach potentials of cat (Papasova *et al.* 1968). Reduction of sodium to 1·5 mM decreased slow-spike amplitude and increased frequency. Altering sodium and calcium levels such that the ratio of $\sqrt{(Ca^{2+})}/Na^{+}$ was constant did not result in constant amplitude slow-spikes as has been observed for cat intestinal spikes (Connor & Prosser, 1974), but the effects of decreased calcium levels were potentiated by sodium reduction. This suggests that sodium and calcium may be synergistic rather than antagonistic in the toad stomach.

The rhythmic electrical activity of toad intestine differs from that of toad stomach in consisting of higher-frequency near-sinusoidal slow-waves much like those of mammalian small intestine (Connor, Prosser & Weems, 1974). Unlike the stomach potentials, those of the toad intestine are reduced and may be abolished by ouabain, as are the slow-waves of longitudinal muscle of intestine of mammals such as cat (Connor *et al.* 1974; Liu *et al.* 1969).

Phylogenetic comparisons are difficult, for the results of the present study emphasize the diversity of electrogenesis in gastrointestinal muscles. It is noteworthy that the longitudinal muscle is the pacemaker layer in toad, frog and skate stomach, as in most mammalian intestines, and that the rhythmic waves appear to spread into underlying circular muscle. The spontaneous waves of stomach in ectothermic

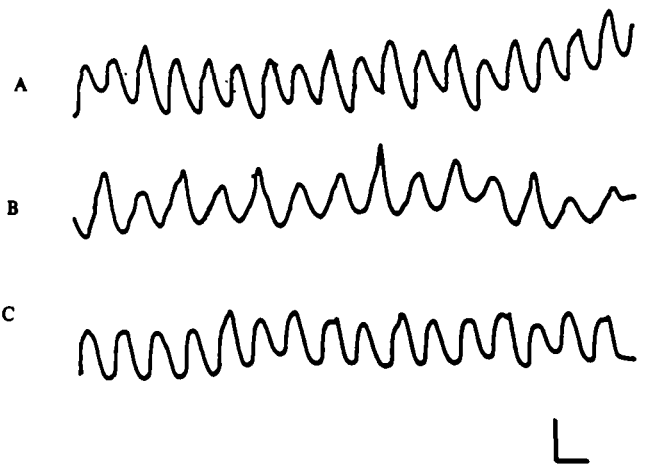

Fig. 11. Effects of calcium free solution on slow-waves of toad intestine as recorded with pressure electrodes. (A) Control; (B) incubation for 20 min in calcium-free solution; (C) return to control (cal. bar, 0·1 mV, 6 s).

vertebrates resemble those of circular intestinal muscle of many mammals in being spike-like (but longer) and dependent on calcium conductance. In contrast, the intestinal waves of toad resemble those of intestinal longitudinal muscle of mammals in being continuously oscillating slow-waves which are sensitive to Na-pump inhibitors such as ouabain and potassium free solution. It is, therefore, not possible from present evidence to present a simple phylogenetic sequence.

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