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RHYTHMIC ELECTRICAL ACTIVITY IN RABBIT AORTA INDUCED BY EGTA

BY ALLEN MANGEL AND CORNELIS VAN BREEMEN

Department of Pharmacology,
University of Miami School of Medicine,
P.O. Box 016189,
Miami, Florida 33101,
U.S.A.

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Normal electrical activity in smooth muscle is dependent on the presence of calcium in the extracellular fluid (Bennett, 1967; Brading, Bülbring & Tomita, 1969; Mangel & Prosser, 1980; Weigel, Connor & Prosser, 1979). However, it has recently been demonstrated that calcium-free solutions containing EGTA induce two types of spontaneous electrical activity in visceral smooth muscle. Prolonged depolarizations of about 17 s were recorded under these conditions from segments of cat small intestine and the stomach of the skate, toad or frog (Prosser *et al.* 1977), while rat small intestine produced fast electrical potentials of approximately 0·85 s duration (Mangel & Nelson, 1978). Since both potential types were eliminated by removal of external sodium or by addition of calcium channel blockers, it was concluded that their mechanism of generation consisted of sodium ions traversing channels normally used by calcium. In this report we examine whether EGTA is capable of inducing electrical activity in a vascular smooth muscle which does not exhibit spontaneous activity in normal saline.

Electrical activity was recorded with pressure electrodes (Bortoff, 1961) from segments of aorta removed from rabbits killed by concussion. The muscle segments were placed immediately into Krebs saline of the following composition (mM): NaCl, 118·5; KCl, 4·7; $MgCl_2$, 1·2; $NaHCO_3$, 23·8; KH_2PO_4, 1·2; $CaCl_2$, 2·5; and glucose, 5·5. The calcium-free saline used in this study had no added calcium plus 3 mM EGTA. Normal Krebs saline and calcium-free Krebs saline were aerated with a 95% O_2–5% CO_2 gas mixture. When manganese was tested, the saline was buffered with MOPS. MOPS saline was as follows (mM): NaCl, 142·3; KCl, 5·9; $CaCl_2$, 2·5; $MgCl_2$, 1·2; glucose, 5·5 and MOPS, 4·0. This solution was bubbled with 100% O_2. All experiments were at 35–37 °C.

Electrical recordings made from rabbit aorta in normal saline showed no spontaneous electrical activity (Fig. 1A). Following a 21 min incubation period in calcium-free saline, fast rhythmic potentials developed (Figs. 1B, C). In other preparations incubation period between 11–72 min was necessary before the initiation of activity. The mean duration of the fast potentials was $1·02 \pm 0·01$ s. ($n = 38$) with

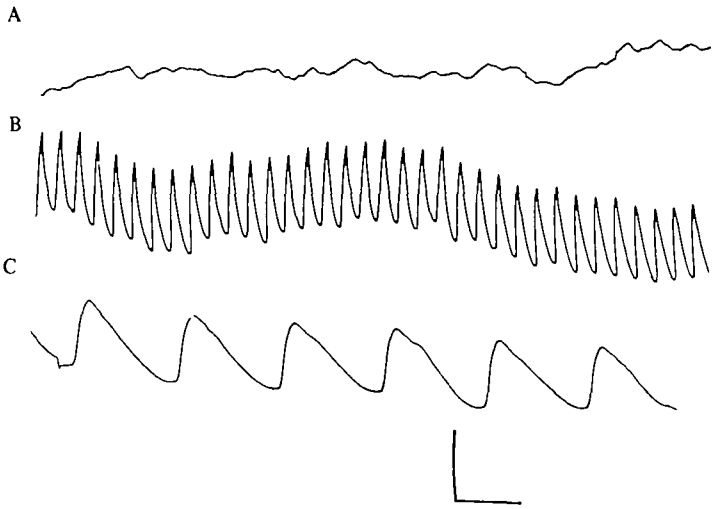

Fig. 1. Electrical activity recorded from rabbit aorta with pressure electrodes. (A) Control saline. (B) 23 min in calcium-free saline (calibration bar: A and B: 1·0 mV, 4 s). (C) 27 min in calcium-free saline. Recording is at faster chart speed (calibration bar: 1·0 mV, 0·8 s).

a range from 0·55 to 2·5 sec. Typically, there was considerable variation in the wave form and duration of fast potentials within a single preparation. In several cases a slow depolarization, similar to intestinal prepotentials (Brading *et al.* 1969; Liu, Prosser & Job, 1969), preceded each fast potential. Fast potentials were often superimposed upon a slower underlying rhythm (Fig. 1 B). There were no consistent changes observed in fast potential activity during the rising or falling phase of the slower rhythm. No rhythmic mechanical activity was found to be associated with fast potentials as has been shown to correspond to prolonged electrical potentials in cat small intestine (Mangel *et al.* 1979).

The ionic mechanism responsible for generation of fast potentials was studied next. Replacement of saline NaCl with LiCl resulted in reversible elimination of fast potentials (Fig. 2). However, in the presence of 5 μM tetrodotoxin or 100 μM ouabain activity persisted. Following the addition of calcium channel blockers (manganese, D600) activity was eliminated within 3 min (see Fig. 3). Reduction in external magnesium concentrations did not result in elimination of fast potentials.

In the calcium-free saline used in this study, an inward-directed calcium gradient would not exist. Therefore, fast potentials cannot result from an influx of calcium. Elimination of fast potentials in sodium-deficient saline suggests that sodium ions are the current carriers responsible for fast potential generation. From the failure of tetrodotoxin to eliminate fast potentials, along with their sensitivity to calcium channel blockers, the most probable mechanism responsible for the production of fast potentials is sodium ions traversing calcium channels.

Prosser *et al.* (1977) concluded that in preparations which show spontaneous activity in normal saline, EGTA induction of rhythmic electrical activity is a general property of smooth muscle membranes. We have extended that observation by showing that rabbit aorta, a preparation quiescent in normal saline, will also produce rhythm

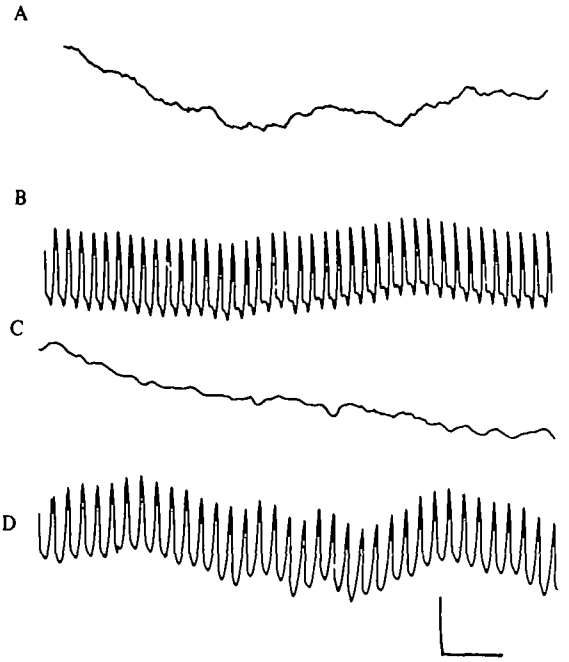

Fig. 2. Effect of sodium removal on fast potentials. (A) Control saline. (B) 17 min in calcium-free saline. (C) Lithium chloride replacement of sodium chloride in calcium-free saline. (D) Return to sodium containing calcium-free saline (calibration bar: 1·0 mV, 3 s).

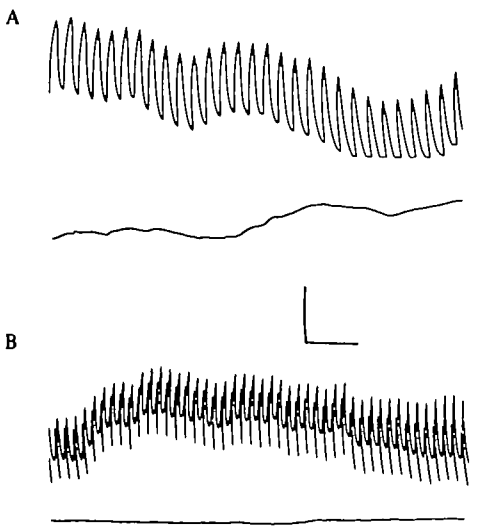

Fig. 3. Effect of calcium channel blockers on fast potentials. (A) Upper record: control fast potential activity in calcium-free saline. Lower record: 2 min after the addition of 5×10^{-5} M D600 (calibration bar: 0·4 mV, 4 s). (B) Upper record: control fast potential activity in calcium-free saline. Lower record: 1 min after the addition of 1 mM manganese (calibration bar: 1·0 mV, 4 s).

activity following EGTA treatment. Under physiological conditions, smooth muscle membranes will not be exposed to a calcium-free environment; thus, fast potentials would not be expected to be present. However, from the present study, it can be concluded that the cellular machinery to generate rhythmic electrical activity is inherent in smooth muscle membranes. Under *in vivo* conditions where neural connections are present recordings from the aorta may show rhythmic activity.

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