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The spontaneous electrical and mechanical activity of the longitudinal smooth muscle of the rabbit duodenum and its modification by drugs and temperature changes

R. C. SMALL AND A. H. WESTON

Department of Pharmacology, University of Manchester, Manchester M13 9PL, U.K.

The electrical and mechanical activity of strips of longitudinal smooth muscle from the rabbit duodenum was recorded using the sucrose gap method. The muscle exhibited rhythmic tension changes each of which was associated with a slow potential wave of electric depolarization surmounted by a burst of spike activity (a multispike complex). Cooling the tissue reduced the frequency of tension waves and multispike complexes. Catecholamines produced a reduction in the amplitude of tension waves. This was associated with hyperpolarization and decreased spike activity. Slow wave frequency and amplitude were unaffected. Acetylcholine, methacholine and histamine produced tension wave fusion associated with depolarization and increased spike activity.

In spite of the widespread use of isolated preparations of rabbit duodenum for both research and teaching purposes, the electrical activity of the longitudinal muscle layer of this tissue has been described only briefly (Bortoff, 1961a; Gonella, 1965). This paper presents the results of experiments designed to examine the effects of drugs and temperature changes on the spontaneous electrical and mechanical activity of rabbit duodenum longitudinal muscle using the sucrose gap method.

Some of the results have been presented to the British Pharmacological Society (Small & Weston, 1969).

MATERIALS AND METHODS

Preparation of the muscle strip

New Zealand White rabbits (Hyline), 2–3 kg, of either sex, were stunned and bled. The duodenum (defined for the purposes of these experiments as the 15 cm of small intestine adjacent to the pyloric sphincter) was removed and strips of the longitudinal muscle layer were prepared according to Ambache (1954). Histologically, the strips consisted of the longitudinal muscle layer together with an adhering nerve plexus. After removal of the damaged ends, 2 cm lengths of the muscle strip were prepared for mounting in the sucrose gap apparatus.

Sucrose gap apparatus

The apparatus used was similar to that described by Bülbring & Burnstock (1960). The potential difference across the gap was recorded by means of a pair of Ag/AgCl wick electrodes attached to a Grass P17 high impedance probe. The output of the probe was recorded on one channel of a Grass polygraph. The frequency response of the recording system was such that a sine wave calibration signal of 12 Hz was reduced in amplitude by 50%.

One side of the gap was perfused with a physiological salt solution (PSS) at 37·5° equilibrated with 5% carbon dioxide in oxygen. The solution had the following composition (mM): Na^+, 143; K^+, 5·93; Ca^{2+}, 2·55; Mg^{2+}, 1·2; Cl^-, 125; HCO_3^-, 25; SO_4^{2-}, 1·2; $H_2PO_4^-$, 1·18; dextrose, 11·1. The free end of the perfused muscle strip in this side of the gap was attached by cotton thread to a force-displacement transducer (Grass FTO3C) for the simultaneous recording of mechanical activity under isometric conditions at a resting tension of 1 g.

The other side of the gap was perfused with potassium sulphate solution (100 mM) at room temperature. The sucrose solution (300 mM) perfusing the gap itself was passed through a deionizing column before use and had a specific resistance greater than 1MΩ cm.

Constant flow rates were maintained for all solutions during an experiment and ranged from 2 to 3 ml/min.

Addition of drugs

(−)-Adrenaline, (−)-noradrenaline, (−)-isoprenaline, acetylcholine, methacholine and histamine were diluted in PSS and 0·05 ml volumes were introduced into the flow of PSS by injection.

Temperature changes

The temperature of the PSS was measured with an accuracy of 0·1° using an electric thermometer (Light Laboratories). Cooling experiments were performed by switching off the warm water circulating system.

RESULTS

Spontaneous activity

Records were made of the spontaneous mechanical and electrical activity of muscle strip preparations from a total of 42 rabbits. The mechanical activity of these strips, i.e. waves of tension of variable amplitude and frequency, was similar to that of pieces of intact duodenum mounted in a tissue bath. In general, three types of mechanical activity could be distinguished:

1. *Regular.* The waves of tension were relatively constant in their shape and amplitude (55% of the preparations, Fig. 1a).
2. *Intermittently regular.* Large, regular tension waves were interposed with periods of smaller, irregular activity (25% of preparations, Fig. 1c).
3. *Irregular.* The size, shape and frequency of the tension waves varied continuously (20% of preparations, Fig. 1b, d).

In the preparations examined, the frequency of tension waves ranged from 11 to 24/min (mean 17/min) whilst their range of amplitude (measured from the foot to the peak of the tension wave) was 0·05 to 10 g (mean 1·7 g). The pattern of electrical activity exhibited by the muscle strips also varied, but usually consisted of slow waves of depolarization with each peak surmounted by a burst of spike activity (Fig. 1a,c). Electrical events such as these have been described for the rabbit portal vein by Holman, Kasby & others (1968) who termed them multispike complexes (MSCs). The same terminology is used in this paper.

The total amplitude of the MSCs ranged from 0·2 to 6·5 mV (mean 1·6 mV). The rise time of the slow potential wave was shorter than its decay time. Indeed, the rate

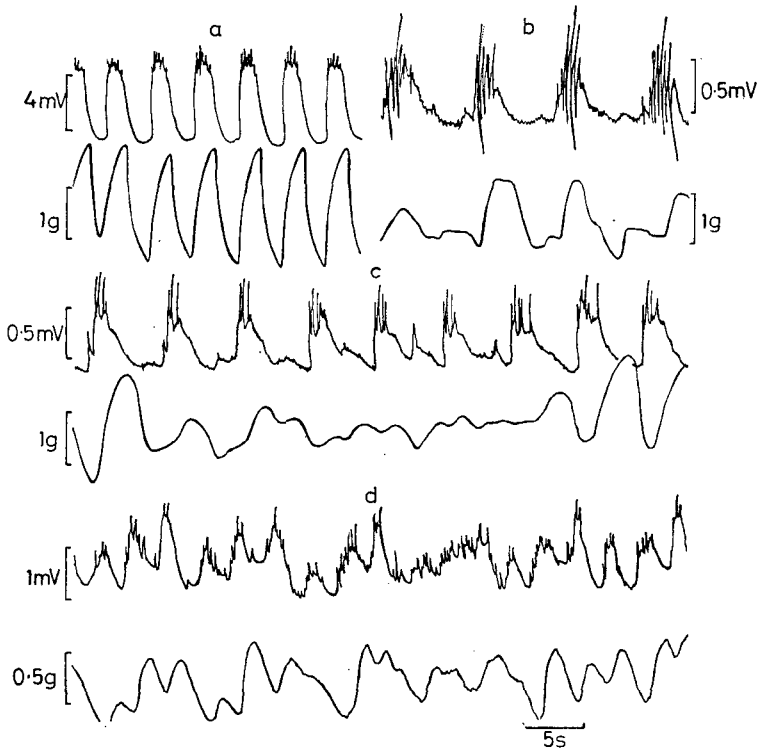

FIG. 1. Spontaneous electrical and mechanical activity at 37·5° in four different preparations. (a) regular mechanical activity; (b) irregular mechanical activity, spikes show positive undershoots; (c) intermittently regular mechanical activity; (d) irregular mechanical activity, spikes not always associated with slow waves.

of rise of the slow waves was often comparable to that of the spikes. Each MSC contained from 1 to 20 spikes whose amplitude ranged from 0·2 to 2·5 mV (mean 0·75 mV). The duration of the fastest spikes measured at half total amplitude was 30 ms. Occasionally, the spikes exhibited a rapidly developing phase of repolarization which extended below the point of initiation of the spike. This is illustrated in Fig. 1b. In most preparations, there was a discrete period between successive slow waves during which the potential remained stable or showed only a very slow rate of change compared to the repolarization phase of the slow waves.

Usually, the rising phase of the slow wave occurred either immediately before or simultaneously with the onset of a tension wave. The correlation between the frequency of MSC generation and the frequency of tension wave development is illustrated in Fig. 2.

Effects of cooling

When the warm water circulating system was switched off, the tissue cooled from 37·5° to 25° in 16 min. The effects of cooling were investigated on ten preparations. Of these, six showed an overall resting hyperpolarization of up to 5 mV. Three preparations exhibited no change in mean potential level and one was depolarized by 4 mV. Cooling also produced a reduction in the resting tension of seven preparations but the other three showed no change.

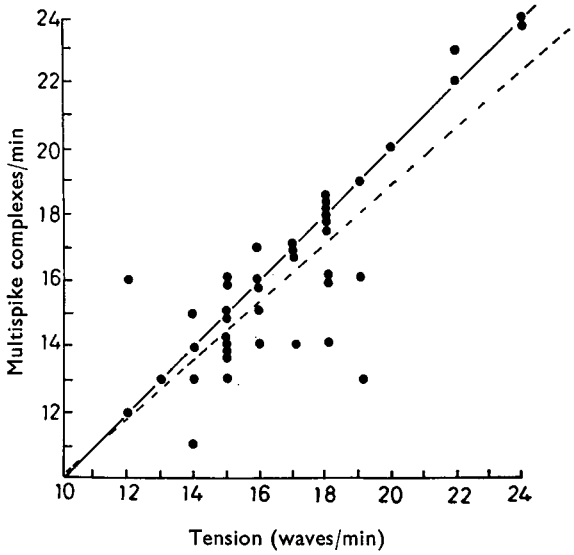

FIG. 2. The correlation between MSC frequency and tension wave frequency. Each point represents a single preparation. The broken line indicates the calculated regression line with $r = 0{\cdot}84$ ($P < 0{\cdot}001$). The continuous line represents a perfect positive correlation.

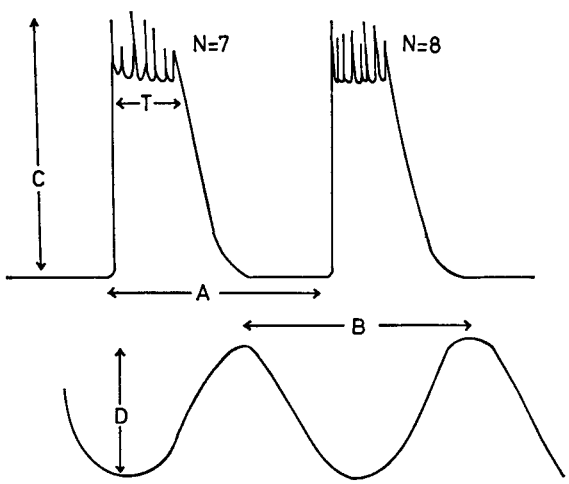

FIG. 3. Parameters of electrical and mechanical activity measured to assess the effects of cooling. A: MSC cycle time; B: tension wave cycle time; C: MSC amplitude; D: tension wave amplitude; N: number of spikes/MSC; T: duration of spiking; N/T: intracomplex spike frequency.

In order to examine the effects of cooling in detail, the MSC cycle time, tension wave cycle time, MSC amplitude, tension wave amplitude and intracomplex spike frequency were measured at 2·5° intervals between 37·5° and 25°. These parameters are defined in Fig. 3. At a given temperature, measurements of each parameter were made upon four consecutive MSCs or tension waves and the mean value was calculated. In three preparations, spike activity disappeared at temperatures above 25° thus reducing the size of the group in which intracomplex spike frequency measurements were made.

The most marked effect of cooling was a parallel increase in the MSC and tension wave cycle times. The amplitude of tension waves decreased but this was not accompanied by a corresponding reduction in MSC amplitude. However, there was a reduction in intracomplex spike frequency. These results are illustrated in Fig. 4.

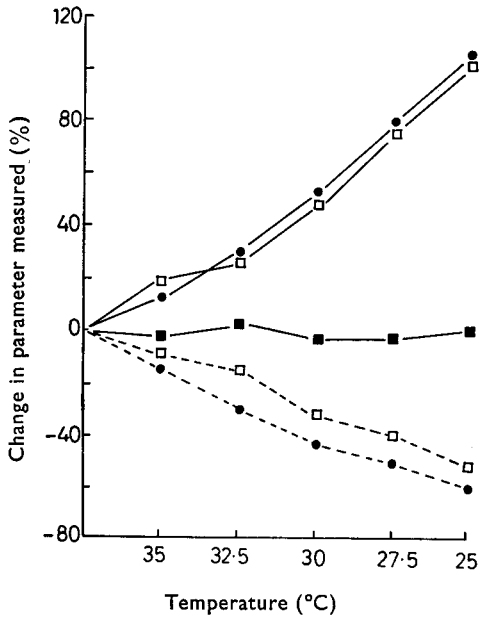

FIG. 4. The effect of cooling on MSC cycle time ●——●, tension wave cycle time □——□, MSC amplitude ■——■, tension wave amplitude □ - - - □, and intracomplex spike frequency ●- - -●. These parameters are defined in Fig. 4. Ordinate, percentage change in the parameter measured taking the change at 37·5°C as zero. Each point represents the mean of measurements from ten preparations.

Effects of rewarming

When the warm water circulating system was switched on again, the temperature of the PSS rose from 25° to 37·5° in 3 min. Such experiments showed that the effects of cooling were reversible. In one experiment, a complete inhibition of both MSCs and tension waves was observed in the initial rewarming period.

Effects of drugs

Catecholamines such as adrenaline, noradrenaline and isoprenaline all caused a reduction in the amplitude of tension waves together with a decrease in the resting tension of the tissue. These effects were associated with an overall hyperpolarization. Spike activity was reduced or abolished by these agents but slow wave frequency and amplitude of the rhythmical electrical changes were unaffected. Moreover, the slow waves retained their fast rising phase in the absence of spike activity. The effects of increasing concentrations of isoprenaline are shown in Fig.5.

Acetylcholine, methacholine and histamine all caused an increase in the resting tension of the preparation. With large doses of these drugs, individual tension waves were lost in a fused tension response. When tension waves remained discrete, slow waves of electrical potential change were still discernible but these were associated with increased superimposed spike activity. With the larger, fused

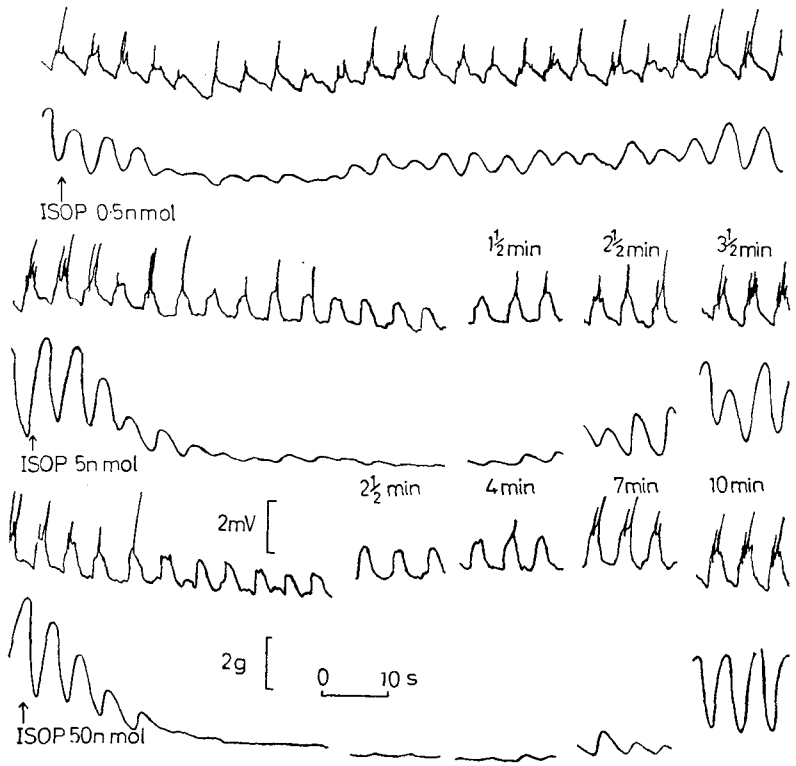

FIG. 5. The effect of increasing doses of isoprenaline on electrical and mechanical activity. All records from the same preparation.

tension responses, electrical spike activity occurred both on the crests and in the troughs of the slow waves and there was a greater overall depolarization. The effects of increasing doses of acetylcholine are shown in Fig. 6.

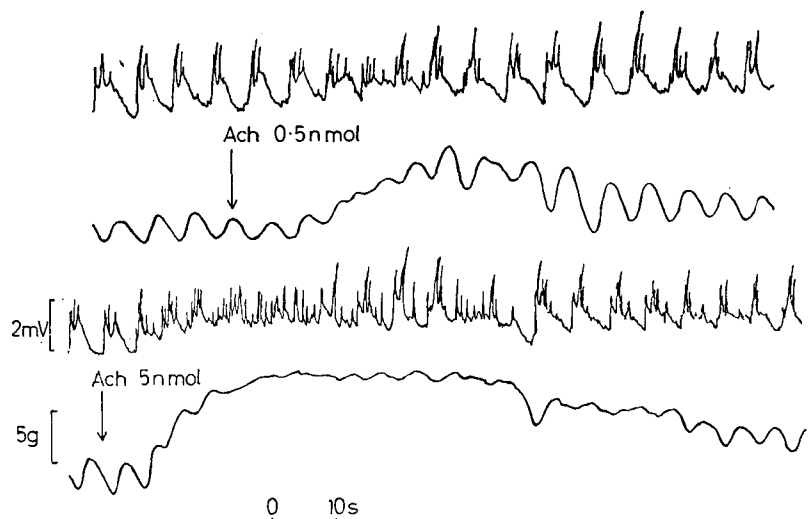

FIG. 6. The effect of increasing doses of acetylcholine on electrical and mechanical activity. Both records from the same preparation.

DISCUSSION

The electrical activity of the longitudinal smooth muscle of the rabbit duodenum was seen to consist of a series of MSCs i.e. slow waves of depolarization each surmounted by a burst of spike activity. This pattern of electrical events is similar to that obtained by the intracellular recording technique applied to this tissue by Bortoff (1961a) and Gonella (1965). Most preparations produced MSCs in a 1 to 1 relation with tension waves, suggesting a correlation between these two events. Since the rising phase of the MSC occurred immediately before or simultaneously with an increase in tension, it seems probable that the electrical events trigger the mechanical response of the tissue.

In contrast to most preparations examined, some showed no regular pattern of either electrical or mechanical activity. Such preparations may have been damaged during dissection or mounting in the apparatus, thus destroying the functional integrity of the smooth muscle syncytium and permitting random excitation of cells. This explanation might not suffice for those preparations exhibiting intermittently regular mechanical activity. Here, the electrical record maintained its regularity despite the transient loss of regular mechanical events. This might suggest that, at these times, the part of the tissue from which the electrical record was obtained contributed very little to the overall mechanical record.

The ability of catecholamines to abolish spikes and reduce the amplitude of tension waves whilst leaving slow waves intact suggests that, as in guinea-pig taenia coli, tension development is closely related to spike activity (Bülbring, 1955). Our cooling experiments and those in which the spasmogens acetylcholine, methacholine and histamine were used also demonstrate the importance of spike activity in determining the magnitude of the mechanical response.

Catecholamines did not affect slow wave frequency or amplitude. A similar observation has been made by Bortoff (1961b) for the action of adrenaline on cat jejunum. Low concentrations of spasmogens were also without effect on slow wave frequency although at higher concentrations, individual slow waves became difficult to distinguish because of the overall depolarization and continuous spiking. Cooling was the only procedure to affect slow wave frequency; this was reduced in parallel with the frequency of tension waves.

The failure of spasmogens and spasmolytics to alter MSC frequency in rabbit intestinal muscle contrasts with the observations of Holman, Kasby & others (1968) on the smooth muscle of rabbit portal vein. Although the MSCs of the latter preparation were similar in shape to those recorded from rabbit duodenum, their frequency was markedly affected by drugs; it was increased by spasmogens and decreased by spasmolytics. Furthermore, spasmolytic agents did not selectively abolish spike activity. This suggests that the slow component of the vascular MSC may be produced by the same mechanism as that responsible for spike generation. Alternatively, the slow waves of vascular smooth muscle may not represent discrete entities but merely result from residual depolarization produced by repetitive high frequency spiking (Holman, 1968).

The ability of drugs to dissociate slow waves from spikes in visceral smooth muscle supports the hypothesis of Tamai & Prosser (1966) that these two events are produced by different electrogenic processes. These authors showed that the slow waves and spikes of the cat small intestine exhibited marked differences in their sensitivity to changes in the ionic environment.

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