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PAPAVERINE-INDUCED INHIBITION OF ELECTRICAL AND MECHANICAL ACTIVITY AND CALCIUM MOVEMENTS OF RAT ILEAL SMOOTH MUSCLE

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SUMMARY

1. Papaverine, in the concentration range 50–150 μM, inhibited rhythmic spontaneous contraction and caused relaxation of KCl-induced contractures of rat ileal smooth muscle.

2. Spontaneous slow waves and spikes declined at low papaverine concentrations.

3. The normal ^{45}Ca uptake of the ileum was reduced by papaverine. Although papaverine caused only a small inhibition of normal ^{45}Ca efflux from the ileum, it strongly inhibited the KCl-induced stimulation of ^{45}Ca efflux.

4. Calcium uptake by the isolated ileal smooth-muscle microsomal fraction was slightly increased by papaverine, but mitochondrial calcium uptake was unaffected.

5. Ileal smooth muscle incubated in calcium-free media showed little response to either the reintroduction of calcium or KCl in the presence of papaverine.

6. It is suggested that the inhibitory actions of papaverine on ileal smooth muscle result from a blockade of the calcium influx which triggers the excitation–contraction coupling sequence.

INTRODUCTION

Papaverine, the major benzylisoquinoline alkaloid of opium, possesses potent relaxatory action on a number of smooth muscles (Santi, Ferrari & Contessa, 1964; Diamond & Marshall, 1969; Tashiro & Tomita, 1970; Bauer & Capek, 1971; Poch & Kukovetz, 1971; Bauer, Kadlek & Seferna, 1974; Reinhardt, Wagner & Schumann, 1974; Uruno *et al.* 1974; Miyamoto *et al.* 1976). For a number of years, papaverine has been employed as a vasodilator agent because of its relaxatory effect on vascular smooth muscle (Kukovetz & Poch, 1970; Triner *et al.* 1971) and as an antiarrhythmic agent on the heart (Reinhardt *et al.* 1977; Whipple, 1977). However, there is no concensus of opinion regarding the mechanism of papaverine-induced relaxation of these various visceral muscles.

There is evidence that papaverine inhibits phosphodiesterases in visceral muscle (Poch & Kukovetz, 1971; Urono *et al.* 1974; Miyamoto *et al.* 1976; Takayanagi,

Yamashita & Kasuya, 1978), implicating an increase in cell cyclic AMP level as the mediator of the relaxatory mechanism (Triner *et al.* 1971; Poch & Umfahrer, 1976; Honeyman, Merriam & Fay, 1978) as is thought to be the case in methylxanthine action on smooth muscle (Robison, Butcher & Sutherland, 1971; Aberg & Andersson, 1972; Berridge, 1975; Theobald, Syson & Burrin, 1978). However, the role of cyclic AMP as the secondary regulator of drug-induced contraction and relaxation of smooth muscle is far from clear. A number of inconsistencies in the relationship between inhibition of phosphodiesterases and the relaxatory effect of papaverine have been found (Andersson *et al.* 1977; Polson *et al.* 1978).

Although papaverine increased cyclic AMP levels in cardiac muscle and in arterial smooth muscle, no correlation could be found between cyclic AMP level and contractility (Kukovetz & Poch, 1970; Reinhardt *et al.* 1977). In contrast, papaverine was found to be without effect on cyclic AMP level in seminal vesicle smooth muscle, but caused strong relaxations of KCl-induced contractures (Gamo *et al.* 1977). In addition, cyclic AMP was not involved in phosphorylase activation nor in the relaxation of uterine smooth muscle caused by papaverine and a number of other relaxants (Diamond, 1973; Diamond & Holmes, 1975). Cyclic GMP has also been implicated in contraction and relaxation of a number of smooth muscles (Schultz *et al.* 1973; Bulbring & Hardman, 1976; Schultz & Hardman, 1975; Andersson & Djarv, 1978). Some correlation does exist between development of tension and increase in cyclic GMP levels, especially in potassium depolarized muscle (Andersson & Djarv, 1978), although evidence suggests that cyclic GMP may not be involved in contraction and relaxation of taenia coli and uterus in response to catecholamines (Bulbring & Hardman, 1976).

There is, however, one major drawback in implicating cyclic nucleotides in the major immediate effects of papaverine on smooth muscle. The very speed of action of papaverine must cast doubt on direct mediation by the rather circuitous cyclic AMP/cyclic GMP/phosphodiesterase route. The speed of papaverine-induced responses could well be due to a much more direct action on the muscle, possibly at some stage in the early excitation–contraction coupling sequence, although this possibility has not been investigated.

In this study, the direct actions of papaverine on electrical and mechanical activity of ileal smooth muscle have been examined, and attempts have been made to correlate these actions with calcium influx and efflux. The most immediate actions of papaverine on this smooth muscle seem more related to an action on calcium influx than on cyclic nucleotide effects (Saad & Huddart, 1979).

METHODS

Wistar-strain albino rats were used throughout this study. The animals were killed by a blow to the head, the ileum was removed immediately and placed in aerated Krebs saline at 37 °C. This saline contained (in mM): NaCl, 120·7; KCl, 5·9; $CaCl_2$, 2·5; $MgCl_2$, 1·2; Na_2HPO_4, 1·2; $NaHCO_3$, 15·5 and glucose, 11·5 at pH 7·3. A concentrated stock solution of papaverine in Krebs saline was prepared, and appropriate amounts were added directly to the organ bath, efflux salines and incuba-

ion media. For studies in calcium-free media, calcium was omitted, but chelating agents such as EDTA and EGTA were not employed in order to prevent undesirable artificial removal of intracellular free Ca by these agents.

For tension recording, 2 cm ileal strips were mounted vertically in a 50 ml organ bath and were connected to a Washington Instruments isotonic strain gauge which fed into a curvilinear pen recorder. For simultaneous recording of tension and membrane potentials, preparations were mounted horizontally in an organ bath. Membrane potentials were recorded with glass capillary intracellular microelectrodes filled with 3 M-KCl. Signals were fed via a high-impedance probe for display on a Tektronix 502A dual-beam oscilloscope and they were recorded on a Bell & Howell ultraviolet oscillograph connected in parallel with the oscilloscope. Preparations were continuously aerated throughout the experiments.

The techniques for estimating ^{45}Ca influx and efflux of ileal smooth muscle strips have been described in detail elsewhere (Huddart & Syson, 1975; Huddart & Saad, 1977, 1978). Mitochondria and membrane vesicles (microsomes) of ileal smooth muscle were prepared for ^{45}Ca uptake studies as follows. Sections of ileal muscle were homogenized in ice-cold extraction medium (100 mM-KCl and 1 mM imadazole, pH 6·8) using a Teflon coaxial homogenizer. The homogenate was first centrifuged at 1000 ***g*** for 30 min to remove cell debris and the mitochondria were sedimented from the supernatant by centrifugation for 30 min at 8000 ***g***. Microsomes were sedimented from the supernatant by centrifugation for 60 min at 28000 ***g***. All centrifugations were carried out at 2 °C. The mitochondrial and microsomal pellets were resuspended in extraction medium and adjusted to 1 mg protein/ml following biuret analysis.

To determine ^{45}Ca uptake by mitochondria and microsomes, 1 ml of suspension was mixed with 1 ml of incubation medium containing (in mM): $MgCl_2$, 4; $CaCl_2$, 2·5; ATP, 2; tris maleate buffer, 40 at pH 7·0 with 0·1 μCi ^{45}Ca/ml. The reaction proceeded at room temperature and was stopped at various time intervals by passage through membrane filters of pore size 0·45 μm. The suspensions on the filters were washed to remove extraneous ^{45}Ca by passing 20 ml of Krebs saline through the filter. After drying, ^{45}Ca activity retained on the filters was estimated in a Packard Tricarb liquid scintilation counter. Background ^{45}Ca contamination of blank control filters was subtracted from the experimental counts.

RESULTS

Mechanical and electrical activity

In the concentration range 10–150 μM, papaverine caused relaxation of the spontaneous rhythmic contractions of the rat ileum, accompanied by a fall in resting tension (Fig. 1). The concentration threshold for consistently observable relaxation was remarkably low at only 10 μM, and the dose-dependency of papaverine-induced relaxation of this intestinal smooth muscle is shown in Fig. 2. The inhibition of contraction was measured simply as the percentage reduction in height of spontaneous contractions. In addition to altering contractile activity, papaverine also slowed the spontaneous rhythm.

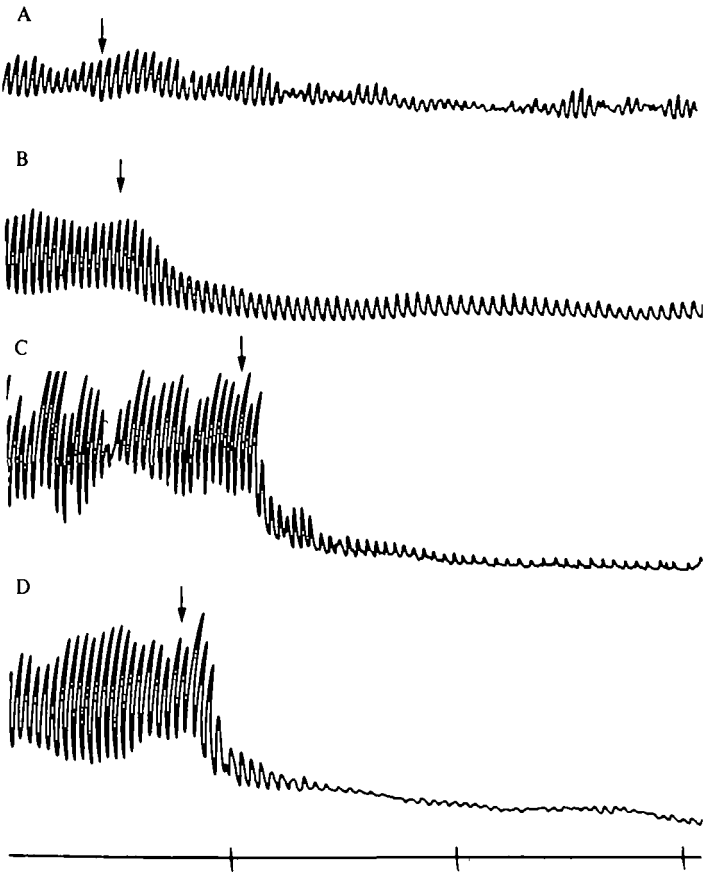

Fig. 1. Papaverine-induced relaxation of spontaneous contractility in rat ileal smooth muscle. Papaverine was added at the arrowed point in the following concentrations: (*a*) 0·01 mM, (*b*) 0·05 mM, (*c*) 0·1 mM, (*d*) 0·15 mM. Time calibration, 1 min.

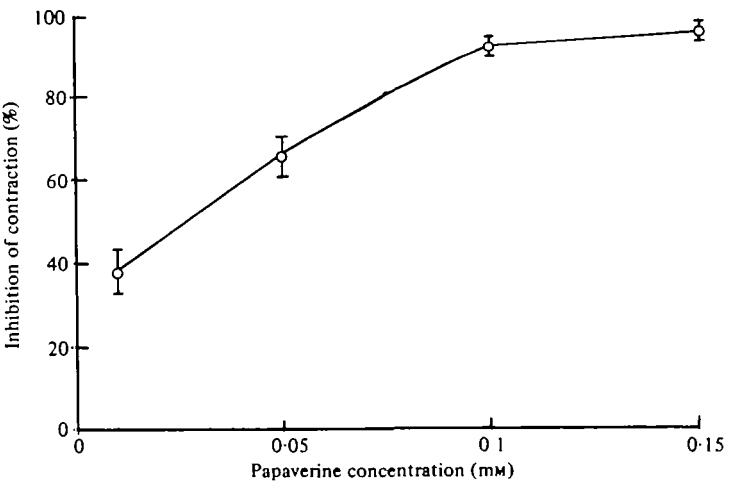

Fig. 2. Dose–response curve of papaverine-induced relaxation of spontaneously active rat ileal smooth muscle. Vertical bars represent ±S.E. of mean, $n = 4$.

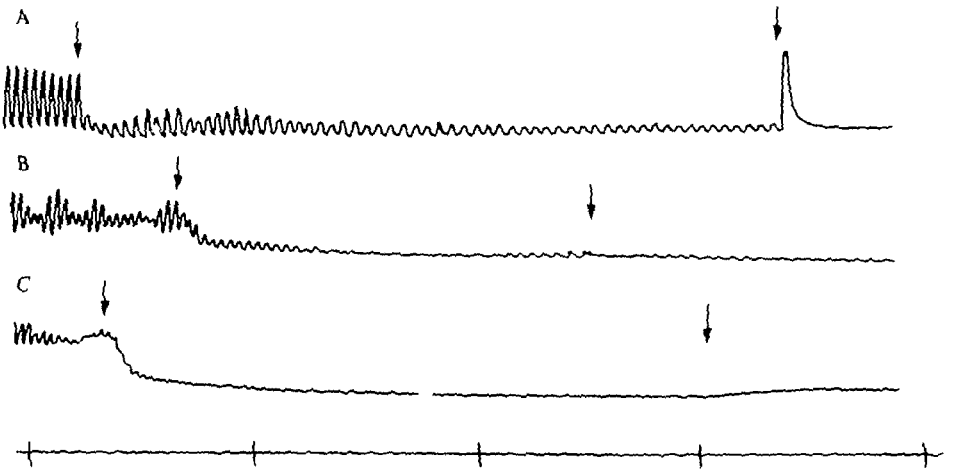

Fig. 3. The effect of papaverine (first arrow) on the response of ileal smooth muscle to KCl-induced depolarization (second arrow). (*a*) 0·01 mM papaverine then 50 mM-KCl, (*b*) 0·05 mM papaverine then 50 mM-KCl and (*c*) 0·15 mM papaverine then 100 mM-KCl. Note the clear inhibition of KCl-induced tension. Time calibration, 1 min.

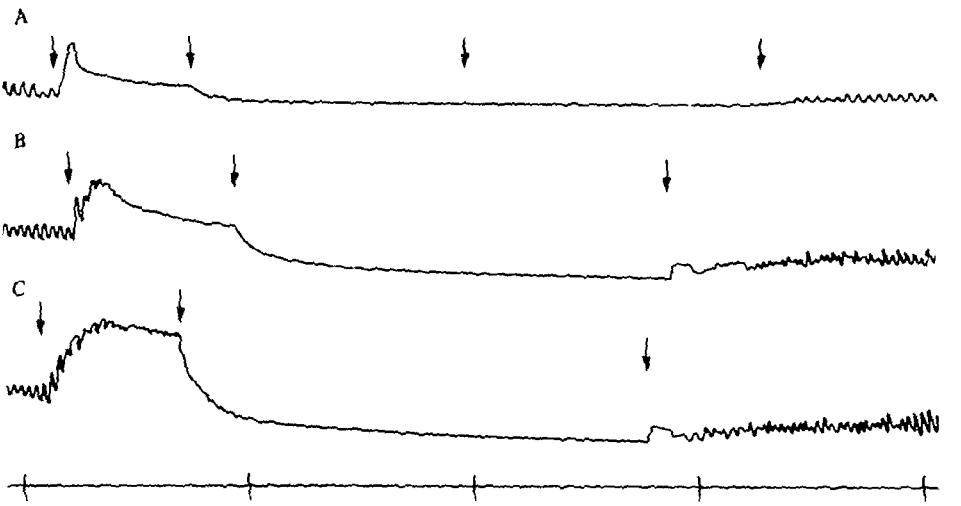

Fig. 4. Papaverine-induced relaxation of KCl contractures of rat ileal smooth muscle. (a) 25 mM-KCl (first arrow) followed by 0·1 mM papaverine (second arrow). A further 25 mM-KCl was added at the third arrow followed by control saline (fourth arrow). (*b*) 40 mM-KCl (first arrow) followed by 0·1 mM papaverine (second arrow) then control saline (third arrow). (*c*) 60 mM-KCl (first arrow) followed by 0·1 mM papaverine (second arrow) then control saline (third arrow). Time calibration, 1 min.

Pre-treatment of ileal smooth muscle with papaverine salines for periods as short as 2 min caused a dose-dependent inhibition of subsequent KCl-induced contracture tension. At 10 μM, the phasic part of the contracture was greatly reduced while the tonic part of the contracture was completely inhibited. At all higher concentrations both the phasic and the tonic KCl contracture were virtually abolished (Fig. 3). To check whether papaverine could influence preparations in which calcium influx had been stimulated, papaverine was applied to muscles induced into contracture with high KCl salines. In such preparations, papaverine was found to strongly inhibit the KCl-induced tonic contracture (Fig. 4). Papaverine inhibition was completely reversible even at the highest concentrations used. All preparations showed a return to normal resting tension and spontaneous contractility on perfusion with control Krebs saline (Fig. 4).

Papaverine, in the concentration range 10–150 μM, caused a decline in spontaneous electrical activity of the ileum. Electrical activity of ileal smooth muscle of the rat consists of phases of slow waves of 8–14 mV amplitude with superimposed spikes of 8–15 mV amplitude (Syson & Huddart, 1976), separated by periods of small slow wave activity only. These events correspond to the cyclical peristalsis of the preparation. It is usually not possible with simultaneous recording to achieve a perfect register of the electrical and mechanical events since electrical activity is recorded from a single cell of the preparation while tension has to be recorded from the whole muscle strip. However, reasonable overall correlation can often be achieved.

Typical spikes and slow waves recorded from the ileum are shown in Fig. 5. At low concentrations of papaverine (10–50 μM) spikes were slowly abolished while some small slow wave activity continued (Fig. 5*a*). In this trace, papaverine has clearly inhibited the cyclical burst of slow waves and spikes compared with the previous cyclical burst 60 s earlier. At higher concentrations both spikes and slow waves declined in a matter of 10–20 s, accompanied by a corresponding decline in mechanical activity (Fig. 5*b*). The inhibition of spontaneous electrical activity was not accompanied by any significant change in the resting membrane potential. The mean resting potential of this particular smooth muscle was -41.2 ± 2.3 mV. After 5 min treatment with 50 μM papaverine the resting potential was -39.8 ± 3.6 mV. The effect of papaverine on electrical activity was reversible. On return to control Krebs saline, spontaneous slow waves and spikes were slowly restored (Fig. 5*c*).

Calcium influx and efflux

The relationship between inward calcium movement and contractile activation of smooth muscle has been firmly established (Joiner, 1972; Huddart & Syson, 1975; Casteels & Van Breemen, 1975; Barratt & Huddart, 1978). Any agent that blocks inward calcium movement or competes with calcium at surface binding sites impairs normal spontaneous contractile activity (Van Breemen *et al.* 1972; Reinhardt *et al.* 1974; Saida, Yanaura & Chujyo, 1974; Godfraind, 1976; Huddart & Saad, 1977; Deth, 1978). The clear inhibition of ileal smooth muscle spikes by papaverine suggested that this agent may block inward calcium movement since spikes are calcium-dependent (Job, 1969; Huddart & Hunt, 1975). It was therefore thought pertinent to examine the effect of papaverine on calcium influx into ileal smooth muscle strips.

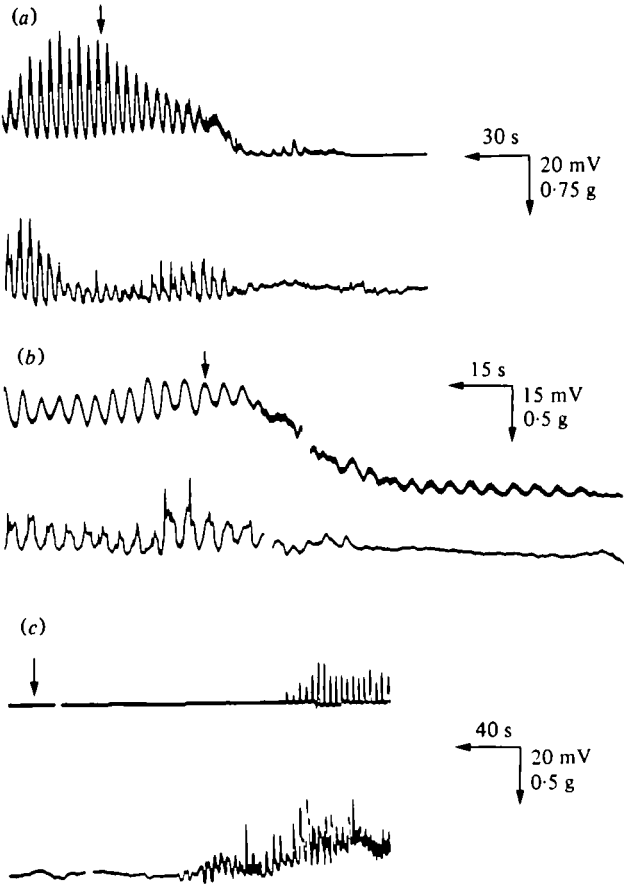

Fig. 5. The effect of papaverine on spontaneous electrical activity of rat ileal smooth muscle. (*a*) Effect of 50 μM papaverine (added at arrow), showing decline then elimination of spikes and severe inhibition of slow waves with corresponding inhibition of tension. (*b*) Rapid inhibition of electrical and mechanical activity by 100 μM papaverine (added at arrow). (*c*) Restoration of first the electrical and then mechanical activity after 100 μM papaverine treatment on return to control saline. In all traces the upper panel is mechanical activity, the lower trace is electrical activity and arrows apply to both traces. In (*a*), the papaverine was added when the cell from which the electrical record was taken was entering a low spike activity phase. The papaverine has prevented the development of subsequent high spike activity cycles. In (*b*) the papaverine was added during a high spike activity cycle, where it causes almost instant spike abolition.

The results of these experiments are summarized in Fig. 6, where a clear inhibition of calcium uptake of between 30–40% is seen in papaverine-containing media.

It is possible to test whether this level of inhibition of calcium influx by papaverine is of real significance in the excitation–contraction coupling cycle. If ileal smooth muscle preparations are incubated in calcium-free Krebs saline, spontaneous activity is greatly reduced, the rhythm is slowed, and finally abolished. When calcium-containing media are then introduced to these preparations, normal activity is quickly established by rapid calcium influx (Huddart & Saad, 1977, 1978). When such preparations are again exposed to calcium-free media, activity is again reduced and

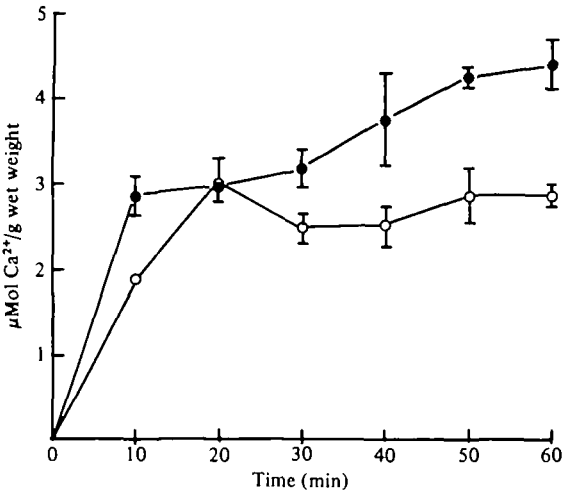

Fig. 6. Uptake of ^{45}Ca by rat ileal smooth muscle incubated in control media (solid circles) and in media containing 0·1 mM papaverine (open circles). Vertical bars represent ±S.E. of the mean, $n = 6$.

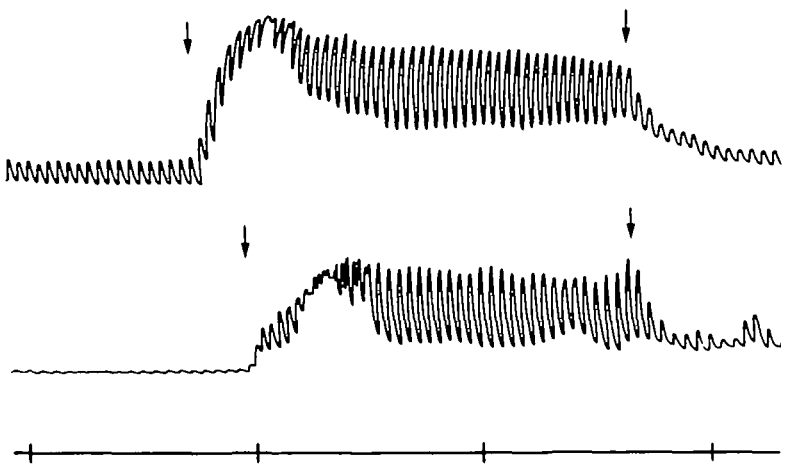

Fig. 7. The effect of reintroduction of 2 mM calcium (first arrow) on the contractile activity of ileal smooth muscle preparations maintained in calcium-free media without EDT or EGTA. On return to calcium-free media (second arrow) activity is again inhibited. Time calibration, 1 min.

the rhythm slowed (Fig. 7). These results show just how labile contractile activity is in this smooth muscle and how dependent it is on extracellular calcium.

If these experiments are repeated in papaverine-containing media, quite different results are obtained (Fig. 8). In Fig. 8(*a*), papaverine (0·05 mM) has inhibited spontaneous activity of the ileum maintained in normal calcium media. When extra calcium is added to this preparation in the form of three successive additions of 2 mM calcium (total Ca content 8·5 mM), this still fails to restore normal activity in contrast to the situation seen in Fig. 7. In Fig. 8(*b–d*), preparations were first exposed to calcium-free media (at first arrow) that caused a reduction in activity, as seen in the experiments

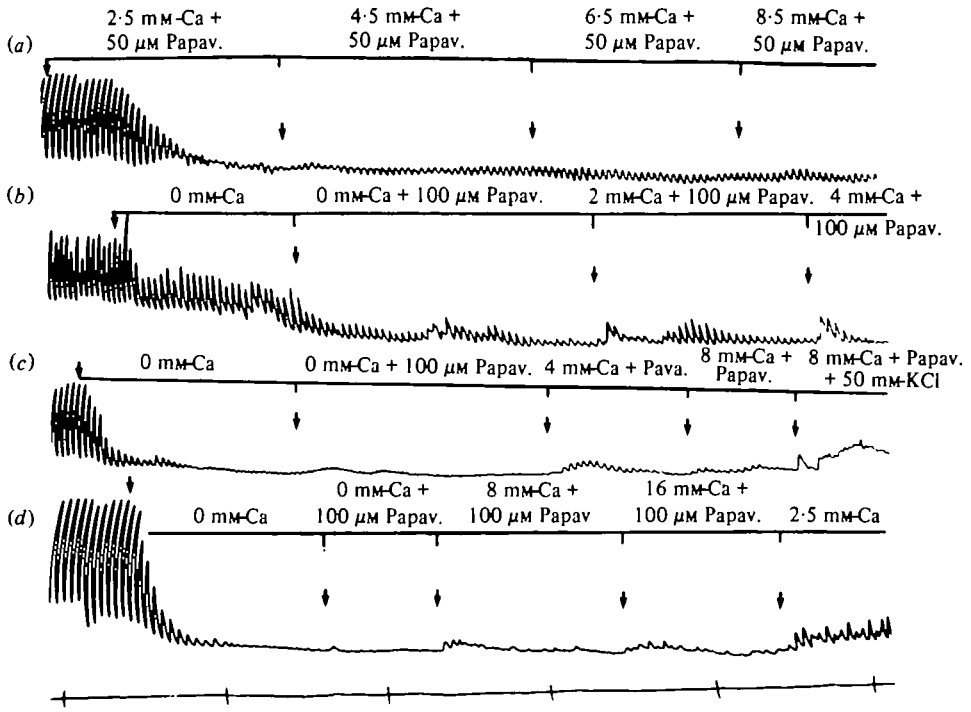

Fig. 8. The effect of papaverine on the reintroduction of calcium to ileal smooth muscle preparations incubated in normal calcium media (*a*) and in calcium-free media (*b*, *c* and *d*). The experimental protocol was as follows: (*a*) 0·05 mM papaverine (first arrow) followed by three stepwise increases (2nd to 4th arrows) in the level of calcium (2 mM/step). (*b*) calcium-free saline (first arrow), 0·1 mM papaverine (second arrow), followed by two successive additions of 2 mM calcium (3rd and 4th arrows). (*c*) calcium-free saline (first arrow) followed by 0·1 mM papaverine (second arrow) then two successive additions of 4 mM calcium (3rd and 4th arrows) and finally 50 mM-KCl (fifth arrow). (*d*) calcium-free saline (first arrow) followed by 0·1 mM papaverine (second arrow) then two successive additions of 8 mM calcium (3rd and 4th arrows) and then return to normal physiological saline (fifth arrow). In the presence of papaverine addition of calcium fails to restore normal activity nor was there any response to KCl in the presence of calcium and papaverine. Time calibration, 1 min.

in Fig. 7. Papaverine was then added to the preparations at the second arrow. Successive additions of calcium to these preparations at the third and fourth arrows completely failed to restore normal spontaneous activity in the presence of papaverine, in contrast to reintroduction of calcium in the control experiment in Fig. 7, where papaverine was not present. With papaverine present, the ileum failed to respond to KCl even in high-calcium media (Fig. 8(*c*), fifth arrow), but when papaverine saline was replaced by normal physiological saline, spontaneous activity was slowly restored (Fig. 8(*d*), fifth arrow). The reintroduction of calcium to preparations in either normal or calcium-free media failed to restore normal contractile activity as long as papaverine was present. These results strongly suggest that papaverine has inhibited calcium influx to such an extent as to prevent a sufficient inward calcium signal to trigger normal contractions, and they correlate well with the results of ^{45}Ca uptake by the ileum in the presence of papaverine. This inhibition of calcium influx by papaverine is very reminiscent of quinine action on smooth muscle (Huddart & Saad, 1977).

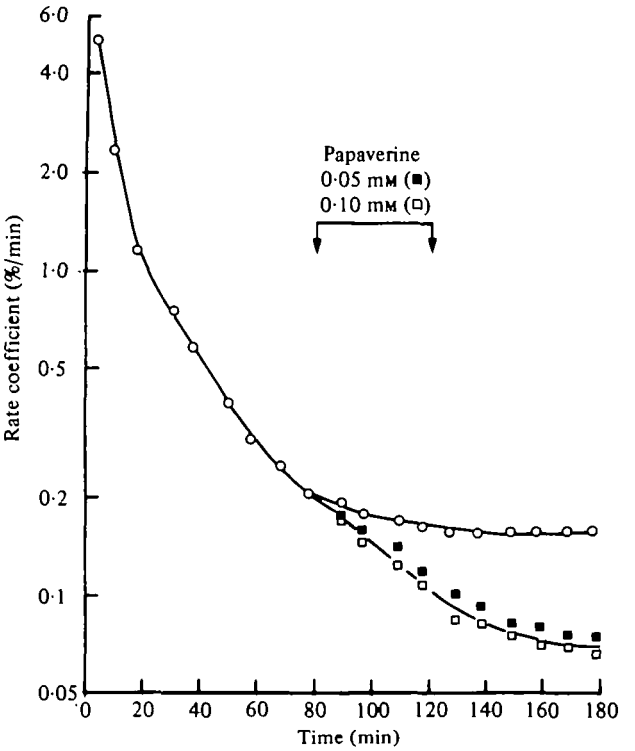

Fig. 9. ^{45}Ca efflux from rat ileal smooth muscle in control conditions (open circles) and with papaverine treatment (0·05 mM, solid squares; 0·1 mM open squares) as indicated during the slow efflux phase. Each point is the mean of six determinations.

To test any actions by papaverine on gross cellular calcium movements, ^{45}Ca efflux from ileal smooth muscle strips was examined. The normal ^{45}Ca efflux curve of ileal smooth muscle is shown in Fig. 9. When papaverine is added to the efflux saline during the slow, presumably intracellular, efflux phase, a significant inhibition of efflux is seen, from 0·17%/min to about 0·07%/min. This represents a sharp fall in myoplasmic free calcium level in the presence of papaverine. That this fall is almost certainly related to inhibition of calcium influx is confirmed when ^{45}Ca efflux in high KCl salines is examined. KCl-induced depolarization of smooth muscle causes a sharp rise in calcium influx (Sperelakis, 1962; Briggs, 1962; Urakawa & Holland, 1964), and this can be seen clearly as a large stimulation of ^{45}Ca efflux in Fig. 10, as has previously been shown in this preparation (Huddart & Syson, 1975). In the presence of papaverine this KCl-induced stimulation of efflux is greatly inhibited.

Intracellular calcium binding

The effects of papaverine so far described on calcium influx and efflux do not rule out additional effects on the subcellular structures involved in calcium binding. Various membrane vesicles (microsomes) and mitochondria have been implicated in calcium regulation during the excitation–contraction coupling cycle of a number of visceral muscles (Huddart & Syson, 1975; Heumann, 1976; Uchida, 1976; Huddart, Hunt & Oates, 1977; Janis & Daniel, 1977; Raeymaekers *et al.*1977; Nilsson *et al*

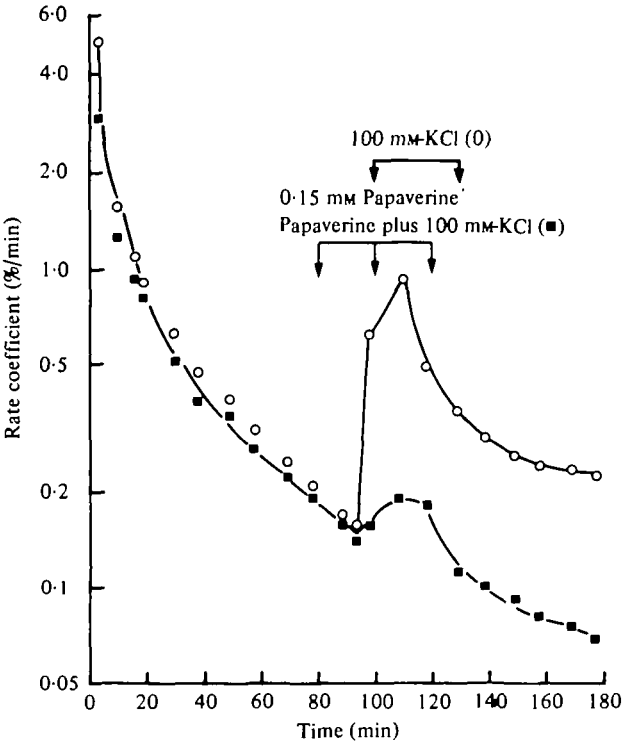

Fig. 10. The effect of KCl depolarization during the slow phase of ^{45}Ca efflux from rat ileal smooth muscle (open circles). Note the large stimulation of efflux. In the parallel experiments (filled squares), preparations were first exposed to 0·15 mM papaverine saline (first arrow) and then papaverine plus 100 mM-KCl (second arrow). Test salines were replaced with control saline at the final arrow. Note the strong inhibition of KCl-stimulated ^{45}Ca efflux by papaverine. Each point was the mean of six determinations.

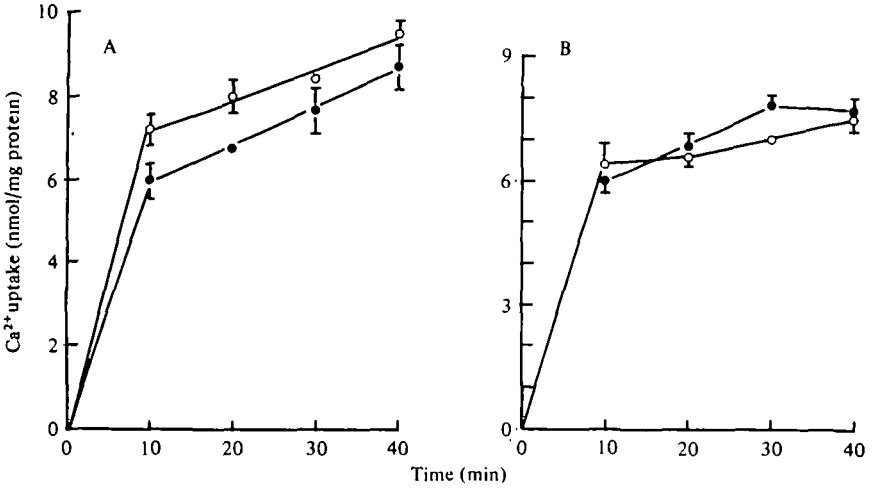

Fig. 11. ^{45}Ca uptake by isolated microsomes (*a*) and mitochondria (*b*) isolated from rat ileal smooth muscle. In both cases control conditions are represented by solid circles and preparations incubated with 0·05 mM papaverine by open circles. Note the slight increase in calcium uptake by microsomes in the presence of papaverine. Vertical bars represent ±S.E. of mean, n = 6.

1978; Theobald *et al.* 1978). To check for any involvement of these subcellular fractions in papaverine responses of the ileum, calcium uptake by isolated microsomes and mitochondria was investigated. The results of these experiments are summarized in Fig. 11. Papaverine was found to be without effect on calcium uptake by ileal smooth muscle mitochondria, although it caused a very slight stimulation of calcium uptake by the microsomal fraction *in vitro*.

DISCUSSION

The confusion surrounding the mechanism of papaverine-induced relaxation of smooth muscle stems from the fact that there has been no single comprehensive study of papaverine action on all stages of the excitation–contraction coupling mechanism in any one muscle.

Tashiro & Tomita (1970) concluded that papaverine decreased mechanical activity by suppressing spontaneous spike activity in the smooth muscle of guinea-pig taenia coli. They claimed that excess calcium (6 mM) could restore mechanical responses but failed to restore electrical activity, a clear inconsistency since it is the spikes which generate thythmic contractions. In the rat ileum studied here, papaverine does indeed suppress spikes and rhythmic contractions, but neither event can be restored by excess calcium in the presence of papaverine. Bauer *et al.* (1974) found that papaverine inhibited the nicotinic cholinoreceptors of the intramural parasympathetic ganglia of guinea-pig taenia coli and ileum. This observation is certainly consistent with the inhibition of spike activity seen in this preparation.

A number of biochemical investigations have linked papaverine-induced relaxation of smooth muscle with cyclic nucleotide metabolism (Kukovetz & Poch, 1970; Nishikori, Takenaka & Maeno, 1977; Fabiato & Fabiato, 1978; Honeyman *et al.* 1978). The simple picture portrayed by these studies is that papaverine-induced inhibition of phosphodiesterases causes a rise in cell cyclic AMP which reduces myoplasmic free calcium by a stimulation of intracellular calcium binding, resulting in relaxation. Such a simple picture is now not tenable. A rise in cell cyclic AMP is not a prerequisite for relaxation in a number of smooth muscles (Diamond, 1973; Diamond & Holmes, 1975; Clyman *et al.* 1976; Gamo *et al.* 1977; Sands, Mascali & Paietta, 1977).

If the primary action of papaverine in inducing relaxation is mediated via a rise in cyclic AMP, then papaverine should stimulate calcium binding by subcellular fractions such as the mitochondria and microsomes (Kukovetz & Poch, 1970). In the rat ileum the concentrations of papaverine that bring about immediate relaxation are without significant effect on mitochondrial or microsomal calcium uptake, as is also the case in vascular smooth muscle (Sands *et al.* 1977). In these smooth muscles, at least, any effects of papaverine on intracellular calcium binding are not significant factors in the relaxatory mechanism. These results do not support the view that papaverine's immediate effect on tension is mediated via cyclic nucleotide metabolism. Direct investigation of cyclic AMP effects on rat ileal smooth muscle also fail to support this simple model (Saad & Huddart, 1979). The incidental observation

that papaverine inhibits oxidative phosphorylation in guinea-pig ileum and duodenum (Santi *et al.* 1964) also provides no satisfactory explanation of the immediate inhibitory effects of papaverine on tension.

There is evidence in the literature that papaverine may directly influence contractility by altering calcium fluxes in smooth muscle. Tomiyama, Takayanagi & Takagi (1973) found that papaverine reduced calcium influx into guinea-pig taenia caecum and relaxed KCl-induced contractures, although they observed a surprisingly anomalous small increase in ^{45}Ca efflux. Their overall view was that papaverine action could be explicable in terms of a fall in cell free calcium without involving cyclic AMP.

The evidence from this present study on rat ileum is that the relaxatory actions of papaverine can all be related to its observed inhibition of calcium influx. The inhibition of calcium-dependent spike activity is consistent with this view. The observation that addition of calcium to ileal muscles in calcium-free salines failed to restore contractility if papaverine was present is further evidence that papaverine has inhibited calcium influx.

Studies on isotopic efflux from tissues have to be viewed with some caution since a number of not easily quantifiable complications make apparently simple results open to a number of different interpretations. Isotopic back-flux may occur and intracellular specific activity constantly falls during the course of the efflux experiment as ^{45}Ca is lost by replacement with ^{40}Ca. The view commonly held from a number of efflux studies is that efflux of calcium is dependent upon the level of calcium within the cell, hence stimulation or inhibition of the slow calcium efflux phase reflects a rise or a fall in cell free calcium (Sperelakis, 1962; Blaustein & Hodgkin, 1969; Deth & Van Breemen, 1977; Deth, 1978). The ^{45}Ca efflux response thus provides a test of the effect of papaverine on intracellular free calcium. The consistent inhibition of ^{45}Ca efflux by papaverine seen here represents a clear fall in myoplasmic free calcium. The experiments summarized in Fig. 6 show that papaverine inhibits calcium influx and would thus reduce entry of ^{40}Ca to the cell. While this may increase cytoplasmic ^{45}Ca specific activity, the fall in cell free calcium would greatly reduce $^{40}Ca/^{45}Ca$ exchangeability, thus a decline in efflux rate coefficient would be expected. An alternative explanation could be that perhaps papaverine stimulated intracellular calcium binding since this would be reflected as a fall in ^{45}Ca efflux rate coefficient. That this explanation is unlikely is indicated by the results summarized in Fig. 11, where papaverine was found to be without effect on mitochondrial calcium binding and to only very slightly stimulate microsomal binding. On balance, the reduction in calcium efflux seen here could not easily be related to stimulation of mitochondrial or microsomal calcium binding but it is perfectly consistent with the observed inhibition of calcium influx by papaverine. This view is strengthened by the observation that KCl-stimulated ^{45}Ca efflux is also inhibited by papaverine, this being related to an inhibition of the calcium influx which accompanies KCl-induced responses.

Undoubtedly papaverine and a number of other alkaloids do have actions on cell cyclic nucleotide metabolism and on calcium binding and release by sub-cellular calcium storage structures. However, the inconsistency of such actions by papaverine in various smooth muscles, coupled with the immediacy of papaverine action suggests that the most obvious effects of this alkaloid are more consistent with an inhibition of

calcium influx which is the trigger stage of the whole excitation-contraction coupling mechanism.

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