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ALTERATION OF SMOOTH MUSCLE CONTRACTILITY AFTER MUSCARINIC AGONIST-INDUCED K^+ LOSS

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1 After stimulation of the longitudinal smooth muscle of the guinea-pig ileum by an optimal dose (2×10^{-7} M) of a muscarinic agent, *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (CD), the muscles failed to regain their normal spontaneous activity for 20 to 30 min. During the recovery period, subsequent contractions induced by either CD or 60 mM KCl were altered, particularly when only short times (15 min or less) were allowed between exposures.

2 Altered responses to CD had depressed phasic but increased tonic tensions and were characteristic of responses induced by lower doses of CD. The altered responsiveness probably represented an early phase of muscle 'desensitization'.

3 In contrast to muscarinic stimulation, the smooth muscles gave identical responses after repeated stimulation by 60 mM KCl, even when only 2 min were allowed between exposures.

4 Whereas K^+ levels increased in muscles exposed to 60 mM KCl, they decreased during contractions to CD. The K^+ levels remained low until the muscles recoverd their normal responsiveness.

5 Increasing the extracellular K^+ concentration (5 to 13 mM) hastened the recovery of the muscle responsiveness after CD, whereas lowering external K^+ concentration to 1.35 mM or the addition of ouabain (5×10^{-7} M) delayed the recovery. The results suggested that the Na^+,K^+-pump is rate-limiting in the recovery of the normal ionic balance of the muscles after stimulation by muscarinic agonists.

Introduction

Muscarinic agents and depolarizing solutions of K^+ induce biphasic contractions of a longitudinal smooth muscle layer of the guinea-pig ileum. Both the initial phasic response and the slower, more sustained tonic tension have been shown to be particularly dependent on the presence of extracellular Ca^{2+} (Hurwitz & Joiner, 1969; Chang & Triggle, 1973a; Rosenberger, Ticku & Triggle, 1979). In addition to increasing Ca^{2+} influx, muscarinic agents and high concentrations of KCl increase K^+ efflux from ileal smooth muscle (Hurwitz, 1960; Hurwitz, Tinsley & Battle 1960; Weiss, Coalson & Hurwitz, 1961; Bergen & Spero, 1968). Doses of muscarinic agents that stimulate K^+ efflux are generally higher than doses required for contraction (Burgen & Spero, 1968).

Exposure of smooth muscle to supramaximal concentrations of muscarinic agonists 'desensitizes' them to subsequent stimulation by muscarinic and other agonists (Cantoni & Eastman, 1946; Rand, 1957; Bülbring & Burnstock, 1960; Chang & Triggle, 1973b). In the present study, exposure of the longitudinal muscle of the guinea-pig ileum to a maximal dose (2×10^{-7} M, less than a supramaximal dose) of a specific muscarinic agent, *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (CD), was shown to alter the responsiveness of the muscle to subsequent doses of agonists and to delay the return of its spontaneous activity. The phasic tension was depressed, but the tonic tension was greater or equal to the control responses and reached its maximum tension sooner. The altered responsiveness appeared to represent an early phase of muscle 'desensitization'. These effects were not observed after contraction of the muscles by 60 mM KCl. Many explanations for 'desensitization' of muscles to acetylcholine have been advanced (Cantoni & Eastman, 1946; Rand, 1957; Katz & Thesleff, 1957; Bülbring & Burnstock, 1960; Paton & Rothschild 1965; Nastuk & Parsons, 1970; Chang & Triggle 1973b; Magaribuchi, Ito & Kuriyama, 1973; Bolton, 1979). Although several mechanisms may be working additively to cause 'desensitization', a number of observations suggested that Ca^{2+} uptake and K^+ loss, in part, regulated the responsiveness of the ileal smooth muscle. The purpose of the present study was to investigate this possibility further.

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Methods

Contractile studies

The ileum from male guinea-pigs (250 to 350 g) was removed, cleaned and equilibrated in Tyrode solution (TS) at 37°C until spontaneous activity appeared. The longitudinal layer was removed into a water-jacketed trough containing TS at 37°C, essentially by the method of Rang (1964). Isometric contractions of muscle strips (1 to 2 cm) tied to stainless steel tissue hooks attached to Grass FT.03C transducers in 10 ml water-jacketed baths at 37°C were recorded on a 4 channel Grass 79D polygraph under 350 mg basal tension. The TS (pH 7.2) contained (mM): NaCl 136, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.36, $NaHCO_3$ 11.9 and glucose 5.55 and was gassed with 95% O_2 and 5% CO_2. The standard dose of CD (2×10^{-7} M) was added to the baths in a volume of 0.2 ml. Contractions to high concentrations of KCl were induced by adding 0.5 ml of 1.6 M KCl to bring the final concentration of K^+ to 60 mM. The hyperosmolarity of the 60 mM KCl solutions was not compensated by reduction of the NaCl concentration because tonic tension was not maintained in low Na^+ medium. A Tris-Tyrode solution (TT) was substituted for the TS in studies measuring ion contents by a modified lanthanum method (see below). The TT contained (mM): NaCl 136, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 5.55 and Tris 24, adjusted to pH 7.4 with HCl (at 37°C), and was oxygenated with 100% O_2. Contractions in TS or TT were similar.

Measurement of muscle K^+

Tissues were equlibrated under tension for at least 30 min before the control or experimental levels of K^+ were determined. The method of measurement of cellular K^+ contents by atomic absorption spectrophotometry was described in detail previously (James & Roufogalis, 1977; James-Kracke & Roufogalis, 1980). The muscles were quickly released from the transducers after equilibration or at specific stages of contraction or recovery and plunged into ice-cold $LaCl_3$ (10 mM) in 160 mM Tris-HCl solution (LTS), pH 7.4, for a total time of 30 min. Tissues were dried and weighed and K^+ was extracted and analyzed in a Varian Atomic Absorption Spectrophotometer (AA-5).

All inorganic salts used were reagent grade. CD was a gift from Dr D.J. Triggle, State University of New York at Buffalo, N.Y.

Results

Biphasic contractions

Contraction and recovery of the guinea-pig ileum longitudinal smooth muscle was studied in muscles exposed to CD or 60 mM KCl. CD is a very potent, stable and selective muscarinic receptor agonist (Chang & Triggle, 1973a). Biphasic contractions and $^{45}Ca^{2+}$ uptake induced by CD are blocked by atropine (Rosenberger *et al.*, 1979). Similar results were

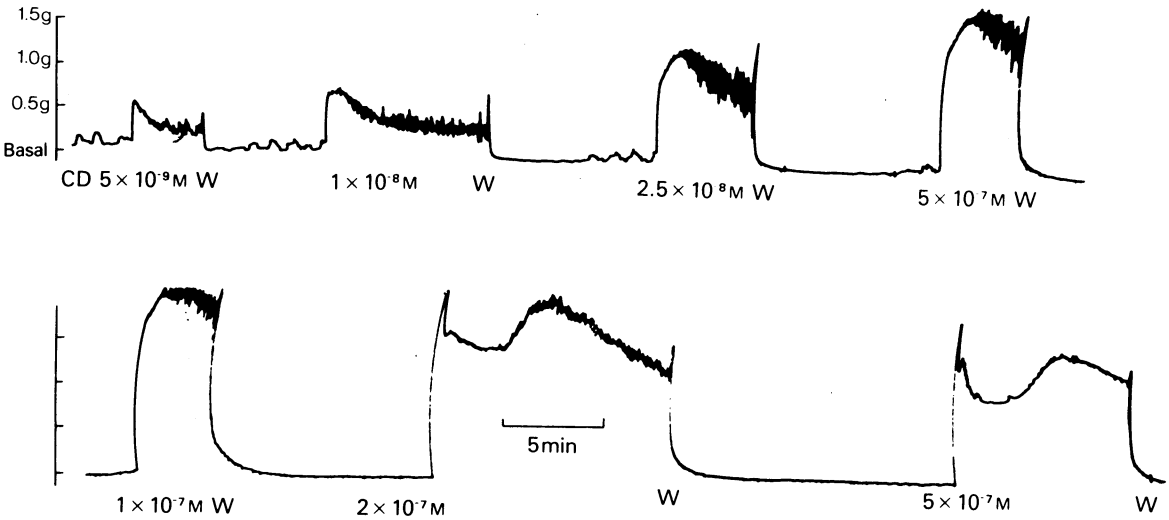

Figure 1 The response of the longitudinal smooth muscle of the guinea-pig ileum to increasing doses of CD. Maximal tonic and phasic contractions were induced by 5 to 10×10^{-8} M and 2×10^{-7} M, respectively. The chart speed was halved during measurement of the final contraction (5×10^{-7} M). CD was washed out (W) and subsequent responses were initiated after the muscles had regained spontaneous activity. Upper and lower traces are from one continuous experiment.

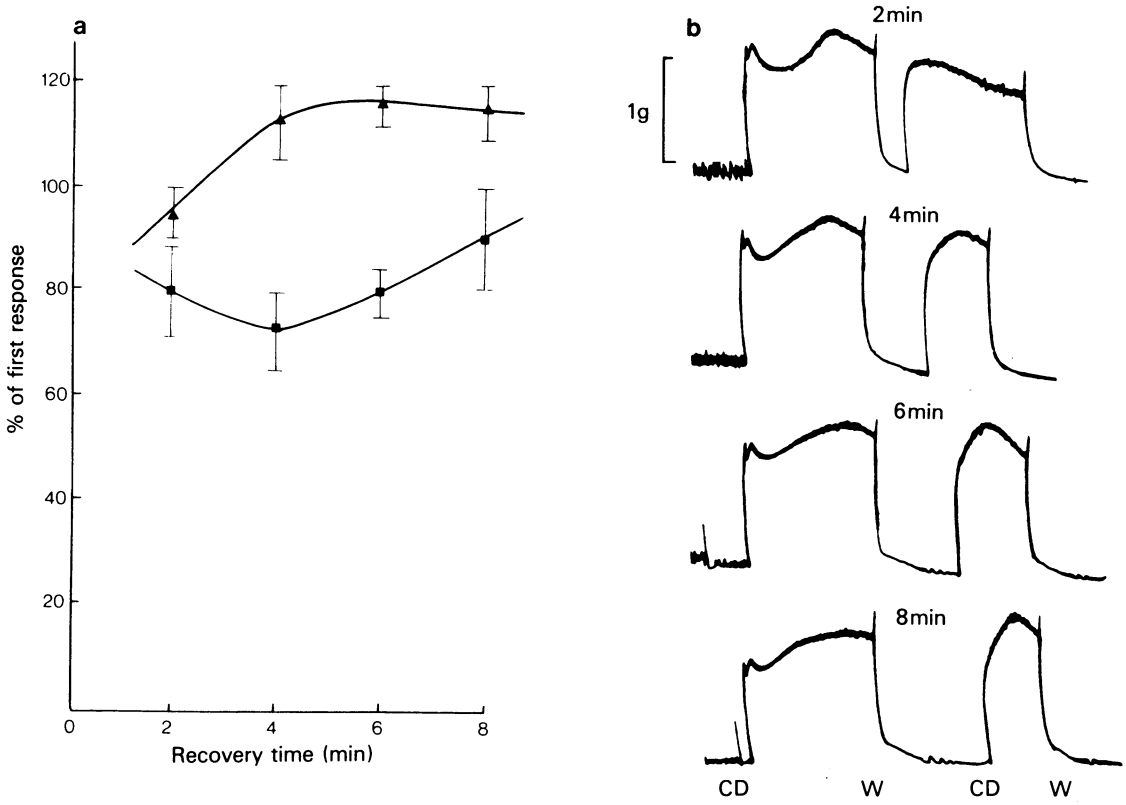

Figure 2 Changes in the biphasic contraction of the longitudinal muscle of the guinea-pig ileum induced by CD with increasing periods of time (2 to 8 min) between contractions. The responses of a single tissue are shown in (b). The response to 2×10^{-7} M CD ( ) was terminated by washout (W) with Tyrode solution. The maximum phasic tension of the second of the two responses was measured as the height of the response after 20 s, and compared to the maximum height of the phasic component of a control response to CD. The maximum tonic tension was determined from the maximum height of the delayed component in both the control and the second response. In (a) the phasic (■) and tonic (▲) tensions are expressed as a % of the respective control tensions, and plotted as a function of time between responses in the left side of the figure. Points show mean values and bars represent the s.e. mean ($n=7$).

obtained with carbachol and methacholine, and with responses measured either isotonically or isometrically. The contractile responses of the muscle induced by various concentrations of CD are shown in Figure 1. The shape of the biphasic response varied with the concentration of CD. At 2×10^{-7} M (the concentration just sufficient to produce a maximal contraction) there was a partial relaxation between the phasic and tonic response. The phasic tension peaked in 10 s while the tonic tension peaked after 5 to 8 min. At lower concentrations of CD (5×10^{-9} M to 10^{-7} M), the tonic tension appeared to rise more rapidly and immediately after the initial phasic response. A dose of 2×10^{-7} M CD was used routinely in these studies as it induced a maximal phasic tension easily distinguishable from the tonic tension. Depolarization of muscles by 60 mM KCl produced phasic and tonic contractions of similar magnitude to those induced by 2×10^{-7} M CD, but the phasic response was larger than the tonic response, and the interval between them much shorter (see Figure 3).

The effect of exposure to CD on subsequent responses to CD

The longitudinal smooth muscle of the ileum is spontaneously active. However, following exposure to 2×10^{-7} M CD for 10 min the muscles lacked spontaneous activity for 20 to 30 min following washout of the agonist (Figures 2 and 4). Subsequent responses induced by the same dose of CD during the 'quiescent' recovery phase were altered (Figure 2), and appeared more like those induced by lower concentrations of CD (10^{-7} M or less) (see Figure 1). As under these

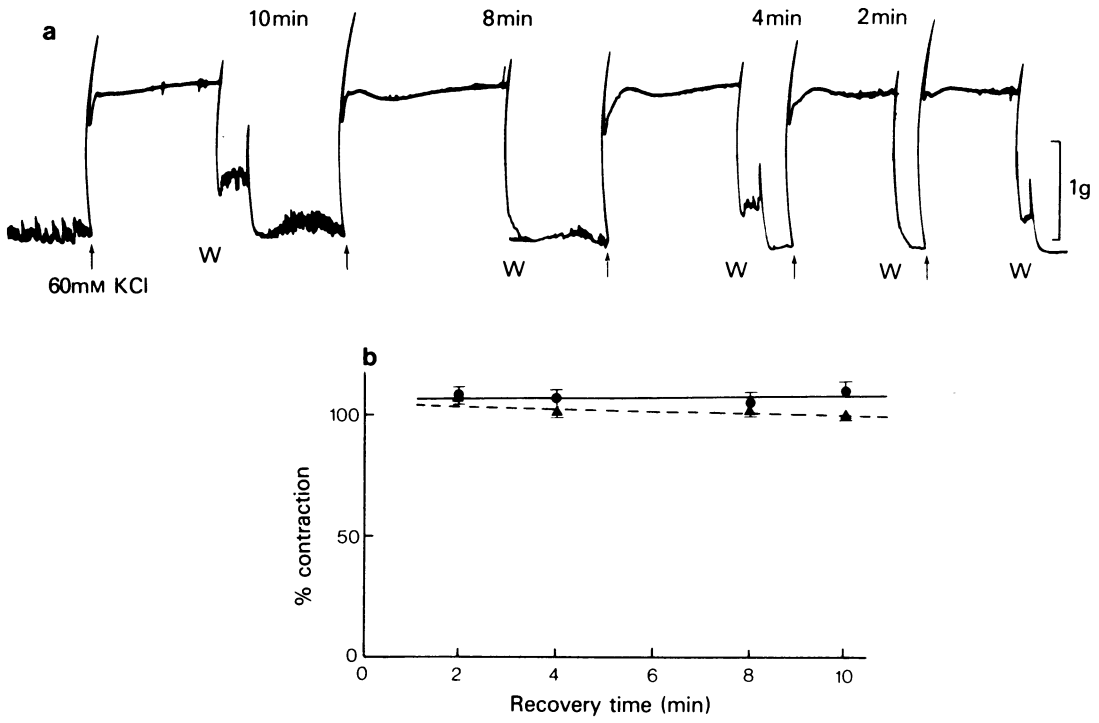

Figure 3 The effect of the recovery interval between consecutive responses of the longitudinal muscle of the guinea-pig ileum to 60 mM KCl on the biphasic shape of the responses. In (a) muscles were washed (W) with Tyrode solution between each stimulation by 60 mM KCl (↑); the responses of a single tissue are shown. In (b) the maximum heights of the rapid phasic and delayed tonic tensions of the second of the two responses to 60 mM KCl were measured and compared to the respective tensions of the control response. The phasic (●) and tonic (▲) tensions were expressed as a % of the respective control tensions and plotted as a function of the time betwen KCl responses (recovery time). Points are mean values and bars represent s.e. mean (n=4).

conditions development of the phasic tension was slightly delayed and its peak was indistinct, its magnitude was estimated 20 s after stimulation. The magnitude of the phasic tension decreased as the interval between consecutive CD contractions was shortened (Figure 2). In contrast, the magnitude of the tonic tensions were somewhat greater (approx. 115% of control). The maximum tonic tension was reached more quickly as the interval between CD contractions was shortened, and the usual pattern of partial relaxation between the phasic and tonic component disappeared. When only a 2 min interval was allowed between contractions, the second response to CD appeared as a continuation of the first response to CD, its progress interrupted but not appreciably affected by the washout.

The effect of exposure to high KCl on subsequent responses to 60 mM KCl

In contrast to CD, the biphasic responses to 60 mM KCl were not altered when the interval between KCl contractions was shortened (Figure 3). The magnitude of both the phasic and tonic components was independent of the recovery time between contractions.

Recovery of muscles after phasic and tonic responses

Experiments were performed to determine how much exposure to CD was necessary before the subsequent response was altered. Both the time for return of spontaneous activity and the shape of the second response were used as criteria for estimating the extent of alteration of the responsiveness of the muscle. CD was washed out either (1) after the maximum phasic response was attained, (2) at the point of least tension between phasic and tonic maxima, or (3) just after the peak of the tonic component (Figure 4). Spontaneous activity returned more slowly the longer the muscles were exposed to CD (Figure 4a). When CD was washed out after the maximum tonic response was reached, the second response generated 4 min later was depressed (Figure 4b). When CD was

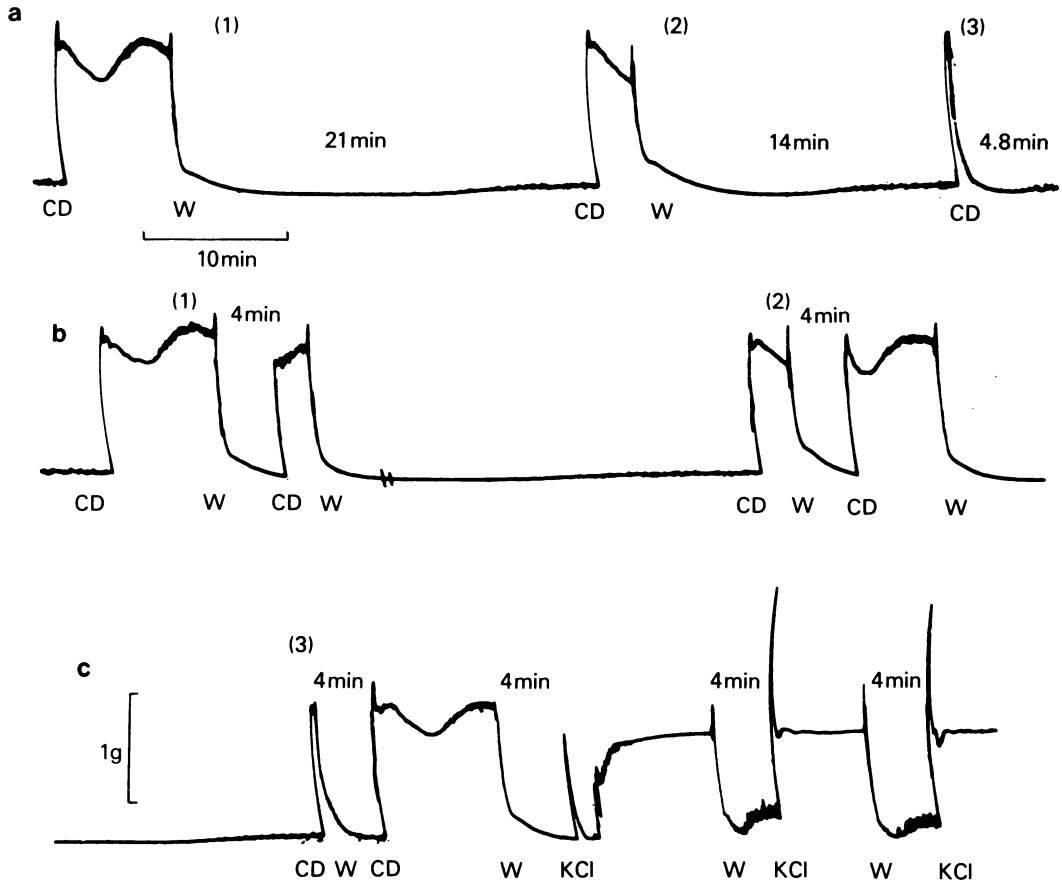

Figure 4 The effect of the duration of exposure to CD (2×10^{-7} M) on the time required for recovery of the longitudinal muscle of the guinea-pig ileum, monitored by the return of spontaneous activity (a) and the return of the normal biphasic contractile pattern (b and c). CD was washed out (W) (1) after the tonic maximum, (2) at the point of least tension between the phasic and tonic components and (3) immediately after the peak of the phasic component; 4 min were allowed between contractions. Tracings represent one continuous record of an experiment typical of the responses of 4 individual muscle strips.

washed out at the point of least tension between components, the second response to CD after 4 min was similar to the control response (Figure 4b), except that the tonic component peaked earlier than in the control response. When the CD contraction was terminated just after the peak of the phasic response was reached, the second response after 4 min matched its control response (Figure 4c).

The effect of exposure to CD on subsequent responses to 60 mM KCl

Following a 10 min exposure to CD, the muscles were allowed to recover for 4 min before being stimulated by 60 mM KCl. The phasic response to high KCl was reduced and the muscle tension relaxed completely to baseline for approx. 1 min before the tonic tension developed. Therefore, stimulation by CD affected the subsequent responses to both CD and 60 mM KCl. Further responses to 60 mM KCl applied 4 min later were completely normal (Figure 4c).

The effect of CD on subsequent responses to 60 mM KCl induced at various intervals was further investigated. After stimulation of the muscle by CD for 10 min, the tonic component induced by 60 mM KCl 2 min later was essentially unaffected, but the phasic component was reduced to 60% of its control (Figure 5). Although the muscles should have been depolarized by application of 60 mM KCl, they completely relaxed between the phasic and tonic components for periods as long as 1 min. Thus the phasic and tonic components can be clearly separated and probably are dissociable events.

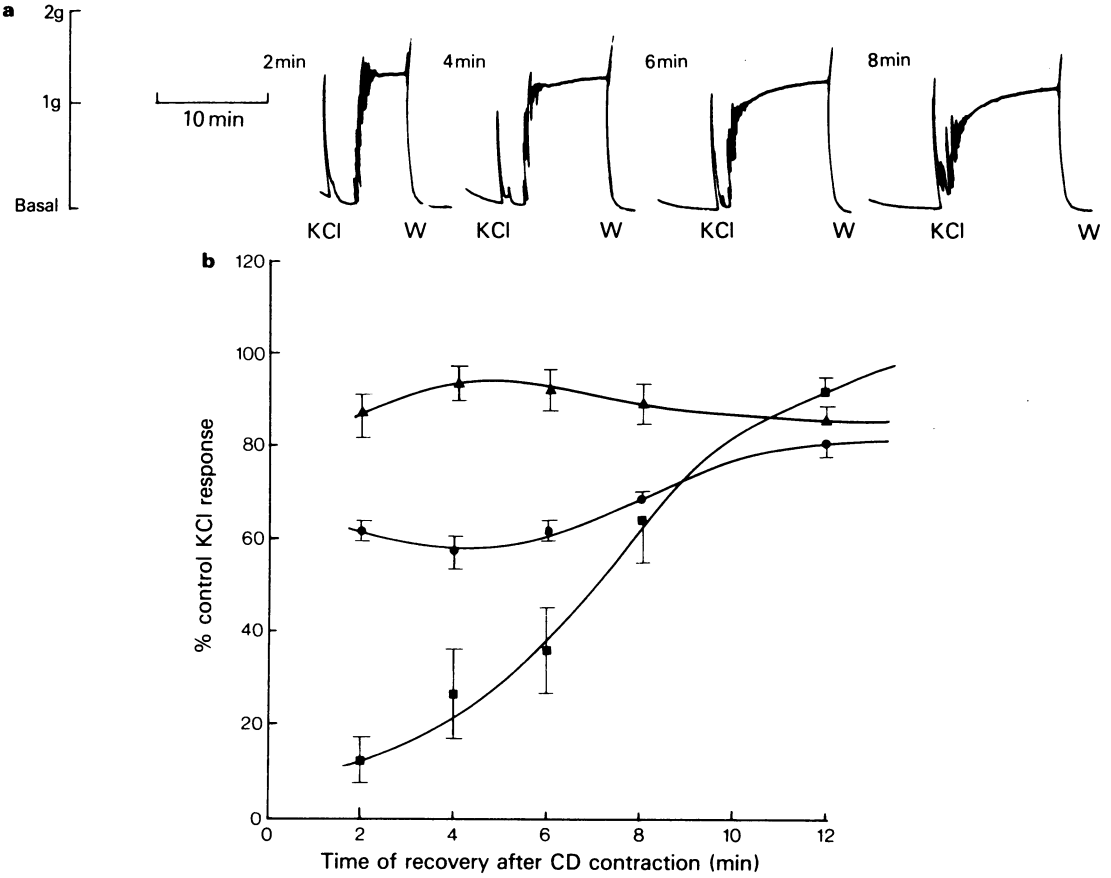

Figure 5 The effect of a previous 10 min exposure to CD (2×10^{-7} M) on subsequent responses to 60 mM KCl, 2 to 12 min after the initial response to CD was washed out. Tracings obtained in one tissue are shown in (a). Muscles were exposed to CD before a second KCl response was obtained at the times shown. Responses of each component (phasic and tonic) are expressed as a % of the corresponding component of the initial control responses to 60 mM KCl, determined at the beginning of each experiment (not shown); the control responses were similar to those shown in Figure 3. In (b) the change in magnitude of the phasic (●) and tonic (▲) components as a function of the time of recovery between washout of the initial CD response and the subsequent KCl contraction are shown. The magnitude of the least tension (relaxation) between phasic and tonic responses (■) was compared to the relaxation of a control KCl response as a function of recovery time between CD and KCl responses. Points are mean values and bars represent the s.e. mean ($n=4$).

The effect of varying the external KCl concentration on the recovery of spontaneous activity

In the following experiments the time taken for muscles to recover their spontaneous activity was used to measure the effect of CD on the subsequent muscle contractility (see Figure 4). Increasing the KCl concentration in the TS used to wash out the response to CD enabled the muscles to regain spontaneous activity more quickly (Figure 6). Contractions to 2×10^{-7} M CD (for 10 min) were terminated by washing out the CD in TS containing 0.5 to 5 times the normal (2.7 mM) concentration of KCl (i.e. containing 1.35 to 13.5 mM KCl). Spontaneous activity did not return for more than 60 min in 1.35 mM KCl. Spontaneous activity of the muscle returned in half the usual time (10 min) in 5.4 mM KCl.

K^+ appeared to be specific for hastening the recovery time. Raising the NaCl concentration by an equivalent amount (8 mM) or even by 70 mM had no effect on the recovery period. Lowering the Na^+ to 0.5 times its concentration hindered the recovery process, while the rate of recovery was normal at 0.75 times the normal NaCl concentration. Raising the extracellular Ca^{2+} concentration to 5 times its normal level and Mg to 3 times its normal concentration did not alter the recovery time noticeably.

The effect of ouabain on the recovery of the muscle

When the response to CD was washed out with TS containing 5×10^{-7} M ouabain (a concentration which

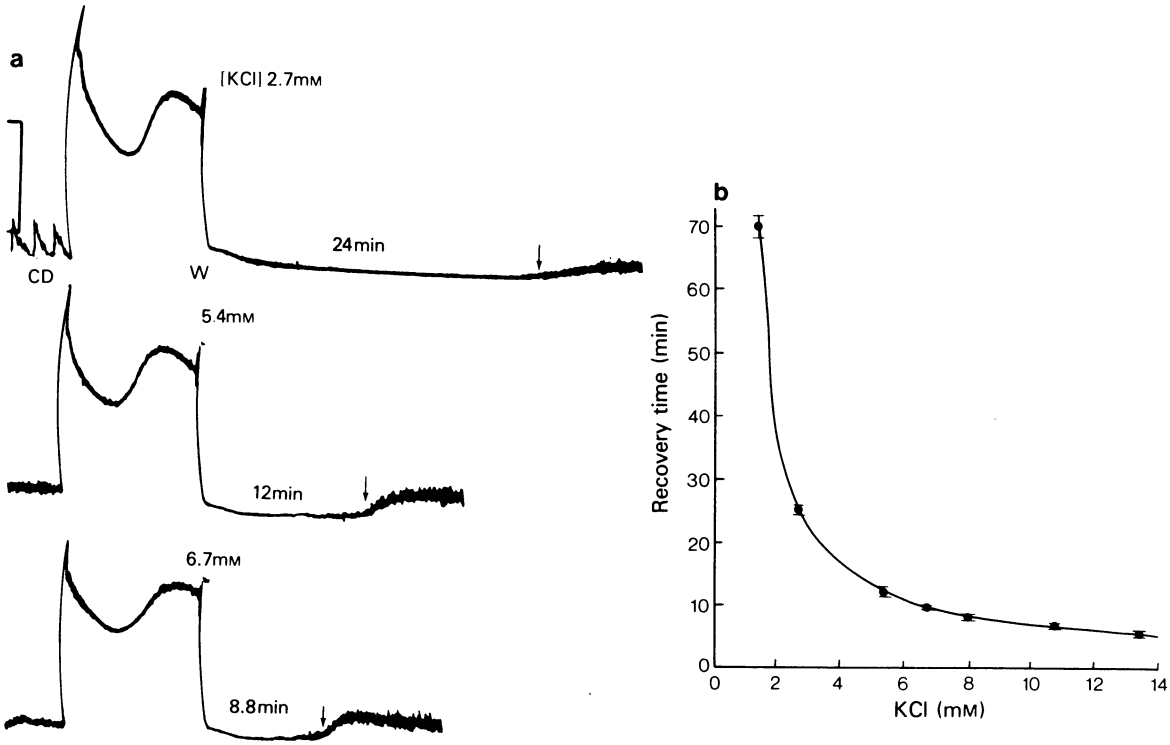

Figure 6 The effect of various KCl concentrations on the time taken for spontaneous activity to return ('recovery time') following contractions by CD (2×10^{-7} M) in the longitudinal muscle of the guinea-pig ileum (b). The response to CD was washed out (W) after 10 min with Tyrode solution containing various KCl concentrations ranging from 1.35 mM to 13.5 mM. An example showing the time taken for spontaneous activity to return after washout of the CD response using a concentration of 2.7 mM, 5.4 mM and 6.7 mM KCl (control, 2 and 2.5 times control concentration, respectively) is shown in (a). Recovery (↓) following the washout of a 10 min muscle response to 2×10^{-7} M CD was estimated as the point at which muscles had regained spontaneous activity after a quiescent period and a small rise in tension had begun. Points are mean values and the bars represent s.e. mean (n=4–8).

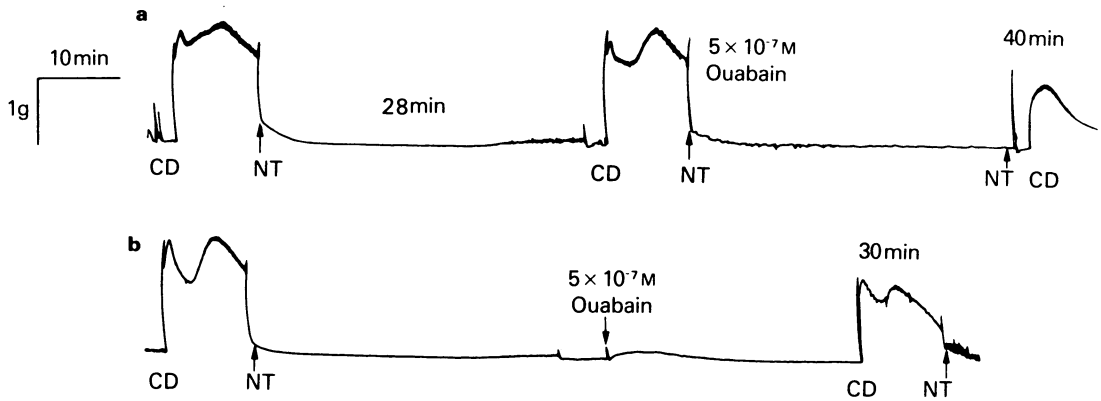

Figure 7 The effect of ouabain on the recovery of responsiveness of the longitudinal muscle of the guinea-pig ileum after a contraction induced by CD. (a) Ouabain (5×10^{-7} M) (↓) was added to the wash solution after a 10 min exposure to CD (2×10^{-7} M). The muscles were then washed for 1 min in normal Tyrode solution (NT) (↑) and a subsequent response to CD (2×10^{-7} M) determined. (b) Response, in a separate experiment, to CD (2×10^{-7} M) in a muscle preincubated with 5×10^{-7} M ouabain for 30 min.

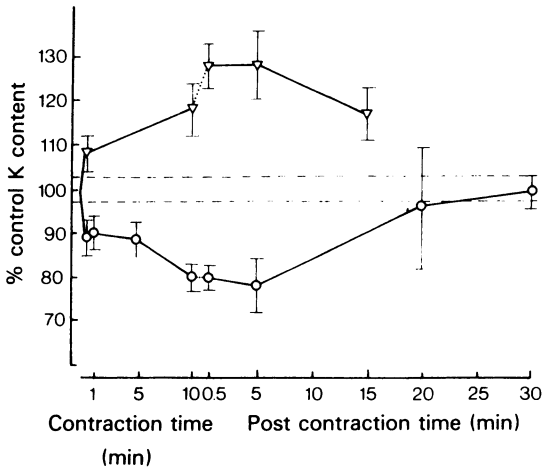

Figure 8 'Intracellular' K^+ levels during (0 to 10 min) and after washout (0.5 to 30 min) of contractions of the longitudinal muscle of the guinea-pig ileum induced by 2×10^{-7} M CD (○) and 60 mM KCl (▽) in Tris-Tyrode solution (TT). The control intracellular K^+ was 212.3 ± 6.1 nmol/mg dry wt. ($n=73$) and the total K^+ content was 250.8 ± 11 nmol/mg dry wt. ($n=33$). The area between the dotted lines indicates the normal concentrations (100% ± 3%). Results represent the mean ($n=$ 13–55, contraction phase and $n=15$–25, recovery phase, except at time 20 min when only 4 observations were made) and vertical bars show s.e. mean. The increases in K^+ (▽) after KCl contractions are significant ($P < 0.05$) at 10 min (contraction time) and 0.5 and 5 min (post-contraction time). The decreases in K^+ (○) after CD contractions are significant ($P < 0.05$) at 0.17, 1, 5 and 10 min (contraction time) and 0.5 and 5 min (post-contraction time).

is insufficient to induce contraction) both the phasic and tonic contractions induced by 2×10^{-7} M CD, applied 40 min later, were much reduced. Pretreatment of muscles with the same dose of ouabain somewhat reduced the magnitude of the response to 2×10^{-7} M CD induced 30 min later, but had little effect on the biphasic pattern (Figure 7b). Thus ouabain mainly affected the recovery of normal contractility of the muscle following a CD contraction. The delay of the recovery process by ouabain implied that ouabain further prevented recovery of the ion levels already disturbed by CD.

Measurement of intracellular K^+ levels during and after contraction

Changes of 'intracellular' K^+ content during contraction and recovery of the guinea-pig ileum longitudinal smooth muscle were estimated by the modified lanthanum method. K^+ levels increased in muscles exposed to 60 mM KCl but decreased and remained low during and after contractions to CD (Figure 8). The K^+ content slowly returned to control levels 20 to 30 min after washout of CD. In contrast to the K^+ changes, changes of Ca^{2+}, Na^+ and Mg^{2+} concentrations were qualitatively similar whether muscles were contracted by CD or 60 mM KCl (James-Kracke & Roufogalis, 1980) and did not explain the difference in responsiveness after exposure to CD but not to a high concentration of KCl.

Discussion

The altered responsiveness of the longitudinal smooth muscle of the guinea-pig ileum after stimulation by the muscarinic agent, CD, and the phenomenon of desensitization (Cantoni & Eastman, 1946) may represent the same process at different stages. The altered responsiveness occurred at a CD concentration just sufficient to produce a maximum contraction, whereas, previously, desensitization was studied at supramaximal doses of muscarinic agents (Cantoni & Eastman 1946). In the present study the concentration of CD used appeared to be at the threshold for producing muscle 'desensitization' and was studied to characterize the earliest events underlying the phenomenon.

Stimulation of the ileum by 2×10^{-7} M CD produced a net loss of K^+, probably by the muscarinic agonist-induced K^+-efflux mechanism (Born & Bülbring, 1956; Weiss *et al.*, 1961; Bannerjee & Lewis; 1964; Burgen & Spero, 1968). A number of observations suggest that the loss of K^+ from the muscle may account for the altered responsiveness to subsequent doses of CD or KCl, and the delay in the return of spontaneous activity. Whereas the phasic and tonic tensions were separated clearly by a partial relaxation phase after stimulation by 2×10^{-7} M CD, this separation was indistinct at lower doses of CD. The separation was also lost when the muscles were stimulated by CD soon after they had been contracted previously by $2-10^{-7}$ M CD. The second response was characteristic of responses obtained at lower doses of CD, where the phasic tension was lower and phasic and tonic tensions were not clearly separated. At lower doses of muscarinic agents, measurable K^+ efflux does not accompany muscle contraction (Burgen & Spero, 1968). After full recovery of the contraction to 2×10^{-7} M CD (about 20 to 30 min), when the K^+ content and spontaneous activity has been restored, the response to 2×10^{-7} M CD was again normal and showed the partial relaxation betweeen the phasic and tonic components.

The loss of responsiveness was greater the longer the muscle was in contact with CD, and tended to correspond to the extent of K^+ loss from the muscle. The recovery of the spontaneous activity of the muscle after stimulation by 2×10^{-7} M CD was faster when the KCl concentration of the washout medium was increased and was delayed when the KCl concentration was reduced. Raising the external KCl concentration was shown previously to protect

muscle against desensitization to acetylcholine (Cantoni & Eastman, 1946; Rand, 1957; Magaribuchi *et al.*, 1973). Halving the Na^+concentration of the washout medium, or addition of 5×10^{-7} M ouabain, delayed recovery of the muscle responsiveness. Recovery of the normal responsiveness of the muscle appears to coincide with the return of the cellular K^+ to normal levels. The rate of recovery appeared to be dependent on and to be rate-limited by, the operation of the Na^+,K^+-pump.

An enhanced K^+ efflux also occurs from muscles contracted by high KCl (Weiss *et al.*, 1961; Burgen & Spero, 1968). This K^+ efflux may also account for the relaxation which always occurred between the phasic and tonic responses to 60 mM KCl. However, under these conditions, the K^+ efflux was probably matched or overcompensated by an enhanced K^+ influx. This was confirmed by the atomic absorption measurements showing no net loss, but rather a net gain, of cell K^+ following a response to 60 mM KCl. As a consequence of the maintained high internal K^+ contents, the muscles did not appear to undergo any changes in responsiveness to further stimulation despite repeated exposures to 60 mM KCl.

The responses to 60 mM KCl were very unusual after the muscle responsiveness had been altered by previous exposure to 2×10^{-7} M CD for 10 min. This obervation ruled out a conformational change of the acetylcholine receptor (see Katz & Thesleff, 1957) as a means of explaining the altered responsiveness, because 60 mM KCl obviously does not act via muscarinic receptors. The phasic tension of the response to 60 mM KCl was reduced and the muscles usually relaxed to baseline before the tonic tension began. Under these conditions, the phasic and tonic components can be clearly separated, indicating that they probably are dissociable events. A number of explanations could be given for the prolonged relaxation. The 60 mM external K^+ may have stimulated the Na^+,K^+-pump to pump K^+ rapidly into the K^+-depleted muscles (Casteels, Droogman & Hendrickx, 1973), possibly causing a hyperpolarization due to an electrogenic action of the Na^+,K^+-pump. Alternatively, CD may have depleted an internal store of Ca^{2+} (Casteels & Raeymaekers, 1979), which may have been rapidly refilled on the application of depolarizing K^+, thus assisting relaxation (A.F. Brading, personal communication). Clearly, the operation of the Na^+,K^+-pump is not rate-limiting under these conditions.

The mechanism of the muscarinic agonist-induced K^+ efflux is not clear. It may result from stimulation of a population of low affinity muscarinic receptors (Burgen & Spero, 1968; Birdsall, Burgen & Hulme, 1978) or could possibly result from stimulation of a Ca^{2+}-dependent K^+-efflux mechanism (for review see Meech, 1978). Mirroneau, Savineau & Rahety (1977) have shown that a component of the delayed outward K^+ current in uterine smooth muscle is dependent on a Ca^{2+} influx and suggested that it was due to a transient increase of Ca^{2+} at the inner side of the membrane. Extracellular Ca^{2+} concentration influences both muscarinic agonist-induced K^+-efflux (Hurwitz *et al*, 1960; Durbin & Jenkinson, 1961; Weiss *et al.*, 1961) and muscle desensitization (Magaribuchi *et al.*, 1973; Chang & Triggle, 1973b) and carbachol-induced K^+-efflux in the rat parotid gland (Keryer & Rossignol, 1981). Intracellular Ca^{2+} levels increase in ileal muscles stimulated by CD or 60 mM KCl (James-Kracke & Roufogalis, 1980) (see also Triggle & Triggle, 1976; Rosenberger *et al.*, 1979). At maximal agonist concentrations, the uptake of Ca^{2+} exceeds that required to cause maximal contraction of smooth muscles (Marshall & Kroeger, 1973; Daniel & Janis, 1975; van Breemen, 1977), perhaps allowing the excess Ca^{2+} to bind to the inside of the sarcolemma and cause either an increase of the permeability of the membrane to K^+ (Tomita & Watanabe, 1973; Brading, 1978), resulting in a net K^+ loss, or a stabilization of the membrane to ion permeability (Nastuk & Parsons, 1970).

The loss of K^+ from muscle cells could explain the altered responsiveness of the muscle in a number of ways. Contraction of muscles by CD may deplete or redistribute a pool of Ca^{2+} essential for the phasic tension and its restoration may be in some way dependent on the recovery of normal levels of K^+. The recovery of cellular levels of Ca^{2+} following washout of CD seems to correspond to restoration of cellular K^+ (James-Kracke & Roufogalis, 1980). The loss of K^+ may have affected metabolic changes by inhibiting the enzymes of glycolysis (Bygrave, 1967) and oxidative phosphorylation (Blond & Whittam, 1965), thereby reducing energy stores during the period of increased demand for transport activity. Alternatively, and probably more likely, the low internal K^+ concentrations during recovery would be expected to reduce the membrane potential and cause hypoexcitability through accommodation. In support of this mechanism are the observations of Bolton (1973) and Bülbring & Burnstock (1960) that longitudinal ileal muscles, depolarized by short exposures to acetylcholine, repolarized very slowly and then hyperpolarized because of the stimulated activity of the Na^+ pump (blocked by ouabain). Bülbring & Burnstock (1960) found that sensitivity to acetylcholine was reduced during the repolarization and hyperpolarization. By contrast, hyperpolarization did not occur after depolarizing solutions of KCl were washed out (Burnstock & Straub, 1958); this may explain why the responsiveness was not altered after KCl contractions.

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References

BANNERJEE, A.K. & LEWIS, J.J. (1964). Effect of smooth muscle stimulants and their antagonists upon potassium ion uptake and release in strips of guinea-pig ileum. *J. Pharm. Pharmac.*, **16**, 134–136.

BIRDSALL, N.J.M., BURGEN, A.S.V. & HULME, E.C. (1978). The binding of agonists to brain muscarinic receptors. *Molec. Pharmac.*, **14**, 723–736.

BLOND, D.M. & WHITTAM, R. (1965). Effects of sodium and potassium ions on oxidative phosphorylation in relation to respiratory control by a cell-membrane adenosine triphosphatase. *Biochem. J.*, **97**, 523–531.

BOLTON, T.B. (1973). The role of electrogenic sodium pumping in the response of smooth muscle to acetylcholine. *J. Physiol.*, **228**, 713–731.

BOLTON, T.B. (1979). Mechanism of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

BORN, G.V.R. & BÜLBRING, E. (1956). The movement of potassium between smooth muscle and the surrounding medium. *J. Physiol.*, **131**, 690–703.

BRADING, A.F. (1978). Calcium-induced increase in membrane permeability in the guinea-pig taenia coli: evidence for involvement of a sodium-calcium exchange mechanism. *J. Physiol.*, **275**, 65–84.

BÜLBRING, E. & BURNSTOCK, G. (1960). Membrane potential changes associated with tachyphylaxis and potentiation of the response to stimulating drugs in smooth muscle. *Br. J. Pharmac. Chemother.*, **15**, 611–624.

BURGEN, A.S.V. & SPERO, L. (1968). The action of acetylcholine and other drugs on the efflux of potassium and rubidium from smooth muscle of the guinea-pig intestine. *Br. J. Pharmac.*, **36**, 99–115.

BURNSTOCK, G. & STRAUB, R.W. (1958). A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes. *J. Physiol.*, **140**, 156–167.

BYGRAVE, F.D. (1967). The ionic environment and metabolic control. *Nature Lond.*, **214**, 667–671.

CANTONI, G.L. & EASTMAN, G. (1946). On the response of the intestine to smooth muscle stimulants. *J. Pharmac. exp. Ther.*, **87**, 392–399.

CASTEELS, R., DROOGMAN, G. & HENDRICKX, H. (1973). Active ion transport and resting potential in smooth muscle cells. *Phil. Trans. R. Soc. Lond. B.*, **265**, 47–56.

CASTEELS, R. & RAEYMAEKERS, L. (1979). The action of acetylcholine and catecholamines on an intracellular calcium store in the smooth muscle cells of the guinea-pig taenia coli. *J. Physiol.*, **294**, 51–68.

CHANG, K.-J. & TRIGGLE, D.J. (1973a). Quantitative aspects of drug-receptor interactions I. Ca and cholinergic receptor activation in smooth muscle: a basic model for drug receptor interactions. *J. Theor. Biol.*, **40**, 125–154.

CHANG, K.-J. & TRIGGLE, D.J. (1973b). Quantitative aspects of drug-receptor interactions II. The role of Ca^{2+} in desensitization and spasmolytic activity. *J. Theor. Biol.*, **40**, 155–172.

DANIEL, E.E. & JANIS, R.A. (1975). Calcium regulation in the uterus. *Pharmac. Ther. B.*, **1**, 695–729.

DURBIN, R.P. & JENKINSON, P.H. (1961). The effect of carbachol on the permeability of depolarized smooth muscle to inorganic ions. *J. Physiol.*, **157**, 74–89.

HURWITZ, L. (1960). Potassium transport in isolated guinea pig ileum. *Am. J. Physiol.*, **198**, 94–98.

HURWITZ, L. & JOINER, P.D. (1969). Excitation-contraction coupling in smooth muscle. *Fedn Proc.*, **28**, 1629–1633.

HURWITZ, L., TINSLEY, B. & BATTLE, F. (1960). Dissociation of contraction and potassium efflux in smooth muscle. *Am. J. Physiol.*, **199**, 107–111.

JAMES, M.R. & ROUFOGALIS, B.D. (1977). The effect of ouabain on the guinea pig ileum longitudinal smooth muscle. 2. Intracellular levels of Ca, Na, K and Mg during the ouabain response and the dependence of the response on extracellular Ca. *Can. J. Physiol. Pharmac.*, **55**, 1197–1203.

JAMES-KRACKE, M.R. & ROUFOGALIS, B.D. (1980). Estimation of the levels of calcium and other electrolytes during various phases of contraction of the longitudinal smooth muscle of the guinea pig ileum. *Cell Calcium*, **1**, 37–48.

KATZ, B. & THESLEFF, S. (1957). A study of the 'desensitization' produced by acetylcholine at the motor endplate. *J. Physiol.*, **138**, 63–80.

KERYER, G. & ROSSIGNOL, B. (1978). Lanthanum as a tool to study the role of phosphatidylinositol in the calcium transport in rat parotid glands upon cholinergic stimulation. *Eur. J. Biochem.*, **85**, 77–83.

MAGARIBUCHI, T., ITO, Y. & KURIYAMA, H. (1973). Desensitization of smooth muscle cells in the guinea pig taenia coli to prolonged application of carbachol. *Jap. J. Physiol.*, **23**, 447–464.

MARSHALL, J.M. & KROEGER, E.A. (1973). Adrenergic influences on uterine smooth muscle. *Phil. Trans. R. Soc. B.*, **265**, 135–148.

MEECH, R.W. (1978). Calcium-dependent potassium activation in nervous tissues. *A. Rev. Biophys. Bioeng.*, **7**, 1–18.

MIRONNEAU, J., SAVINEAU, J.P. & RAHETY, A. (1977). Evidence for a component of the outward current mediated by calcium influx in uterine smooth muscle. In *Excitation-Contraction Coupling in Smooth Muscle.* ed. Casteels, R., Godfraind, T. & Rüegg J.C. pp. 117–122. Elsevier/North-Holland: Amsterdam, New York.

NASTUK, W.L. & PARSONS, R.L. (1970). Factors in the inactivation of post-junctional membrane receptors of frog skeletal muscle. *J. gen. Physiol.*, **56**, 218–249.

PATON, W.D.M. & ROTHSCHILD, A.M. (1965). The changes in response and in ionic content of smooth muscle produced by acetylcholine action and by calcium deficiency. *Br. J. Pharmac. Chemother.*, **24**, 437–448.

RAND, M.J. (1957). The effect of potassium and acetylcholine on the response of the guinea pig jejunum to histamine. *Austr. J. exp. Biol. Med. Sci.*, **35**, 79–82.

RANG, H.P. (1964). Stimulant actions of volatile anesthetics on smooth muscle. *Br. J. Pharmac. Chemother.*, **22**, 356–365.

ROSENBERGER, L.B., TICKU, M.K. & TRIGGLE, D.J. (1979). The effects of Ca^{2+} antagonists on mechanical responses and Ca^{2+} movements in guinea pig ileal longitudinal smooth muscle. *Can. J. Physiol. Pharmac.*, **57**, 333–347.

TOMITA, T.& WATANABE, H. (1973). Factors controlling myogenic activity in smooth muscle. *Phil. Trans. R. Soc. B*, **265**, 73–85.

TRIGGLE, C.R. & TRIGGLE, D.J. (1976). Analysis of the action of cations of the lanthanide series on the mechanical response of guinea pig ileal longitudinal muscle. *J. Physiol.*, **254**, 39–54.

VAN BREEMEN, C. (1977). Calcium requirement for activation of intact aortic smooth muscle. *J. Physiol.*, **272**, 317–329.

WEISS, G.B., COALSON, R.E. & HURWITZ, L. (1961). K transport and mechanical responses of isolated longitudinal smooth muscle from guinea pig ileum. *Am J. Physiol.*, **200**, 789–793.

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