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Different effects of depolarization and muscarinic stimulation on the Ca^{2+}/force relationship during the contraction-relaxation cycle in the guinea pig ileum

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Abstract. The effects of K^+ depolarization and of the muscarinic agonist carbachol on $[Ca^{2+}]_i$ and force were investigated in smooth muscle sheets of the longitudinal layer of the ileum loaded with Fura-2. K^+-rich solutions increased $[Ca^{2+}]_i$ and force to an initial peak value, which was determined by the concentration of $[K^+]_o$. Thereafter, $[Ca^{2+}]_i$ and force declined to a lower maintained level. The Ca^{2+}/force relationship observed during this contraction-relaxation cycle is represented by a clockwise hysteresis loop. At 140 mM $[K^+]_o$, this loop consisted of three components while at lower $[K^+]_o$ a two-component loop was observed. The stimulation with 0.1 mM carbachol resulted in a transient increase of $[Ca^{2+}]_i$ and force followed by a continuous decline of these parameters despite the presence of the drug. Its EC_{50} of relaxation was around 270 nM $[Ca^{2+}]_i$. The Ca^{2+}/force relationship proceeded along a counterclockwise hysteresis loop during the contraction-relaxation cycle. The extent of this loop decreased but remained unaltered in its direction during repeated stimulation with carbachol. These results suggest that (a) both agonists increase force and $[Ca^{2+}]_i$ during stimulation; (b) during depolarization with K^+, desensitization to Ca^{2+} occurs resulting in a clockwise hysteresis loop; (c) during carbachol stimulation, a counterclockwise hysteresis is observed. This could be due to an increased sensitivity to Ca^{2+} mainly in tonic smooth muscle. These observations might be explained by a modulation of the Ca^{2+} sensitivity by sensitizing and desensitizing mechanisms. These modulations during different stimuli could be due to different myosin light-chain kinase / myosin light-chain phosphatase ratios.

Key words: $[Ca^{2+}]_i$ – Force development – Visceral smooth muscle – Fura-2 – Carbachol – K^+ depolarization

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Introduction

$[Ca^{2+}]_i$ is a key regulator of smooth muscle contraction. The activation of smooth muscle requires an increase of $[Ca^{2+}]_i$, which then induces a phosphorylation of the myosin light chain by the complex of Ca^{2+}-calmodulin and myosin light-chain kinase (Hartshorne 1987). This allows an activation of the myosin ATPase by actin and the contractile activity. Many aspects of the exact relationship between Ca^{2+} and tension still remain unclear. The knowledge of the type of the relationship between $[Ca^{2+}]_i$ and force is, however, central in the interpretation of contraction and relaxation. It has up till now been tacitly assumed that this relationship was unique for any given set of experimental conditions. During the last years it has, however, been suggested that counterclockwise hysteresis existed in barnacle (Ridgway et al. 1983) and cardiac (Hibberd and Jewell 1982; Harrison et al. 1988) muscle and in skinned smooth muscle of the hog carotid arteries (Chatterjee and Murphy 1983). This counterclockwise hysteresis could be due to a higher sensitivity of the regulatory/contractile proteins during a period of decreasing $[Ca^{2+}]_i$ than during a phase of increasing $[Ca^{2+}]_i$.

Studies with Ca^{2+} indicators have shown that the relation between Ca^{2+} and force (Morgan and Morgan 1984; Bradley and Morgan 1987; Himpens and Casteels 1987; Rembold and Murphy 1988) differs between phasic and tonic smooth muscle cells (Himpens et al. 1988, 1989). Himpens et al. (1988) observed a very low level of phosphorylation in the longitudinal layer of the guinea-pig ileum and Somlyo et al. (1989) proposed an important role for the phosphatases in the regulation of the level of the phosphorylation of phasic smooth muscle. In this study the Ca^{2+}/force relationship will be investigated in the ileum by simultaneously measuring $[Ca^{2+}]_i$ and force (Himpens and Somlyo 1988). The effect of agonist stimulation will be compared to the stimulation induced by K^+-rich solutions and their influence on the Ca^{2+}/force relationship during contraction and relaxation will be

investigated. It will be demonstrated that besides a counterclockwise hysteresis, a clockwise hysteresis also appears indicating that under certain conditions, at later stages of the contraction, submaximal $[Ca^{2+}]_i$ elicits a lower tension than during the initial stages.

It is proposed that the Ca^{2+}/force relationship during the different stimuli can be modulated by the interference of sensitizing and desensitizing mechanismus resulting in different myosin light-chain kinase / myosin light-chain phosphatase ratios.

Material and methods

Isolated longitudinal muscle strip from the terminal portion of the guinea-pig ileum were dissected and loaded with 2 μM Fura-2AM at room temperature as described by Himpens and Somlyo (1988). The description of the apparatus used for the simultaneous measurements of fluorescence and force and the method of mounting the strip in the apparatus, have been published (Himpens and Somlyo 1988). The alternating excitation light of 340 nm and 380 nm was obtained by passing the light from a 100 W mercury arc lamp through interference filters mounted in an air-driven wheel. The UV light of the two wavelengths was passed through one end of a bifurcated light pipe to the muscle sheet, which was mounted in the tissue chamber and attached by one end to a force transducer. The fluorescence emitted by the specimen was guided to the photomultiplier through the other end of the light pipe and an interference filter of 510 nm. The signals due to excitation at 340 nm and 380 nm were separated, digitized and stored in a computer for further data analysis.

Cytosolic Ca^{2+} concentrations were calculated as described by Himpens et al. (1988, 1989). The maximum (R_{max}) and minimum (R_{min}) ratios were determined using an internal calibration procedure performed at the end of the experiment. The minimum fluorescence was obtained by superfusing the muscle with a 140 mM K^+, Ca^{2+}-free solution containing 2 mM EGTA at pH 8.6 to optimize the ionomycin effect. Five minutes after superfusion with this solution, 50 μM ionomycin was added. After determining the R_{min}, the tissue was superfused with an excess of calcium (10 mM Ca^{2+} solution at pH 8.6), which gave the maximal signal ratio, R_{max}. The autofluorescence was determined after each experiment at the two excitation wavelengths, in order to subtracts the values from the total fluorescence and obtain the net Ca^{2+}-sensitive Fura-2 fluorescence. This was done by superfusing the strip with a 20 mM Mn^{2+} Krebs solution after the R_{min} and R_{max} values had been determined. Owing to some uncertainties about the value of the K_d (Somlyo and Himpens 1989) the absolute ratio is also shown in the original recordings in addition to the computed $[Ca^{2+}]_i$. The calculated figures were obtained by averaging the records for 2.5 s every 20 s unless otherwise stated.

$[Ca^{2+}]_i$ values are routinely expressed in nM, while force is expressed in mN on the original traces and as a percentage in the text and figures. In each experiment 0% force was arbitrarily defined as the force of the resting muscle in normal Krebs solution. The initial peak of the force response occurring on exposure to 140 mM K^+ solution was used as a reference (100%) for the contractile response of a specific muscle throughout each individual experiment. All values are means ± SEM, and n is the number of observations. Comparisons were made using Student's t-test. The standard physiological solution ('normal Krebs') was a HEPES-buffered modified Krebs solution at pH 7.3 containing (mM): Na^+ 135.5, K^+ 5.9, Ca^{2+} 1.2, Mg^{2+} 1.2, Cl^- 143.8, HEPES 11.6 and glucose 11.6. Solutions with increased K^+ ('depolarizing or high K^+ solutions') were obtained by replacing Na^+ by an equivalent amount of K^+. In 'Ca^{2+}-free solutions', Ca^{2+} was omitted and 2 mM EGTA was added. The experiments were performed at 25°C. Fura-2 AM was obtained from Molecular Probes (Junction City, Ore), ionomycin from Calbiochem (La Jolla, Calif.) and Pluronic F127 from BASF Wyandotte (Parsippany, NJ). Carbachol and atropine were from Sigma (St. Louis, Mo.).

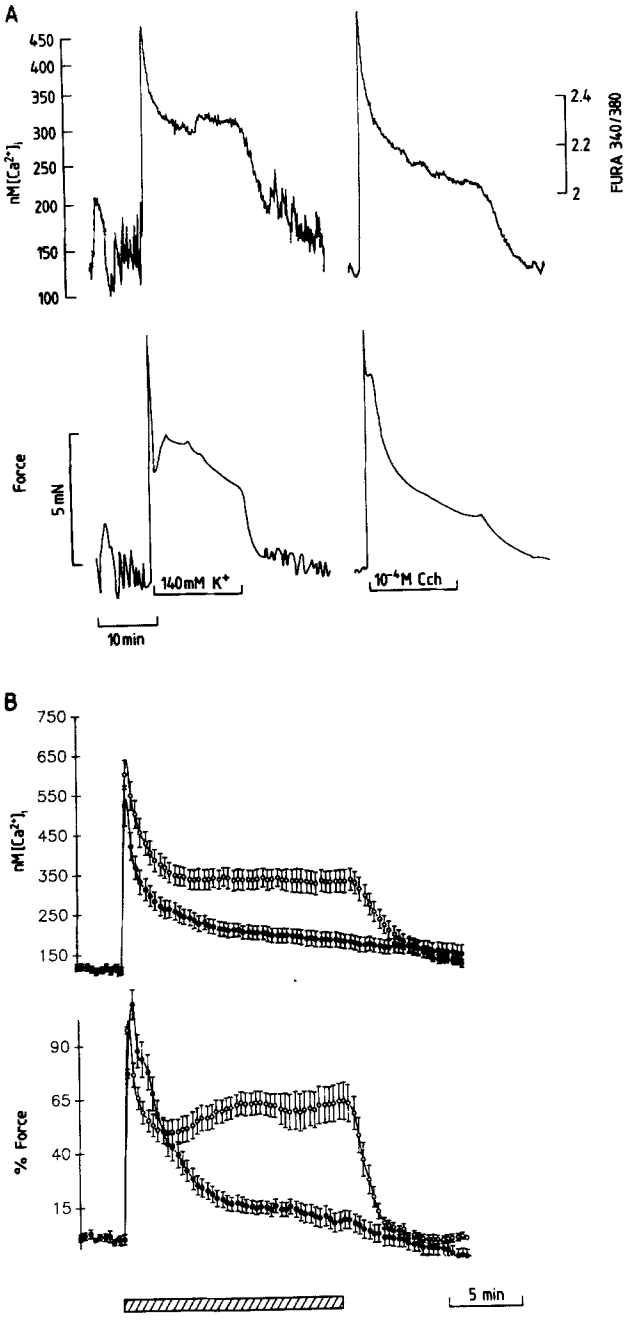

Fig. 1 A, B. Effect of K^+ and carbachol on $[Ca^{2+}]_i$ and force development in the same ileal smooth muscle strip. **A** The changes in the same tissue of $[Ca^{2+}]_i$ (*upper part*) and of force (*lower part*) during a stimulation for 15 min with 140 mM K^+ (*left*) and 0.1 mM carbachol. **B** The mean ± SEM of 11 measurements of the change of $[Ca^{2+}]_i$ (*upper part*) and of the force (*lower part*) as a function of time during stimulation with a depolarizing solution containing 140 mM K^+ and 1.5 mM Ca^{2+} (○) or with 0.1 mM carbachol (●) ($n = 11$). The force is normalized to the peak tension obtained during stimulation with 140 mM K^+

Results

The effect of a stimulation of the longitudinal layer of the guinea-pig ileum for 15 min with a K^+-rich solution

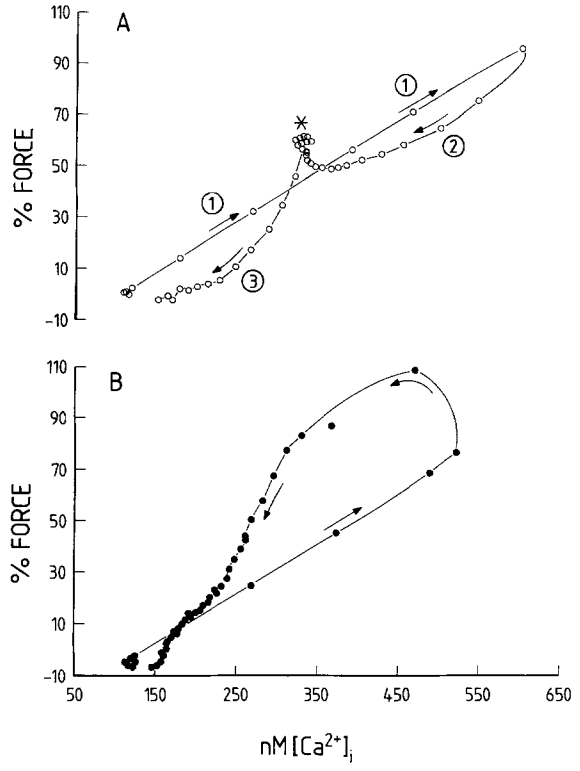

Fig. 2 A, B. The relationship of Ca^{2+} versus force of the ileum during the contraction-relaxation cycle induced by 140 mM K^+ (**A**, ○) and by 0.1 mM carbachol (**B**, ●). The *arrows* in **A** and **B** indicate the sequence of the contraction and the relaxation during these stimuli. During the initial rise in **A** and **B** the points represent the average of the values obtained in the individual experiments and sampled every 5 s. Later on the interval between the sampling was 20 s. The *numbers* in **A** indicate the three components of the contraction-relaxation cycle (*1*) initial fast rise; *2*) decline to steady-state level during maintained stimulation; *3*) relaxation). The *asterisk* in **A** (*) indicates the value of Ca^{2+}/force during maintained stimulation with the K^+-rich solution

containing 1.5 mM Ca^{2+} and 140 mM K^+ or with a solution containing 0.1 mM carbachol on $[Ca^{2+}]_i$ and on the force development is represented in Fig. 1 ($n = 11$). As already pointed out by Himpens and Somlyo (1988), $[Ca^{2+}]_i$ and force increased within 30 s to an initial peak. The maximum $[Ca^{2+}]_i$ and force values obtained at this stage were 583 ± 37 nM and $97 \pm 3\%$ for the K^+-induced stimulation and 523 ± 51 nM and $109 \pm 7\%$ for the carbachol-induced stimulation. Thereupon $[Ca^{2+}]_i$ and force declined during exposure to the depolarizing solution to a lower but steady level and amounted, after 15 min stimulation, to 330 nM $[Ca^{2+}]_i$ and to 60% of the peak tension respectively. In contrast the $[Ca^{2+}]_i$ and the force declined steadily after the initial peak during the continuous superfusion of the muscle with Krebs solution containing carbachol. The maximum force/$[Ca^{2+}]_i$ ratios (% force / nM Ca^{2+}) obtained by a K^+-rich solution and by carbachol amount to 0.16 and 0.21 respectively.

Figure 2 represents the Ca^{2+}/force relationship that has been determined during these two conditions of prolonged stimulation, and the individual points represent the average values of 11 experiments sampled every 5 s during the upstroke and every 20 s during the ensuing contraction and the relaxation. Figure 2A indicates the pattern followed during stimulation with the depolarizing solution. In all experiments performed ($n = 11$) this Ca^{2+}/force relationship follows a loop composed of three components. The initial component (1) represents the fast rise of $[Ca^{2+}]_i$ and force observed immediately upon stimulation with the K^+-rich solution, as shown in Fig. 1. Thereupon the Ca^{2+}/force relationship declines clockwise (2) until a stable steady-state value is reached corresponding to the plateau level represented in Fig. 1, which is maintained as long as the stimulus is applied. A return to the normal Krebs solution containing 5.9 mM $[K^+]_o$ results in a new clockwise decline of the Ca^{2+}/force relationship (3) towards the resting values.

The Ca^{2+}/force relationship during stimulation with carbachol (Fig. 2B) follows a counterclockwise hysteresis loop consisting of a rising and a declining component. No left shift in the initial increase can be observed, as has been described for the pulmonary artery (Himpens et al. unpublished results). The initial increase of the Ca^{2+}/force relationship representing the rise in $[Ca^{2+}]_i$ and force, as shown in Fig. 1, proceeds similarly to the initial changes in Ca^{2+} and force induced by the K^+-rich solution. Thereupon the force and $[Ca^{2+}]_i$ decline as a function of time and the Ca^{2+}/force relationship proceeds in a counterclockwise direction. During this decline the Ca^{2+}/force relationship decreases rather linearly. After 4 min stimulation with carbachol, the force reached a value of approximately 50% of the peak tension and $[Ca^{2+}]_i$ of 275 nm $[Ca^{2+}]_i$.

Figure 3 represents the pattern of the $[Ca^{2+}]_i$ and of the force development in smooth muscle sheets of the ileum during a stimulation for 15 min with different depolarizing solutions added with 0.01 mM atropine and 1.5 mM Ca^{2+} and containing 140, 60, 40 or 20 mM $[K^+]_o$ respectively ($n = 9$). The different stimuli with these depolarizing solutions were applied to the smooth muscle strips in a random way (Fig. 3A). All identical stimulations of the different strips were then averaged for each specific K^+ concentration and represented as such in Fig. 3B.

The amplitude of $[Ca^{2+}]_i$ during the initial phasic force peak was a function of the applied concentration of $[K^+]_o$. Higher K^+ concentrations elicited a higher value of both the initial $[Ca^{2+}]_i$ transient and of the force peak. $[Ca^{2+}]_i$ and force were, for example, 537 ± 47 nM and $94 \pm 4\%$ for 140 mM K^+ and only 238 ± 30 nM and $37 \pm 2\%$ for 20 mM K^+ respectively. After 15 min superfusion with the different $[K^+]_o$ a maintained level of $[Ca^{2+}]_i$ and force was reached. The $[Ca^{2+}]_i$ at this stage was not significantly different for 140, 60 and 40 mM $[K^+]_o$ but it was significantly lower at 20 mM $[K^+]_o$.

The Ca^{2+}/force relationship, measured under the same conditions as in the protocol used for Fig. 2, is represented in Fig. 4. The individual points (average of 9 experiments) on this figure represent again the Ca^{2+}/force relationship sampled initially every 5 s and thereafter every 20 s during the contraction and relaxation. At all K^+ concentrations a clockwise hysteresis loop was observed, in which the relaxation was shifted to the right

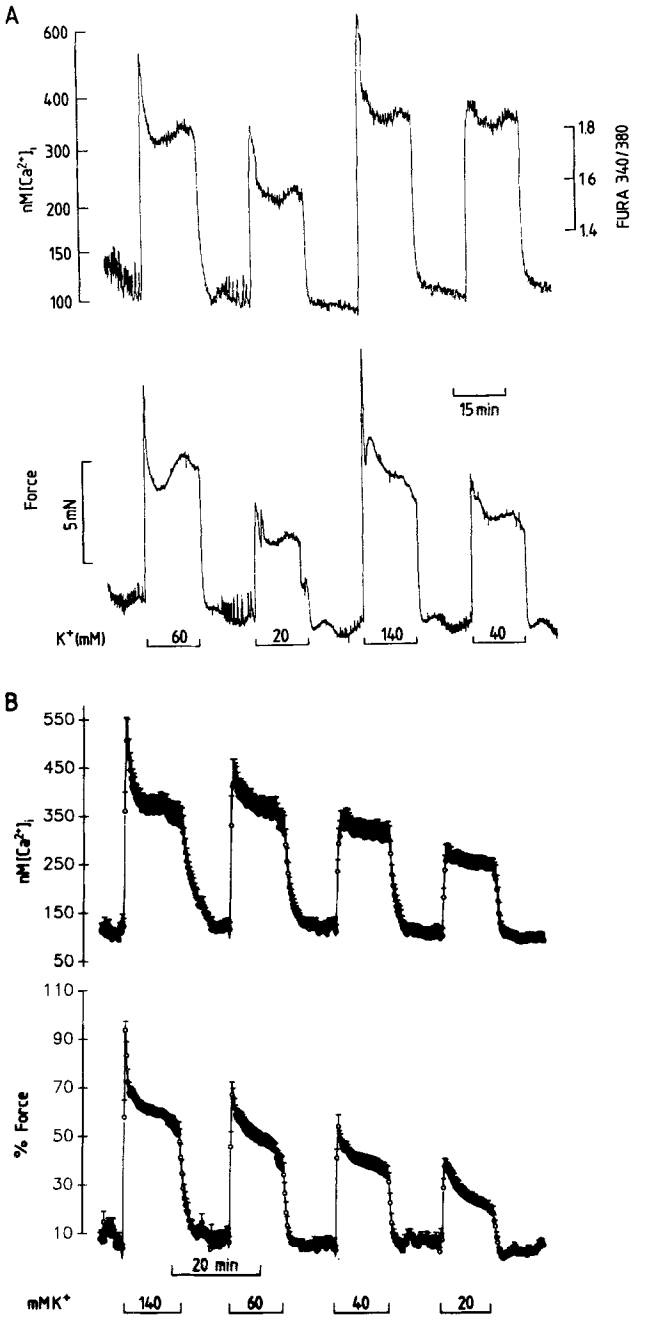

Fig. 3A, B. Effect of solutions with different $[K^+]_o$ on the ileum. **A** The change of $[Ca^{2+}]_i$ in nM and of force (in mN) of the ileum during a stimulation for 15 min with the solutions containing different $[K^+]_o$ as indicated. **B** The effect of the solutions with different $[K^+]_o$ (140; 60; 40 and 20 mM K^+) containing 1.5 mM Ca^{2+} on the mean value of $[Ca^{2+}]_i$ and of force is represented for the nine smooth muscle strips. The *upper trace* represents the $[Ca^{2+}]_i$, the *lower trace* the force. The force is normalized to the peak tension obtained during stimulation with 140 nM K^+

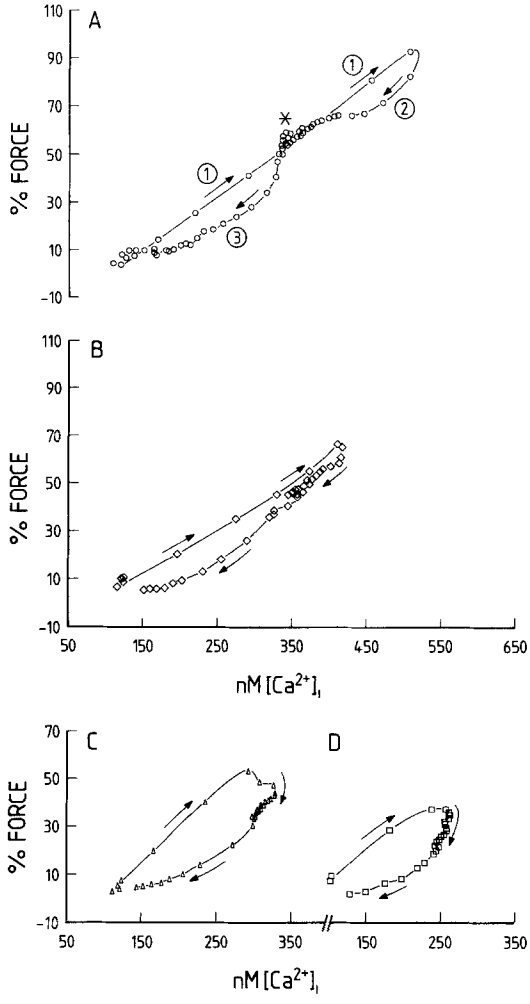

Fig. 4A – D. Ca^{2+} versus force relationship is ileal smooth muscle during stimulation with solutions containing different $[K^+]_o$. **A** The stimulation with 140 mM $[K^+]_o$ (○); **B** 60 mM (◇); **C** 40 mM (△) and **D** 20 mM (□). The *arrows* indicate the contraction and relaxation cycle during the stimulation with the different K^+ concentrations. During the initial rise, samples were taken every 5 s while thereafter the interval between the sampling was 20 s. The numbers (*1, 2, 3*) in **A** indicate the three components of the contraction-relaxation cycle (onset, decline to steady-state level and relaxation) during stimulation with 140 mM $[K^+]_o$. The asterisk in **A** (*) indicates the Ca^{2+}/force value obtained during maintained stimulation with the K^+-rich solution

as compared to the initial transient increase to maximum $[Ca^{2+}]_i$ and force values. The pattern during exposure to the K^+-rich solution containing 140 mM K^+ and 1.5 mM Ca^{2+} is shown in Fig. 4A. Three components can be distinguished in this loop. Stimulation at the lower $[K^+]_o$ [60 mM (Fig. 4B), 40 mM (Fig. 4C), 20 mM (Fig. 4D)] results in a two-component clockwise loop, which becomes smaller for lower K^+ concentrations. The initial part of the hysteresis loop also presents a small shift to the left as compared to that obtained during the 140 mM $[K^+]_o$ stimulation. During washout the Ca^{2+}/force relationship at different K^+ solutions overlapped almost perfectly, indicating that during relaxation at all $[K^+]_o$ tested the force decreased more rapidly.

Figure 5 demonstrates the effect of repeated stimulations of the ileum with a normal Krebs solution containing 0.1 mM carbachol ($n = 9$). The procedure consisted of a stimulation of the muscle for 15 min followed by a washout for the same period of time before starting a renewed superfusion with the carbachol-containing solution (Fig. 5A). This procedure induced a time-dependent decline of the initial maximal $[Ca^{2+}]_i$ transient and force peaks from an initial level of 567 ± 60 nM and $90 \pm 4\%$

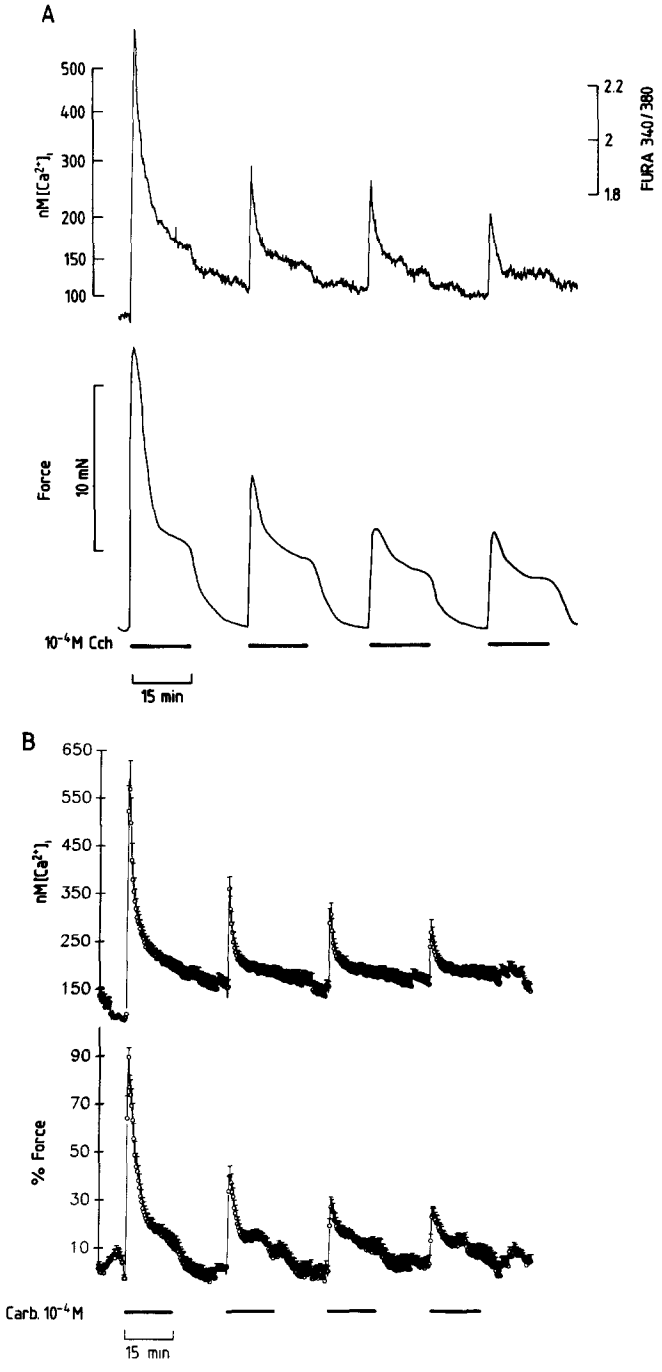

Fig. 5A, B. Effect of repeated stimulation of ileal smooth muscle with 0.1 mM carbachol for 15 min. **A** The change of $[Ca^{2+}]_i$ in nM and of force (in mN) of the ileum during repeated stimulations for 15 min with 0.1 mM carbachol. **B** The effect of repeated stimulations for 15 min with 0.1 mM carbachol on the mean value of $[Ca^{2+}]_i$ and of the force is represented for the nine smooth muscle strips. The *upper trace* represents the $[Ca^{2+}]_i$, the *lower trace* the force; 0% force was arbitrarily defined as the force of the resting muscle during superfusion with normal Krebs solution while 100% was the peak force reached during the first stimulation with 0.1 mM carbachol. After each stimulus the tissue was superfused for 15 min with normal Krebs solution

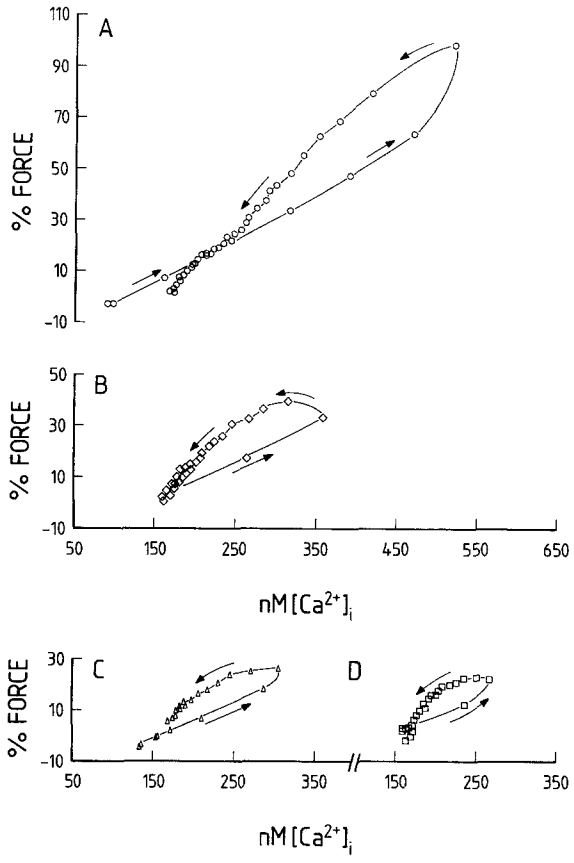

Fig. 6A–D. Averaged Ca^{2+} versus force relationship in the ileum during repeated stimulation with 0.1 mM carbachol for 15 min ($n = 9$). The first stimulation cycle is represented in **A** by ○, the second one in **B** by ◇, the third one in **C** by △ and the fourth stimulation in **D** by □. The *arrows* indicate the direction of the contraction-relaxation cycle

to 268 ± 26 nM and 23 ± 3% during the fourth stimulation (Fig. 5B).

The Ca^{2+}/force relationship determined in these averaged experiments is represented in Fig. 6. The highest value for this relationship is observed for the initial 0.1 mM carbachol stimulation (Fig. 6A). Although the maximum amplitude of the Ca^{2+}/force relationship declined as a function of the number of stimulations, all loops followed the same, albeit smaller, counterclockwise hysteresis loop (Fig. 6B–D).

If a similar sequence of stimuli was applied with a depolarizing solution containing 140 mM K^+ and 1.5 mM Ca^{2+} instead of with an agonist such as carbachol, there was no significant decline of the peak Ca^{2+} or force nor of the tonic part of the contraction (Fig. 7). Successive stimuli with K^+ depolarizing solutions did not, therefore, elicit a shift of the clockwise hysteresis.

Discussion

This study describes the Ca^{2+}/force relationship in the longitudinal layer of the guinea-pig ileum by simultaneous measurements of $[Ca^{2+}]_i$, as determined by Fura-2, and of the tension. Two different types of stimuli were used: K^+-rich solutions and the muscarinic agonist carbachol, and these stimuli resulted in a different type of Ca^{2+}/force relationship during contraction-relax-

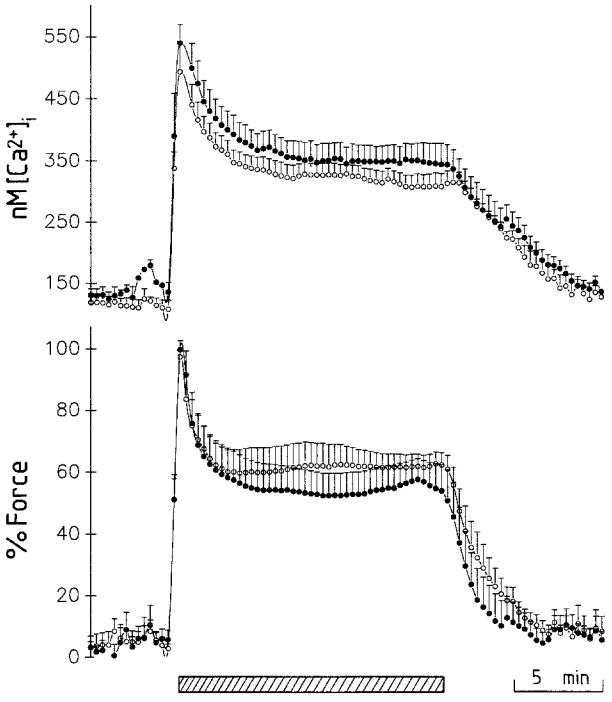

Fig. 7. Effect of different stimulations with a depolarizing solution containing 140 mM $[K^+]_o$ and 1.5 mM $[Ca^{2+}]_o$ on the ileum. In the *upper record* the changes of $[Ca^{2+}]_i$ is represented, in the *lower one* the force. ○, The initial stimulation; ●, the subsequent superfusion with 140 mM $[K^+]_o$ after a 20 min washout in normal Krebs

ation, indicating that this relationship can vary according to the experimental conditions.

The effect of K^+ depolarization on ileal smooth muscle has previously been investigated by Himpens et al. (1988). In that study, the peak and steady-state $[Ca^{2+}]_i$ and force values were determined and correlated to the short-lasting increase of the phosphorylation in that muscle. This investigation has now been extended by measuring dynamically the Ca^{2+}/force relationship not only during contraction but also during relaxation, and using different stimuli. Stimulation with a solution containing 140 mM K^+ and 1.5 mM Ca^{2+} elicited an initial peak of increased Ca^{2+} and force, which was followed by a smaller, but maintained level. Both signals returned to the control value on reexposure of the tissue to the control solution. The Ca^{2+}/force relationship determined during this procedure performed a loop consisting of three components. Initially there was a rise of the curve, expressing the relationship, which was followed by a clockwise decline to a more steady-state level lasting as long as the stimulus was applied. On washing out the K^+-rich solution a second clockwise decline of the loop ocurred. The $[Ca^{2+}]_i$ and force responses of a specific tissue were reproducible if the preparation was reexposed to normal Krebs solution between the different stimuli with high K^+ solutions. We did not observe a disappearance of the clockwise hysteresis loop, as has been reported for isolated cells of the *Bufo marinus* stomach upon repeated electrical stimulation (Yagi et al. 1988). We cannot explain this discrepancy. It may be related to tissue difference, to the use of isolated smooth muscle cells instead of intact smooth muscle, to the difference of stimulation etc. The slope of this Ca^{2+}/force relationship during the ascending phase is similar to the data obtained with aequorin in the ferret portal vein (De Feo and Morgan 1985) and with Fura-2 in isolated smooth muscle cells of the stomach (Yagi et al. 1988). All these studies on intact smooth muscle give a much steeper Ca^{2+}/force relationship than that observed previously in chemically skinned smooth muscle (Endo et al. 1977; Chatterjee and Murphy 1983). The maximal $[Ca^{2+}]_i$ values obtained in this study furthermore agree with the $[Ca^{2+}]_i$ values observed in the same smooth muscle cells of the ileum, which were permeabilized with *Staphylococcus* α toxin during maximal force development (Somlyo and Himpens 1989). The linear relationship between $[Ca^{2+}]_i$ and force observed during the inital steep phase of the force development indicates that under the given experimental conditions the force/$[Ca^{2+}]_i$ ratios during the onset of the contraction proceed linearly.

De Feo and Morgan (1985) could not observe hysteresis in the ferret portal vein during K^+ depolarization. It was suggested that this was due to the phasic properties of this vein. However, the present study demonstrates that hysteresis can also occur in a phasic muscle such as the ileum. This clockwise hysteresis occurring in ileum became more obvious if the Ca^{2+}/force relationship at different K^+ concentrations (140, 60, 40, 20 mM) was compared in the same muscle. Especially at the lower $[K^+]_o$ the clockwise hysteresis loop became pronounced. The loss of sensitivity to Ca^{2+} during K^+ contractions, as indicated by this hysteresis, could be related to the desensitization to Ca^{2+} observed in the ileum (Himpens et al. 1989) and might finally be due to the high phosphatase activity of these cells because it can be reversed by using the phosphatase inhibitor, okadaic acid (Somlyo et al. 1989). The difference between the observations with aequorin and those with Fura-2 could be related to the difference between the muscle type and the procedure used in determining the Ca^{2+}/force relationship. The data obtained with aequorin were collected during steady-state conditions while the Fura-2 data have been obtained over the whole contraction-relaxation cycle at different K^+ concentrations. An additional factor leading to this difference might be the non-linear relation between aequorin luminescence and the $[Ca^{2+}]_i$. Finally this counterclockwise hysteresis loop contrasts with the clockwise observed with the same method and K^+-rich solution in the rabbit pulmonary artery (Himpens et al. unpublished results).

The direction and the shape of the hysteresis loop during muscarinic stimulation is different from the pattern obtained during K^+ stimulation. The Ca^{2+}/force relationship during the contraction-relaxation follows a counterclockwise hysteresis loop. The initial peak values coincide rather with the data obtained during K^+ depolarization. The maximum $[Ca^{2+}]_i$/force ratio was 0.16 for K^+-depolarization and 0.21 for carbachol stimulation. After reaching its peak contraction, the ileal muscle relaxes in spite of the presence of carbachol. The counterclockwise hysteresis loop indicates that a high

tension can be maintained at a submaximal $[Ca^{2+}]_i$ level. The increase of the sensitivity is, however, not as dramatic as that observed in the tonic pulmonary artery. In the latter tissue, the Ca^{2+}/force relationship was shifted to the left during stimulation with an agonist if compared to the Ca^{2+}/force relationship during K^+ stimulation. This contrasts with the force/Ca^{2+} ratio in the pulmonary artery, for which the value increased from 0.27 during K^+ stimulation to 0.48 during superfusion with the thromboxane analog U 46619 (Himpens et al. unpublished results). It has been suggested that the contractile/regulatory apparatus of the ileum is more sensitive to Ca^{2+} during agonist stimulation than during K^+ stimulation (Himpens and Casteels 1987). This could be due to G-protein-mediated modulation (Kitazawa et al. (1989) leading to an altered ratio of myosin light-chain kinase over myosin light-chain phosphatase during agonist stimulation. A possible role of protein kinase C (Somlyo et al. 1989; Itoh et al. 1988) has also been proposed in this process. The less potentiating effect of the stimulation of the ileum with an agonist on the Ca^{2+}/force relationship as compared to the pulmonary artery could be due to desensitization to Ca^{2+} by the concomitant high phosphatase activity (Somlyo et al. 1989).

Desensitization to an agonist is generally referred to as a reduced responsiveness to agonist stimulation after a preceding exposure to that agonist. It has been suggested that this process might be due to alterations of the muscarinic receptors at the plasma membrane by phosphorylation (Harden et al. 1985; Kwatra and Hosey 1986) as has been described for the β adrenergic receptor (Stiels et al. 1984). The desensitization could also be partially due to the appearance of protein kinase C because phorbol esters can inhibit the activity of the muscarinic receptors (Orellana et al. 1985; Liels et al. 1986; Vicentini et al. 1985). A repeated stimulation of the longitudinal layer of the ileum with carbachol results in a decline of the maximum $[Ca^{2+}]_i$ and force as a function of the number of stimulations. Such a phenomenon could be caused by a down-regulation of the muscarinic receptors. However, the Ca^{2+}/force relationship under these conditions still presents the counterclockwise hysteresis, suggesting that in spite of the presumed diminished receptor activation and of the decline of $[Ca^{2+}]_i$ the increased sensitivity of the regulatory/contractile mechanism during agonist stimulation can persist. It is therefore likely that, despite desensitization, a similar myosin light-chain kinase/myosin light-chain phosphatase ratio is preserved.

Rembold and Murphy (1988) demonstrated in the hog carotid artery that similar force during different stimulations (e.g. K^+ depolarization, histamine or phenylephrine) was accompanied by similar degrees of phosphorylation and concluded that the force development in this muscle was more closely related to myosin light-chain phosphorylation than to changes of $[Ca^{2+}]_i$. This is in agreement with our results indicating that the relation between $[Ca^{2+}]_i$ and force is not only tissue-dependent but also can be modulated by the type of agonist. This is most likely due to their different effect on the activity of the myosin light-chain kinase and myosin light-chain phosphatase (Somlyo et al. 1989). Other studies (Hai and Murphy 1988; Somlyo et al. 1988; Himpens et al. 1988; Hai and Murphy 1989; Somlyo and Somlyo 1990) address various possible mechanisms for the 'latch' state. In our opinion the regulation of the contractile activity based on myosin light-chain phosphorylation/dephosphorylation is sufficient to account for force maintenance in the presence of changing $[Ca^{2+}]$; if one accepts a variable rate of the myosin light-chain phosphatase activity.

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