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Measurement by Quin2 of changes of the intracellular calcium concentration in strips of the rabbit ear artery and of the guinea-pig ileum

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Abstract. Ca^{2+} transient and force development were investigated in smooth muscle strips of the rabbit ear artery and the longitudinal layer of the guinea-pig ileum by using the fluorescent indicator Quin2. Agonists only transiently increased the fluorescence intensity despite the enhanced contraction while excess potassium resulted in a maintained light signal. In Ca^{2+} free solutions the release by an agonist of Ca^{2+} from an intracellular store can be demonstrated. These observations illustrate the usefullness of the Ca^{2+} indicator Quin2 in the study of the excitation-contraction coupling in smooth muscle under various conditions.

Key words: Intracellular $[Ca^{2+}]$ – Smooth muscle – Quin2 – K^+-depolarization – Stimulation by agonists

Introduction

Changes of the cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_{cyt}$) may play a predominant role in determining the force development of smooth muscle cells. This hypothesis is based on the presumed analogy between smooth muscle and striated muscle, on data obtained in skinned smooth muscle cells (Endo et al. 1982; Saida 1982) and on the analysis of transmembrane ^{45}Ca fluxes in smooth muscle tissues (Deth and Casteels 1977; Casteels and Droogmans 1981). However, accurate measurements of the cytoplasmic free Ca^{2+} concentration have been difficult to obtain due to the lack of a reliable intracellular Ca^{2+} probe and it has therefore tacitly been assumed by most research workers that changes of $[Ca^{2+}]_{cyt}$ during stimulation could be estimated from the changes of the force development.

Although the use of calcium indicators has become well established in skeletal and cardiac muscle (Ashley and Campbell 1979; Blinks 1982) the use of these substances has proven to be technically difficult in smooth muscle cells, mainly because of the high resistance of the electrodes required for microinjection of the Ca^{2+} indicators (Fay et al. 1979; Neering and Morgan 1980). Recently, a fluorescent indicator (Quin2) has become available (Tsien 1981), which can easily penetrate as an acetoxymethyl ester (Quin2 AM) into small cells. By measuring the fluorescence changes at 492 nm it became possible to estimate $[Ca^{2+}]_{cyt}$ under various conditions in isolated cells of different types such as lymphocytes, platelets, myocytes, cultured smooth muscle cells, macrophages etc. (Tsien et al. 1982; Rink et al. 1982; Powell et al. 1984; Capponi et al. 1985; Reynolds and Dubyak 1985; Hirata et al. 1984; Kobayashi et al. 1985). We now wanted to find out whether Quin2 could also be used for investigating the relation between force development and changes of $[Ca^{2+}]_{cyt}$ in intact smooth muscle tissues. Although such studies have already been performed in ferret portal vein using aequorin (Morgan and Morgan 1984), we found it essential to investigate whether also Quin2 could be used and whether the same results were obtained as with tissues in which the cells were loaded with aequorin by making them transiently permeable to this protein. Preliminary results have been presented at a meeting of the Belgian Physiological Society (Himpens et al. 1985) and of The Physiological Society (Casteels and Himpens 1985).

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Methods

Guinea-pigs of either sex weighing approximately 300 g were killed by a blow on the head, and 3–5 cm of the terminal ileum were quickly removed and after rinsing gently slid on a glass rod of 5 mm diameter. The longitudinal layer was dissected free from the circular muscle and thin strips of about 2 mm wide and 10 mm long were prepared and transferred to an oxygenated Krebs solution.

The ear artery of the rabbit was obtained after stunning and bleeding the animals. The artery was cleaned of its periarterial connective tissue under a dissection microscope, while it was continuously superfused with oxygenated Krebs solution at 35° C. The endothelium was removed by gentle rubbing the inside surface of the vessel as described by Furchgott and Zawadzki (1980). Helical strips 1–2 mm wide and 10 mm long were cut from an artery segment. In order to eliminate the effect of release of endogenous noradrenaline, the tissues were functionally denervated by 6-hydroxydopamine as described by Aprigliano and Hermsmeyer (1976).

The standard physiological solution was a Hepes-buffered modified Krebs solution containing: 135.5 mM Na^+; 5.9 mM K^+; 0.2 mM Ca^{2+}; 1.2 mM Mg^{2+}; 143.8 mM Cl^-; 11.6 mM Hepes and 11.6 mM glucose. The solution was bubbled with O_2 and the pH was 7.3. Solutions with increased $[K^+]_o$ were obtained by replacing Na^+ by an equivalent amount of K^+. The Ca^{2+}-free solutions always contained 2 mM EGTA in order to reduce the concentration of Ca^{2+} below ca 10^{-8} M.

Tissues were loaded with Quin2 by exposing them for 1 h to a Krebs solution containing 7.5 μM Quin2 AM with 0.5% DMSO at 37° C The loading of the strip could be

followed by the increase in fluorescence at 492 nm. However, it is not possible to estimate by this method accurately the intracellular concentration of Quin2, because one would have to record simultaneously the Quin2 AM signal of 460 nm, which has to be excited at 360 nm, and the Quin2 signal at 492 nm, for which excitation light has to be of 339 nm. In addition the Quin2 AM which remains extracellular during loading represents a high background fluorescence. Therefore the exact amount of Quin2 in the tissues was estimated by using ^{3}H-labelled Quin2 AM. The strips loaded with Quin2 were transferred to a home-made set up with a tissue chamber of 1 ml which was continuously perfused. The tissue was mounted isometrically at a sufficient stretch to minimize movement artefacts. The force development and the fluorescent light could be measured simultaneously. The light source was an Osram Berlin (West), mercury arc lamp of 200 Watt. The light was passed through a liquid light guide (Oriel) and monochromatic light of 339 nm was obtained by employing an interference filter with a bandpass of 10 nm. A focusing cylindric lens produced a longitudinal light beam, which could be positioned by a micro-manipulator. The emitted light from the tissue was measured with an Hamamatsu (Tokyo, Japan), R269 photomultiplier tube mounted at 90° to the tissue after passing through an interference filter of 492 ± 10 nm. These light signals were not converted to absolute Ca^{2+} concentrations, because leakage of Quin2, photobleaching and the possibility that the fluorescence signals were unevenly distributed over the different cells would make such calculations difficult. Between the different stimulations the exciting beam was interrupted by a shutter to avoid photobleaching. The Quin2 leaking out of the tissue was washed away by a continuous superfusion of the tissue. Tissues which had not been loaded with Quin2 also presented an autofluorescence which is approximately 15% of the Quin2 loaded signal but this remained constant during stimulation.

The stimulant drugs did not present any additional significant autofluorescence at 492 nm at the concentration used in the experiments.

Results

Uptake of Quin2 by the smooth muscle cell

Tissues were exposed for different periods of time to a Krebs solution at 35° C containing 0.2 mM Ca^{2+} and the various concentrations of ^{3}H labelled Quin2 AM. The amount of Quin2 present in the tissue was estimated by determining the radioactivity remaining in the tissue after eliminating the extracellular ^{3}H Quin2 AM by a 1-h wash at 35° C in a non-radioactive Krebs solution. In Fig. 1, the intracellular Quin2 concentrations were determined in the longitudinal layer of the ileum after loading for 0.5, 1, 2, 3 and 4 h with 2.5, 7.5 and 15 μM of ^{3}H labelled Quin2 AM. At all concentrations of ^{3}H Quin2 AM the presence of Quin2 in the cytoplasm due to cytoplasmic esterase activity (Tsien 1981) is time dependent and reaches a plateau after 2 h. This value depends on the external Quin2 AM concentration.

In the following experiments the tissue was loaded with 7.5 μM Quin2 AM for 1 h. The intracellular concentrations of Quin2 obtained in the longitudinal layer by this procedure was 23.4 ± 1.95 μM/kg wet weight ($n = 18$). A similar loading of the ear artery gave 33.3 ± 5.74 μM/kg wet weight

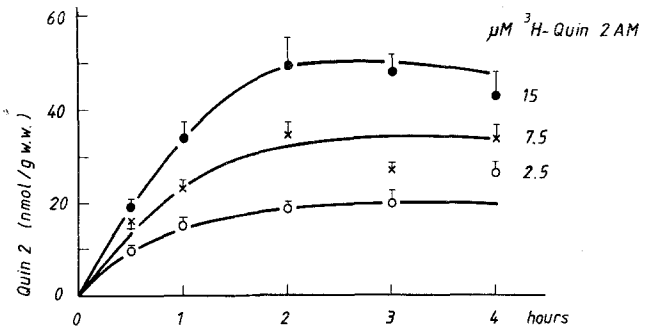

Fig. 1. The intracellular Quin2 concentration (nmol/g wet weight) in the longitudinal layer of the guinea-pig ileum has been estimated by measuring the amount of ^{3}H after loading the tissues for different times with 2.5 μM, 7.5 μM and 15 μM H-Quin2 AM (^{3}H-labelled) and washing out for 1 h in normal Krebs solution at 35° C

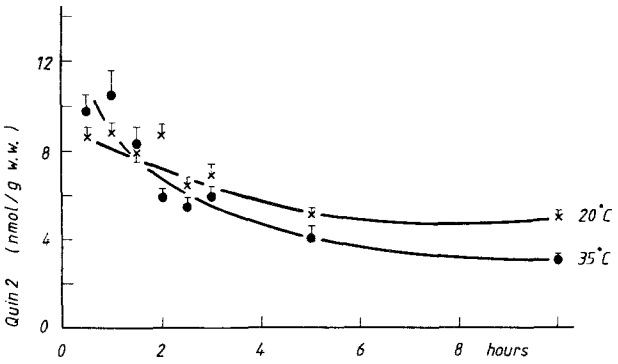

Fig. 2. The intracellular Quin2 concentration (nmol/g wet weight) in the longitudinal layer of the guinea-pig ileum was estimated from the amount of ^{3}H, remaining in the tissue after loading for 1 h with 2.5 μM ^{3}H Quin2 AM and washing out for different times at 20° C and at 35° C

($n = 9$). These presumed intracellular concentrations of Quin2 neither affected the spontaneous force development of the ileal smooth muscle nor the force development elicited by different stimuli in the ileum and ear artery.

However Quin2 also slowly leaks out of the tissue and this can be deduced from Fig. 2. The ^{3}H radioactivity remaining in the ileal strips was determined after loading with 2.5 μM ^{3}H Quin2 AM for 1 h and washing the tissues for different times at 20° C and at 35° C. The radioactivity remaining in the tissue after different times of exposure to non-active Krebs solution declines with a $t_{1/2}$ of 270 min at 35° C and a $t_{1/2}$ exceeding 600 min at 20° C, indicating that there is a slow leakage of Quin2 out of the cells mainly at 35° C. Therefore we have performed our experiments at 20° C and limited their duration to less than 4 h.

Effects of a stimulation with agonists or K^+-rich solution on the fluorescence of Quin2 and force development in the ear artery

A stimulation of the ear artery with 10^{-6} M noradrenaline for 5 min in a solution containing 0.2 mM Ca^{2+} causes a rapid increase of the fluorescence. After reaching a peak value it slowly declines towards its control level. The force development rises more slowly than the fluorescence. It reaches its maximum when the fluorescence is already declining and this tension remains enhanced as long as the

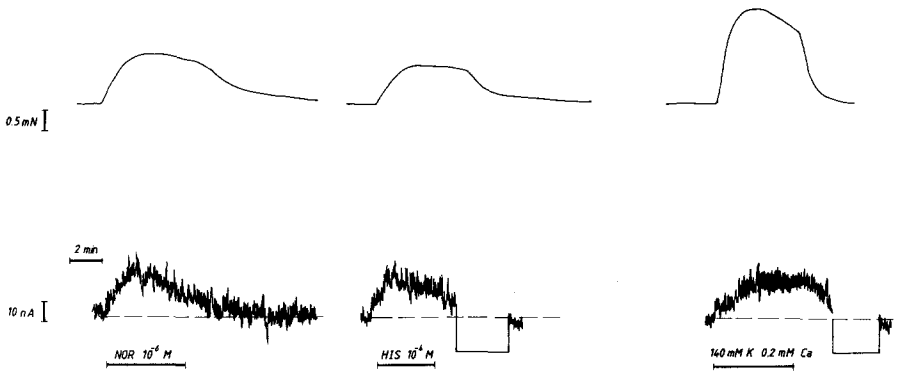

Fig. 3
Typical recordings of force development (*upper trace*) and fluorescence (*lower trace*) in the same strip of the ear artery of the rabbit during stimulation with 10^{-6} M noradrenaline (*left*), 10^{-4} M histamine (*middle*) and 140 mM K^+ (*right*) in a solution containing 0.2 mM Ca^{2+}. Between the different stimuli the exciting light beam was interrupted by closing the shutter (downward deflection in the fluorescence trace). There is a 20 min interval between the different stimulations

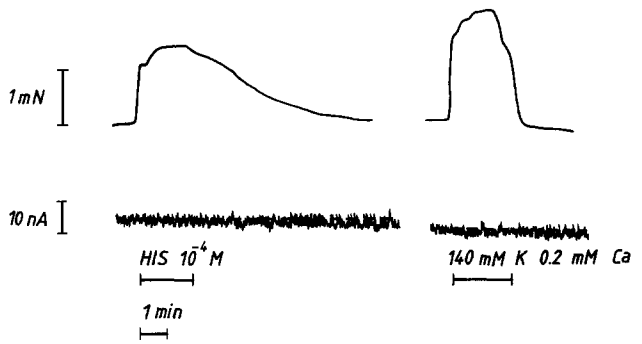

Fig. 4. Simultaneous control recordings of force development (*upper trace*) and fluorescence (*lower trace*) in the same strip of the ear artery which was not loaded with Quin2 during stimulation with 10^{-4} M histamine (*left*) and 140 mM K^+ (*right*) in a solution containing 0.2 mM Ca^{2+}. There is a 20 min interval between the stimulations

agonist is present. On washing out the agonist, the force decrease but the fluorescence remains either at its control value or becomes slightly lower than the initial level due to the photobleaching of Quin2 (Fig. 3). This pattern of fluorescence is not changed by adding 10^{-5}M propanolol. We have not applied higher concentrations of noradrenaline because this substance is then contributing to the fluorescence. A similar configuration of fluorescence and force development is observed during stimulation with 10^{-4} M histamine ($n = 8$).

A stimulation with a solution containing 140 mM $[K^+]_o$ and 0.2 mM $[Ca^{2+}]_o$ induces a rise of the fluorescence and of the force development. However in contrast with the fluorescence signal during agonist stimulation, the light signal remains enhanced as long as the increased force development. On washing out the K^+ rich medium both fluorescence and force return to their control values. This fluorescence pattern indicates that the decrease in fluorescence observed during agonist stimulation cannot simply be the result of photobleaching. Similar experiments have been performed on strips of the ear artery, which had not been loaded with Quin2, in identical conditions as the other experiments. Upon stimulations these tissues develop tension, but changes of the light intensity depending on movement artefacts or on autofluorescence of the tissue could not be detected (Fig. 4).

Effects of a stimulation with agonists or K^+-rich solution on the Quin2 fluorescence and the force development in the ileal smooth muscle

In Fig. 5 the simultaneous changes of Quin2 fluorescence and force development in the longitudinal layer of the guinea-pig ileum are represented ($n = 12$). Stimulation for 6 min with a solution containing 140 mM K^+ and 0.2 mM Ca^{2+} increases both the fluorescence signal and the force development as long as the stimulus is maintained. As in the ear artery both measured parameters are well correlated during stimulation with K^+ rich medium. Stimulation with 10^{-4} M carbachol elicits in the ileal smooth muscle an initial, but transient fluorescence signal. The concomitant force development differs from that observed in the ear artery during application of noradrenaline or histamine by its more rapid rise and by its decline while the agonist is still present. However, as in the ear artery the fluorescence signal does not correlate with the force development which still increases or remains at a high value, while the light signal is already low. Similar observations have been made for a stimulation of ileal smooth muscle with 10^{-4} M histamine.

Effects of agonists on the fluorescence signal during exposure of the tissue to a Ca^{2+} free solution

Earlier studies of the force development and the ^{45}Ca-efflux of the ear artery during exposure to Ca^{2+} free solution have suggested that smooth muscle cells of the ear artery present a compartment from which Ca^{2+} can be released by an agonist during exposure to a Ca^{2+} free medium (Droogmans et al. 1977). It is assumed that the release of this Ca^{2+} results in an increase of the cytoplasmic $[Ca^{2+}]$ which itself then causes a transient force development and an increased fractional loss of Ca^{2+} from the tissue. It seemed worthwhile to study this Ca^{2+} release in tissues loaded with Quin2. Figure 6 shows that exposure of an ear artery strip to a Ca^{2+} free solution added with 2 mM EGTA does not modify the basic fluorescence level. On stimulating the tissue after 5 min exposure to the Ca^{2+} free medium a small transient force development and fluorescence increase occur ($n = 4$). Returning to a solution with 0.2 mM Ca^{2+} after washing out the agonist does not elicit a change of the basal level of fluorescence.

In order to investigate Ca^{2+} release in the ileum, we have taken into account that the depletion of the cellular Ca^{2+} store during exposure to Ca^{2+} free medium proceeds more rapidly (Casteels and Raeymaekers 1979) than in the ear

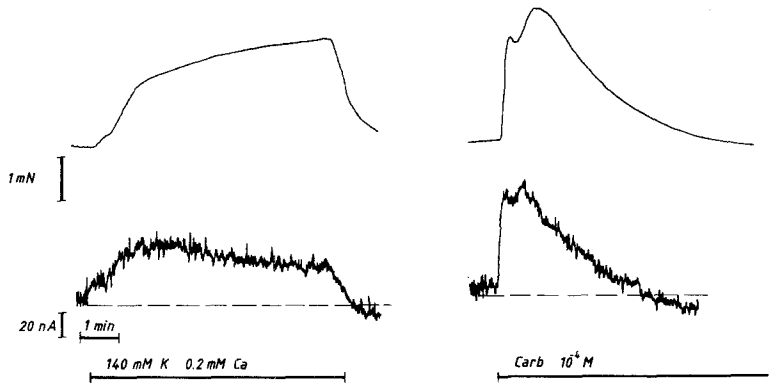

Fig. 5
Typical recording of force development (*upper trace*) and fluorescence (*lower trace*) in the same strip of the longitudinal layer of the guinea-pig ileum during stimulation with 10^{-4} M carbachol (*left*), and 140 mM K^+ (*right*) in a solution containing 0.2 mM Ca^{2+}. There is a 20 min interval between the different stimulations

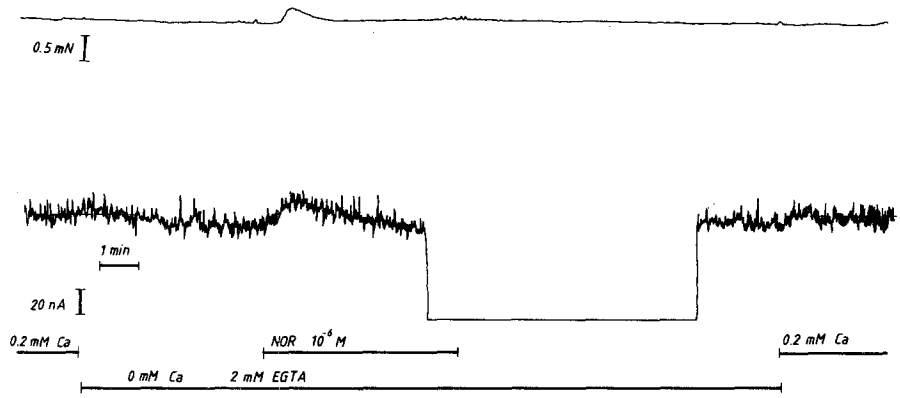

Fig. 6
Simultaneous recording of force development (*upper trace*) and fluorescence (*lower trace*) in the same strip of the ear artery of the rabbit. The solution containing 0.2 mM Ca^{2+} is replaced by a solution without Ca^{2+} and containing 2 mM EGTA. After 5 min 10^{-6} M noradrenaline is added for 5 min. Readmission of 0.2 mM Ca^{2+} does not modify the level of fluorescence. Downward deflection of the fluorescence trace is due to closing the shutter of the exciting light beam

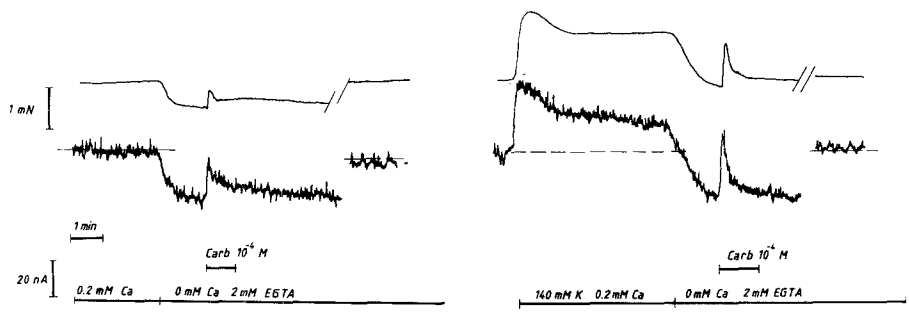

Fig. 7
Typical recordings of force development (*upper trace*) and fluorescence (*lower trace*) in the same strip of the longitudinal layer of the guinea-pig ileum. A 1-min exposure to Ca^{2+}-free solution reduced the spontaneous force development and the fluorescence. Stimulation with 10^{-4} M carbachol elicited a transient increase of both parameters (*right recording*). An exposure to 140 mM K^+ and 0.2 mM Ca^{2+} preceding the 1 min wash with Ca^{2+}-free medium resulted in a larger transient response of both force development and fluorescence

artery (Droogmans et al. 1977). A time of exposure to Ca^{2+} free medium of 1 min is sufficient to eliminate extracellular Ca^{2+} (Casteels and Raeymaekers 1979; Brading and Sneddon 1980). This exposure to Ca^{2+} free medium decreases the force development and the control level of the fluorescence (Fig. 7). On stimulation with 10^{-4} M Carbachol there occurs a small transient increase of the fluorescence and of the force development. If the tissue has been stimulated with a K^+ rich medium before it is exposed to Ca^{2+} free solution both the transient fluorescence signal and the force development induced by carbachol become significantly higher ($n = 4$). These findings substantiate the hypothesis of Casteels and Raeymaekers (1979) that a stimulation of the tissues by K^+ depolarisation results in an additional uptake of Ca^{2+} by the cellular store and a larger subsequent release of Ca^{2+} if the cells are activated thereupon in Ca^{2+} free solution by an agonist.

Discussion

The use of Quin2 to measure changes of $[Ca^{2+}]_{cyt}$ in suspended isolated cells from different tissues is well documented (Tsien et al. 1984). However, up till now Quin2 has not been used to estimate changes of $[Ca^{2+}]_{cyt}$ in whole tissues. Such measurements have been made by Morgan and Morgan (1984) in the ferret portal vein after loading the cells with the photoprotein aequorin. The purpose of the present experiments was to find out whether also Quin2 could be used to determine changes of $[Ca^{2+}]_{cyt}$ in whole smooth muscle tissue and whether similar results were obtained as those described by Morgan and Morgan (1984).

We have on purpose used two different types of smooth muscle tissues: the quiescent ear artery of the rabbit, in which pharmaco-mechanical coupling can be clearly demonstrated, and the longitudinal layer of the guinea-pig ileum,

which is a visceral smooth muscle presenting spontaneous spike discharge and which is a good example of electromechanical coupling. We have first investigated whether the Ca^{2+}-buffering by Quin2 can interfere with the changes $[Ca^{2+}]_{cyt}$ induced by different stimuli. We have loaded the tissues at a Quin2 concentration of 7.5 μM and in order to limit the amount of Ca^{2+} entering the cells during stimulation we have reduced $[Ca^{2+}]_o$ from 1.5 mM to 0.2 mM. Under these experimental conditions the intracellular Quin2 did not effect the force development elicited in the two tissues by either K^+-depolarization or agonist application as compared to the contractions before loading. Also the spontaneous mechanical activity of the ileal smooth muscle remained unchanged.

An important finding was that the changes of $[Ca^{2+}]_{cyt}$ presented a quite different pattern according to the stimulation procedures. Both in the rabbit ear artery and in the ileal smooth muscle of the guinea-pig a stimulation of the cells with K^+-rich solution increase both the fluorescence signal and the force development. For both tissues it can be concluded that during K^+-depolarization the changes of $[Ca^{2+}]_{cyt}$ as estimated with Quin2 fluorescence and the force development are well correlated supporting the hypothesis that during K^+-depolarization $[Ca^{2+}]_{cyt}$ is the main regular of the interaction of actin and myosin.

In contrast the stimulation with an agonist induces a quite different pattern of fluorescence change indicating that only during the initial part of the stimulus, $[Ca^{2+}]_{cyt}$ increase transiently to a high peak, before declining to the control value. The force development of the ear artery is maintained at the same level as long as the stimulus is applied, while it declines progressively in the ileal smooth muscle due to the tachyphylaxis. However, also in the latter tissue the fluorescence signal decreases more rapidly than the force development resulting in a poor correlation between $[Ca^{2+}]_{cyt}$ and force development. These findings on the difference in pattern of changes of $[Ca^{2+}]_{cyt}$ during stimulation with K-rich medium or with agonists confirm the findings in the ferret portal vein of Morgan and Morgan (1984) using aequorin.

We have also studied the changes of $[Ca^{2+}]_{cyt}$ occurring in smooth muscle during stimulation with agonists in Ca^{2+}-free medium. Exposure of the ear artery of the rabbit to such Ca^{2+}-free solution does not modify the control value of fluorescence, but in the ileal smooth muscle this control value of fluorescence decreases significantly. This change is probably related to the disappearance of the spontaneous activity in Ca^{2+}-free medium. Stimulation both of the ear artery of the rabbit and of the guinea-pig ileum with an agonist releases calcium from an intracellular store resulting in a transient increase of the fluorescence and in a transient force development. A preceding exposure to a solution containing 140 mM K^+ and 0.2 mM Ca^{2+} results in the uptake of a larger amount of Ca^{2+} in the sarcoplasmic reticulum (Casteels and Raeymaekers 1979) and in a subsequent larger fluorescence and tension response during stimulation with an agonist in Ca^{2+}-free medium.

We have tried to reduce in these studies the possible artefacts implied in this Quin2 procedure. The intracellular concentrations of Quin2 used in the present experiments are low as compared to the concentrations used by Rink and Pozzan (1985). This low concentration has the advantage of reducing the intracellular buffering of $[Ca^{2+}]_{cyt}$ by Quin2 as suggested by the maintenance of some criteria for a normal cell function in both ileum and ear artery. We can also be confident that the leakage of Quin2 out of the cells is small at 20° C. It cannot completely be excluded that Quin2 enters the endoplasmic reticulum during the leakage of Quin2 out of the cytoplasm. One could assume that the calcium signal could be a mixed signal caused by Ca^{2+}-increase in the cytoplasm and Ca^{2+}-depletion in the SR. However, it is assumed that during maximal stimulation the cytoplasmic $[Ca^{2+}]$ never exceeds 10^{-5} M (Filo et al. 1965; Gordon 1978; Saida and Nonomura 1978; Bond et al. 1984). As a consequence, even when the sarcoplasmic reticulum would be totally depleted of Ca^{2+}, its Ca^{2+} concentration would still be equal to the cytoplasmic Ca^{2+} concentration of 10^{-5} M. Since the dissociation constant of Quin2 is 115 nM, the fully saturated Quin2 signal eventually measured from the sarcoplasmic reticulum during resting conditions would hardly change during even maximum stimulation of the smooth muscle cells. The photobleaching was reduced by limiting the time of exposure to the exciting light. Because in our present work we have been mainly interested in demonstrating for the two types of stimuli differences between fluorescence patterns, both photobleaching and the slow leakage of Quin2 can be considered as of minor importance.

Movement artefacts might be another possible source of error, but some indirect observations lead us to believe that they are of limited importance in our studies. The Quin2 fluorescence could be increased to a maximum by inducing a very high Ca^{2+} influx and this maximum was not modified by an additional force development, suggesting that in our experimental procedure the slight movements of the tissue components occurring during additional force development did not appreciably affect the fluorescence pattern.

The major advantage of this experimental procedure is that it makes possible to study in whole smooth muscle tissues the relation between changes of $[Ca^{2+}]_{cyt}$ and force development with Quin2 and allows us to avoid the procedure of isolating single cells or to use a procedure for making the cell membranes transiently permeable.

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References

Aprigliano O, Hermsmeyer K (1976) In vitro denervation of the portal vein and caudal artery of the rat. J Pharmacol Exp Ther 198:568–577

Ashley CC, Campbell AK (1979) Detection and measurement of free Ca^{2+} in cells. Elsevier/North Holland, Amsterdam Oxford New York, p 461

Blinks JR (1982) The use of photoproteins as calcium indicators in cellular physiology. In: Baker PF (ed) Techniques in cellular physiology, vol P1/II. Elsevier/North-Holland, Amsterdam Oxford New York, pp 126/1–38

Bond M, Kitazawa T, Somlyo AV (1984) Release and recycling of calcium by the sarcoplasmic reticulum in guinea-pig portal vein smooth muscle. J Physiol (Lond) 355:677–695

Brading AF, Sneddon P (1980) Evidence for multiple sources of calcium for activation of the contractile mechanism of guinea-pig taenia coli on stimulation with carbachol. Br J Pharmacol 306:411–429

Capponi AM, Lew PD, Vallotton MD (1985) Cytosolic free calcium levels in monolayers of cultured rat aortic smooth muscle cells. J Biol Chem 260:7836–7842

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

Casteels R, Droogmans G (1981) Exchange characteristics of the noradrenaline-sensitive calcium store in vascular smooth muscle cells of the rabbit ear artery. J Physiol (Lond) 317:263–279

Casteels R, Himpens B (1985) Changes of $[Ca]_i$ estimated by Quin2 during contraction of the longitudinal layer of the guinea-pig ileum. J Physiol 369:96P

Deth R, Casteels R (1977) A study of releasable Ca fractions in smooth muscle cells of the rabbit aorta. J Gen Physiol 69:401–416

Droogmans G, Raeymaekers L, Casteels R (1977) Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. J Gen Physiol 70:129–148

Endo M, Yagi S, Iino M (1982) Tension pCa relation and sarcoplasmic reticulum responses in chemically skinned smooth muscle fibers. Fed Proc 41:2245–2250

Fay FS, Shlevin HH, Granger WC, Taylor SR (1979) Aequorin luminescence during activation of single isolated smooth muscle cells. Nature 280:506–508

Filo RS, Bohr DF, Ruegg JC (1965) Glycerinated skeletal and smooth muscle: Calcium and Magnesium dependence. Science 147:1581–1583

Furchgott RG, Zawadski J (1980) Acetylcholine relaxes arterial smooth muscle by releasing a relaxing substance from endothelial cells. Fed Proc 39:581

Gordon RS (1978) Contraction of detergent-treated smooth muscle. Proc Natl Acad Sci USA 75:3527–3530

Himpens B, Droogmans G, Casteels R (1985) Measurement of changes of intracellular Ca^{2+} in smooth muscle cells by the fluorescent indicator Quin2. Arch Int Phys Pharmac 93:P43–P44

Hirata M, Hashimoto T, Hamachi T, Koga T (1984) Changes of intracellular free Ca^{2+} in macrophages following N-formyl chemotactic peptide stimulation. Direct measurement by the loading of Quin2. J Biochem 96:9–16

Itoh T, Kuriyama H, Suzuki H (1981) Excitation-contraction coupling in smooth muscle cells of the guinea-pig mesenteric artery. J Physiol (Lond) 321:513–535

Kobayashi S, Kanaide H, Nakamura H (1985) Cytosolic calcium transients in cultured vascular smooth muscle cells: microfluorometric measurements. Science 229:553–556

Morgan JP, Morgan KG (1984) Stimulus-specific patterns of intracellular calcium levels in smooth muscle cells of ferret portal vein. J Physiol (Lond) 351:155–167

Neering IR, Morgan KG (1980) Use of aequorin to study excitation-contraction coupling in mammalian smooth muscle. Nature 288:585–587

Powell T, Tatchman PER, Twist VW (1984) Cytoplasmic free calcium measured by Quin2 fluorescence in isolated ventricular myocytes at rest and during potassium-depolarization. Biochem Biophys Res Commun 122:1012–1020

Reynolds EE, Dubyak GR (1985) Activation of calcium mobilization and calcium influx by $alpha_1$-adrenergic receptors in a smooth muscle cell line. Biochem Biophys Res Commun 130:627–632

Rink TJ, Pozzan T (1985) Using Quin2 in cell suspensions. Cell Calcium 6:133–144

Rink TJ, Smith SW, Tsien RY (1982) Cytoplasmic free Ca^{2+} in human platelets: Ca^{2+} threshold and Ca^{2+} dependent activation for shape-change secretion. FEBS Lett 148:21–26

Saida K (1982) Ca release in skinned smooth muscle. J Gen Physiol 80:191–202

Saida K, Nonomura Y (1978) Characteristics of Ca- and Mg-induced tension development in chemically skinned smooth muscle fibers. J Gen Physiol 72:191–202

Stout MA, Diecke FPJ (1983) ^{45}Ca distribution and transport in saponin scinned vascular smooth muscle. J Pharm Exp Ther 225:102–111

Tsien RY (1981) A non-disruptive technique for loading calcium buffers and indicators into cells. Nature 290:527–528

Tsien RY, Pozzan T, Rink TJ (1982) Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new intracellular trapped indicator. J Cell Biol 94:325–334

Tsien RY, Pozzan T, Rink TJC (1984) Measuring and manipulating cytosolic Ca with trapped indicators. TIBS 9:263–266

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