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ALTERATION OF CYTOPLASMIC IONIZED CALCIUM LEVELS IN SMOOTH MUSCLE BY VASODILATORS IN THE FERRET

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SUMMARY

1. Aequorin was used as an indicator of cytoplasmic Ca^{2+} levels during the action of vasodilators in potassium-depolarized strips of ferret portal vein.

2. Moderate concentrations of isoprenaline produced either no change or an increase in cytoplasmic Ca^{2+} levels during smooth muscle relaxation. Only in the presence of very high concentrations of isoprenaline (greater than 10^{-4} M) was a decrease in intracellular Ca^{2+} levels detectable.

3. Both papaverine and forskolin also caused relaxations of the muscle while cytoplasmic Ca^{2+} levels were observed either not to change or to increase.

4. When the muscles were relaxed either by decreasing the calcium concentration in the bathing medium or by the addition of sodium nitroprusside, light and force fell together.

5. The ratio of force to light was greater when the muscle was relaxed with calcium depletion than with the addition of sodium nitroprusside, indicating that sodium nitroprusside was relaxing the muscle by more than just decreasing cytoplasmic Ca^{2+} levels.

6. These results indicate that not all vasodilators cause a lowering of cytoplasmic Ca^{2+} levels and indicate that, in intact cells, agents associated with increases in cytoplasmic cyclic AMP levels can cause an uncoupling of calcium–force relations.

INTRODUCTION

We have previously described the use of the bioluminescent Ca^{2+} indicator aequorin to monitor changes in intracellular ionized calcium levels ($[Ca^{2+}]_i$) in vascular smooth muscle (Morgan & Morgan, 1982; Morgan & Morgan, 1984). We found that increases in contractile force are accompanied by increases in light (a function of $[Ca^{2+}]_i$) but that in the steady state, the ratio of force to light varies with the agonists used to induce the contraction. Differences in the force to light ratio were found even when agonists were compared for the same absolute level of force in the same muscle. This suggested that agonists can modulate the $[Ca^{2+}]_i$–myofilament interaction in intact cells.

Considerable controversy surrounds the mechanism of action of the vasodilators,

especially with respect to the agents reported to cause changes in cytoplasmic cyclic AMP levels. Several laboratories have reported that isoprenaline causes a hyperpolarization of the vascular smooth muscle cell membrane (Somlyo, Haeusler & Somlyo, 1970; von Loh, 1971; Yamaguchi, Honeyman & Fay, 1982; Prehn & Bevan, 1983). However, tissues depolarized by very high $[K^+]$ can still be relaxed by isoprenaline (Meisheri & Van Breemen, 1982) even though there is no change in membrane potential (Somlyo *et al.* 1970) and relaxation can be obtained at concentrations of isoprenaline which cause no change in membrane potential (Ito, Kitamura & Kuriyama, 1980). Meisheri & Van Breemen (1982) reported a significant inhibition of Ca^{2+} influx by isoprenaline in rabbit aortic smooth muscle, but Van Eldere, Raeymaekers & Casteels (1982) reported considerable variability of different arterial muscles in their responses to isoprenaline and demonstrated an effect of isoprenaline to increase calcium uptake into the intracellular stores. An increased ability of microsomes to bind Ca^{2+} has been reported in association with elevated cyclic AMP levels (Baudouin-Legros & Meyer, 1973; Webb & Bhalla, 1976). However, others have suggested that the effect of isoprenaline may be on a later step in the excitation–contraction coupling sequence, i.e. an effect on the $[Ca^{2+}]_i$–myofilament interaction. Based on phosphorylase measurements during relaxation, Paul (1983) has suggested a mechanism of β-adrenergic relaxation in which the sensitivity of the actin–myosin interaction to calcium is decreased. It has been suggested (Adelstein, Conti, Hathaway & Klee, 1978; Silver & DiSalvo, 1979) that phosphorylation of myosin light chain kinase by β-receptor activation may inhibit actin–myosin interactions. Itoh, Izumi & Kuriyama (1982) have suggested that only very high concentrations of cyclic AMP plus cyclic-AMP-dependent protein kinase can suppress Ca^{2+}–myofilament interations in skinned fibres, but Rüegg, Meisheri, Pfitzer & Zeugner (1983) reported an inhibition of calcium-induced tension in skinned muscle by the catalytic subunit of protein kinase alone. Kerrick & Hoar (1981) have relaxed skinned smooth muscle contracted with calcium by adding the catalytic subunit of cyclic-AMP-dependent protein kinase. It still remains to be determined whether β-receptor stimulation alters $[Ca^{2+}]_i$ in intact cells. The recent extension of the aequorin technique to the measurement of $[Ca^{2+}]_i$ in vascular smooth muscle (Morgan & Morgan, 1982) has made such an investigation possible.

The purposes of the present study were (1) to determine whether aequorin is a sufficiently sensitive $[Ca^{2+}]_i$ indicator to detect decreases in $[Ca^{2+}]_i$ when they do occur in vascular smooth muscle; (2) to directly determine the effects on $[Ca^{2+}]_i$ of isoprenaline and other agents which are able to decrease vascular tone, and (3) to see if the force–$[Ca^{2+}]_i$ ratio during vasodilator-induced relaxation varies with different vasodilators, suggesting that the $[Ca^{2+}]_i$–myofilament interaction might be modulated by these agents.

METHODS

Adult ferrets of either sex were anaesthetized with chloroform. The abdomen was opened and the portal vein and surrounding structures removed to a dissection dish filled with oxygenated physiological saline solution. Longitudinal portal vein strips (1 cm by 0·5–1·0 mm) weighing 1·0–5·0 mg were dissected from the area of portal vein near the liver. The endothelium was removed by gentle rubbing of the inside surface of the vessel with a rubber policeman. The strips were placed

in a bath surrounded by an ellipsoidal mirror within a light-tight enclosure. One end of the muscle was held in a clamp and the opposite end was attached to a Gould UC2 transducer. Experiments were run at 22 °C rather than body temperature in order to decrease the rate of aequorin consumption. Additional details of these methods have been described (Blinks, 1982; Morgan & Morgan, 1984).

Solutions containing elevated potassium were obtained by equimolar replacement of NaCl with KCl. The composition of the physiological saline solution was (mM): NaCl, 120; KCl, 5·9; dextrose, 11·5; $NaHCO_3$, 25·0; $MgCl_2$, 1·2; NaH_2PO_4, 1·4; $CaCl_2$, 2·5. The solution was equilibrated with 95% O_2/5% CO_2 and had a pH of 7·4. Aequorin was loaded into cells by a chemical loading procedure previously described (Morgan & Morgan, 1984). Briefly, this procedure consists of incubating the muscle in a series of four solutions at 2 °C for 30–60 min each. The compositions of the solutions were (mM): solution I: EGTA, 10; Na_2ATP, 5; KCl, 120; $MgCl_2$, 2; *N*-tris(hydroxymethyl)methyl-2-aminoethane suphonic acid (TES), 20; solution II: EGTA, 0·1; Na_2ATP, 5; KCl, 120; $MgCl_2$, 2; TES, 20; aequorin, 0·2 mg/ml; solution III: EGTA, 0·1; Na_2ATP, 5; KCl, 120; $MgCl_2$, 10; TES, 20; solution IV: NaCl, 120; KCl, 5·9; dextrose, 11·5; $NaHCO_3$, 15·5; $MgCl_2$, 10; NaH_2PO_4, 1·4. $CaCl_2$ was gradually, in a step-wise manner increased up to 2·5 mM in solution IV. The muscle was then transferred to the normal physiological saline solution. We have previously shown that the chemical loading procedure produces qualitatively similar light signals to those obtained from cells pressure injected with aequorin. Neither the amplitude of contractile responses nor the sensitivity to agonists is significantly different before and after the chemical loading procedure.

Aequorin emits a light signal which is a function of the Ca^{2+} concentration. The signal is reported as nanoamperes (nA) of anode current in an EMI 9635A photomultiplier tube. The aequorin used in this study was prepared from the laboratory of J. R. Blinks by the methods described by Blinks, Wier, Hess & Prendergast (1982). The following drugs were used: phentolomine (Sigma), (−)-isoprenaline (Sigma), forskolin (Calbiochem), papaverine hydrochloride (Sigma), sodium nitroprusside (Sigma).

RESULTS

Effect of isoprenaline on KCl-induced stimulation of the ferret portal vein

The ferret portal vein has little intrinsic tone, as evidenced by the drop in tone of only approximately 100 mg on exposure of the muscle to a 2 mM-EGTA, calcium-free Krebs solution. In order to facilitate the observation of relaxation of this tissue in response to vasodilators, the muscle was first contracted by exposure to a high-potassium solution. Potassium depolarization was chosen as the agonist because of previous work showing that potassium depolarization produces a relatively larger increase in cytoplasmic Ca^{2+} levels than do other agonists (Morgan & Morgan, 1984) and thus the possibility of observing a decrease in calcium levels would be more likely against a background of potassium depolarization. Fig. 1 illustrates the effect of the addition of isoprenaline to a muscle which had already been contracted in the presence of a Krebs solution containing 46 mM-potassium. The contractile response of this tissue to a high-potassium solution generally consisted of an initial 'phasic' component of variable size, followed by a 'tonic' component. The maintenance of tone in the ferret portal vein is not absolute, but rather tone slowly declines with time. As we have described previously, the aequorin signal (reflecting $[Ca^{2+}]_i$) in response to potassium depolarization rises to a plateau level which is maintained as long as is tone. With potassium concentrations less than 40 mM, an initial phasic light component (corresponding to the initial phasic component of the contraction) is sometimes detectable (e.g. Fig. 3) but with higher potassium concentrations it is generally not detectable. The relation between the aequorin signal and the contraction during the period of force development appears to be highly complex, perhaps

reflecting differential effects of $[Ca^{2+}]_i$ on cross-bridge cycling rates and numbers of attached cross-bridges (Aksoy, Mras, Kamm & Murphy, 1983). For the purposes of this study we have primarily investigated changes in the steady-state relation between force and the aequorin signals, i.e. the tonic phase. When isoprenaline

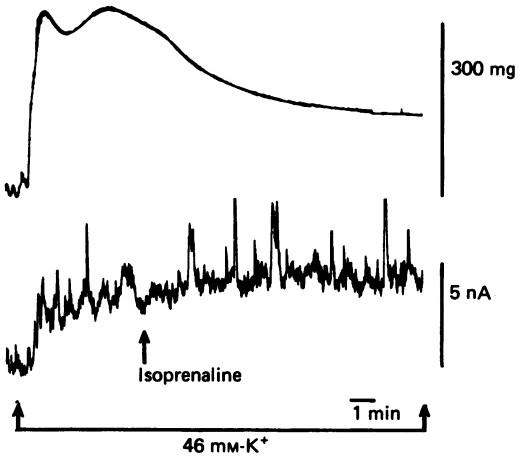

Fig. 1. Force (top trace) and light (bottom trace) in response to exposure of the muscle to 46 mM-potassium physiological saline during the period marked by the arrows. Isoprenaline at 5×10^{-6} M was added at the arrow.

(5×10^{-6} M) was added during the tonic phase of the response to high potassium in Fig. 1, there was an obvious acceleration of the rate of decline of tone (a relaxation) but we were unable to detect any corresponding decrease in cytoplsmic Ca^{2+} levels. Similar results were seen in ten out of ten experiments. In the type of experiment illustrated in Fig. 1, isoprenaline was added after the initiation of a potassium contracture.

We have also reversed the protocol, treating the muscle first with isoprenaline and then adding a high-potassium solution in the continued presence of isoprenaline. Such an experiment is illustrated in Fig. 2. The first set of panels shows the control response to potassium depolarization alone. The muscle was then washed in normal Krebs solution for 1 h and then isoprenaline alone was added to this muscle, causing no detectable change in basal tension or light levels. The addition of the same concentration of potassium in the continued presence of isoprenaline now produced a smaller contraction but a light response which was larger than the control light response. Similar results were seen in thirteen out of thirteen experiments. Thus, we were unable to detect any decrease in cytoplasmic Ca^{2+} levels during relaxation of the muscle with isoprenaline. In 62% of the experiments there was actually a detectable increase in light levels accompanying the relaxation with isoprenaline at 5×10^{-6} M. The responses to this concentration of isoprenaline were unchanged by the addition of 10^{-6} M-phentolamine.

The question arises as to whether the aequorin technique is sufficiently sensitive to detect decreases in $[Ca^{2+}]_i$ when they occur. We have previously (Morgan &

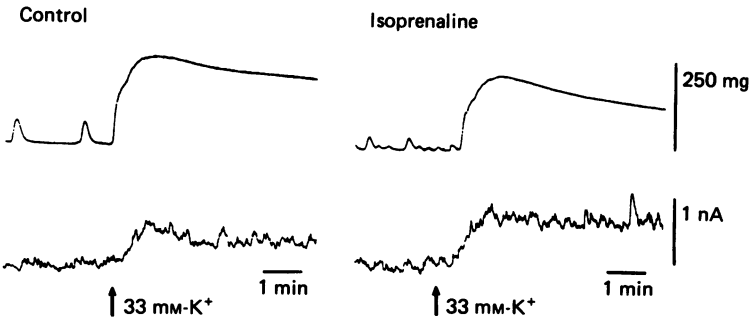

Fig. 2. Force (top trace) and light (bottom trace); both sets of traces are from the same muscle. The left-hand pair of traces shows the control response to 33 mM-potassium Krebs solution added at the arrow. The right-hand pair of traces shows the effect of the same concentrations of potassium when the same muscle was pre-treated with 5×10^{-6} M-isoprenaline for 5 min before the addition of potassium depolarization. Between the two sets of responses the muscle was washed in normal Krebs solution for 1 h.

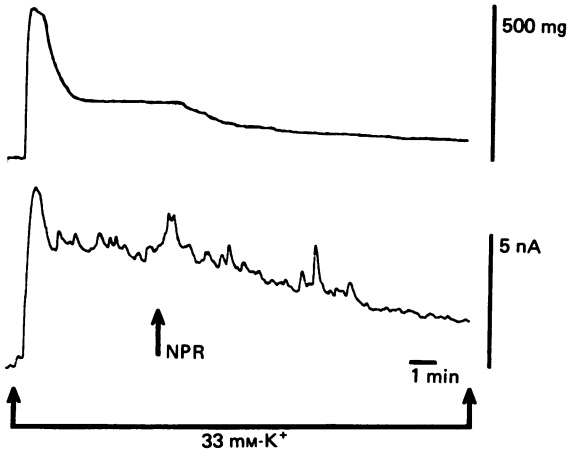

Fig. 3. Force (top trace), light (bottom trace). The muscle was exposed to a 33 mM-potassium physiological saline solution for the period of time marked by the arrows. Sodium nitroprusside (NPR) at 10^{-5} M was added at the arrow.

Morgan, 1984) shown that the aequorin technique is capable of detecting decreases in cytoplasmic calcium levels when the muscle is relaxed by the removal of extracellular calcium during a potassium contracture. This argues against, but does not disprove, the idea that the lack of a fall in the aequorin signal during isoprenaline-induced relaxation is an artifact of the properties of aequorin. We were curious to see whether, using aequorin as a calcium indicator, we could detect decreases in cytoplasmic Ca^{2+} levels with other vasodilator drugs.

Effects of sodium nitroprusside on KCl-induced stimulation of the ferret portal vein

Fig. 3 shows the response to sodium nitroprusside (10^{-5} M) added during a potassium contracture. This muscle produced an especially large phasic tension component in response to potassium depolarization, and in this case there were also a detectable phasic component at the onset of the light response. The sodium

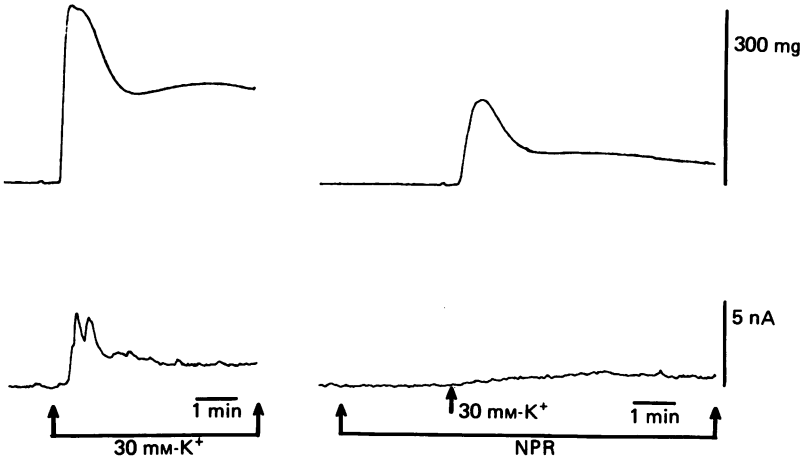

Fig. 4. Force (top trace) and light (bottom trace) in response to 30 mM-potassium physiological saline solution during the period of time marked by the arrows. Both sets of responses are from the same muscle. The muscle was washed in normal Krebs solution for 1 h between the potassium challenges. The pair of traces on the left is the control. The traces on the right show the response of the same muscle pre-treated with sodium nitroprusside for 5 min before the initiation of potassium depolarization.

nitroprusside was added however after a steady state had been established (i.e. during the tonic component), and this produced a fall in the level of tone which was accompanied by a fall in the aequorin signal. The opposite protocol was also used, where the muscle was pre-treated with sodium nitroprusside before the addition of the high-potassium solution. Such an experiment is illustrated in Fig. 4. The first panel of Fig. 4 illustrates the control response to 30 mM-potassium. After an hour of washing the same muscle in normal Krebs solution, it was then pre-treated with sodium nitroprusside, followed by addition of 30 mM-potassium in the continued presence of sodium nitroprusside. With nitroprusside, both the force and light responses to potassium depolarization are decreased in size. Similar effects were seen in fifteen out of fifteen experiments. Thus, sodium nitroprusside produces markedly different effects on light than does isoprenaline even though both agents relax this muscle.

Effects of other agents thought to increase cyclic AMP levels in vascular smooth muscle

Isoprenaline is not a very potent vasodilator in this tissue and we were prevented from using very high concentrations of the drug because of stimulation of α-receptors

at high concentrations of isoprenaline. Others have reported that isoprenaline increases cyclic AMP levels in vascular smooth muscle and have suggested that this effect may be related to the vasodilator properties of the drug (Namm, 1982). For this reason, we investigated the effects on cytoplasmic calcium levels of other agents which have been reported to alter cyclic AMP levels in vascular smooth muscle, and which are more effective vasodilators of this tissue.

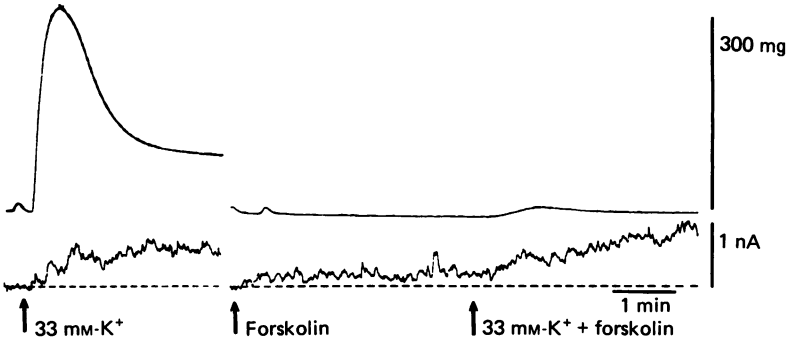

Fig. 5. Force (top trace), light (bottom trace). The dashed line marks the position of the basal light level. The first pair of traces shows the control response to physiological saline solution containing 33 mM-potassium. The second pair of traces shows the response to the same muscle when pre-treated for approximately 4 min with 10^{-5} M-forskolin.

A new drug, forskolin, has recently been introduced, and in a wide variety of tissues has been shown to be a highly specific stimulant of adenylate cyclase. (Seamon, Padgett, & Daley, 1981; Dubey, Srimal, Nityanand & Dhawan, 1981) Fig. 5 illustrates the effect of forskolin pre-treatment on potassium-induced contractions and light signals. The first pair of traces shows the control responses to 33 mM-potassium. The addition of forskolin resulted in an increase in basal light levels, an abolition of spontaneous contractions and a barely detectable drop in basal tone. The addition of 33 mM-potassium in the continued presence of forskolin causes a significant decrease in the size of the potassium contracture, but no change or an increase in the steady-state light response to potassium depolarization. We have seen similar responses in eight out of eight preparations. In four out of five preparations in which forskolin was added before the potassium challenge, the rate of rise of the light signal was slowed by the presence of forskolin.

Another agent reported to increase cyclic AMP levels in vascular smooth muscle is papaverine (Namm, 1982). Fig. 6 illustrates the effects of papaverine on a potassium-induced contraction and light signal in the ferret portal vein. In the first panel, the addition of 33 mM-potassium caused a typical potassium contracture accompanied by a sustained rise in the aequorin signal. The addition of 10^{-6} M-papaverine (botton panel) to the same muscle caused a barely detectable drop in force but a slight increase in light levels. The subsequent addition of 33 mM-potassium in the continued presence of papaverine produced a smaller contraction than the control, but a larger light response, suggesting that papaverine, like isoprenaline and forskolin, is capable of uncoupling changes in cytoplasmic calcium levels from

changes in force. Similar decreases in force accompanied by increases in light were seen in five out of five preparations.

Comparison of effects of sodium nitroprusside to those of decreased calcium entry

In a number of experiments with sodium nitroprusside where this agent decreased both force and light, it appeared that the light signal was not decreased as much as one might expect. For this reason, we compared in the same muscle the effects of sodium

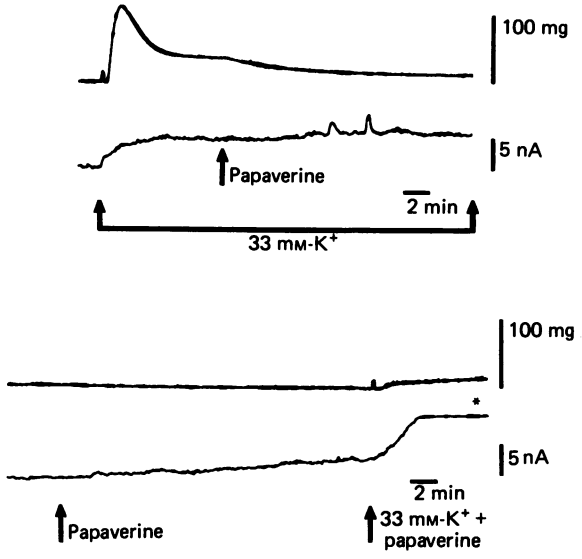

Fig. 6. In each pair of traces, force is on the top, light is on the bottom. In the pair of traces, 33 mM-potassium physiological saline solution was added first to the muscle, then papaverine at 10^{-6} M was added in the continual presence of potassium depolarization. The muscle was then washed for 1 h in normal Krebs solution and papaverine added first, followed by 33 mM-potassium physiological saline solution in the continual presence of papaverine (10^{-6} M). The asterisk indicates that the light response went off scale.

nitroprusside with the effects of a more 'inert' method of decreasing cytoplasmic [Ca^{2+}]. Fig. 7 illustrates such an experiment. The top pair of traces in Fig. 7 shows the control response to 33 mM-potassium. The same muscle was washed for 1 h and then was treated with sodium nitroprusside (middle pair of traces), followed by the same concentration of potassium in the continual presence of sodium nitroprusside. Nitroprusside caused a decrease in the size of the contraction and a decrease in the size of the light response. However, when the muscle was again washed for an hour in normal Krebs solution and then exposed to a Krebs solution containing half the normal concentration of calcium, exposure to 33 mM-potassium (and 1 mM-Ca) produced a smaller contraction than in the control but a larger one than that in the presence of nitroprusside, while the light response was even smaller than that in the presence of nitroprusside. Similar responses were seen in five preparations, and the results were independent of the order in which the solutions were added.

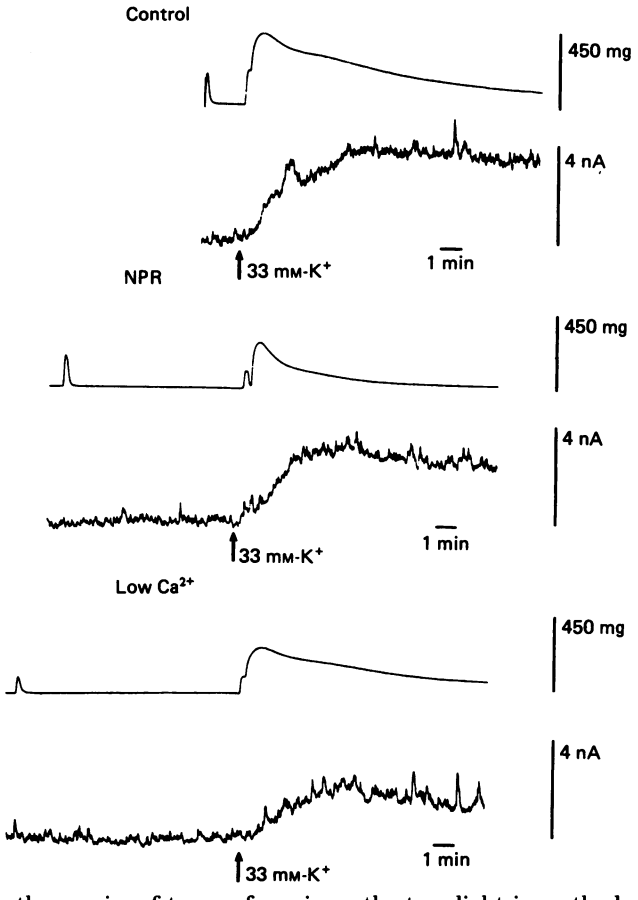

Fig. 7. In the three pairs of traces, force is on the top, light is on the bottom. The top pair of traces shows the control response of this muscle to 33 mM-potassium. The middle pair of traces shows the response when the muscle was pre-treated for 5 min in 10^{-5} M-sodium nitroprusside (NPR) and the bottom panel shows the response when the muscle was pre-treated for 15 min with 1 mM (instead of the usual 2·5 mM) calcium Krebs solution. All three pairs of traces are from the same muscle and between responses the muscle was washed for 1 h in normal Krebs solution.

Effects of high concentrations of isoprenaline on KCl-induced contractions and light signals

The preceding results where concentrations of isoprenaline of 5×10^{-6} M or lower were used, indicate that β-receptor stimulation causes an uncoupling of cytoplasmic calcium levels from force production in this smooth muscle. However, the magnitude of the vasodilator effect of these concentrations of isoprenaline is relatively small in this smooth muscle. For this reason we were anxious to see what effects higher concentrations of isoprenaline might have. The use of higher concentrations required pre-treatment of the muscle with α-receptor blockers. In the presence of 10^{-6} M-phentolamine, we were able to increase the concentration of isoprenaline tenfold to

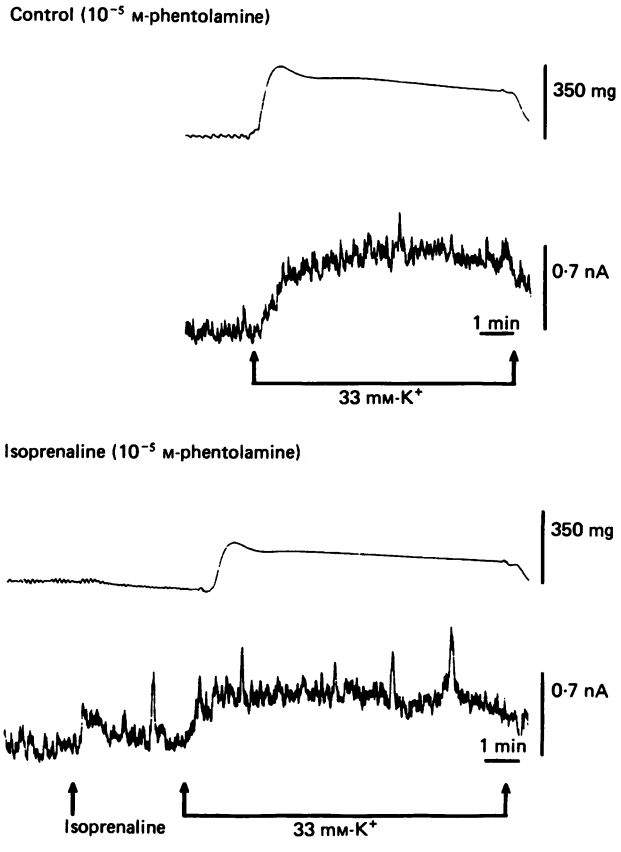

Fig. 8. In both pairs of traces the top panel is force the bottom panel is light. Both pairs of traces were recorded from the same muscle and both are recorded in the presence of 10^{-5} M-phentolamine. In the top pair of traces the control response to 33 mM-potassium is shown. The bottom pair of traces shows the response of the same muscle when pre-treated with 5×10^{-4} M-isoprenaline and then 33 mM-potassium Krebs solution was added in the continual presence of isoprenaline.

5×10^{-5} M, and the results on the aequorin-loaded preparations were qualitatively similar to those presented in Figs. 1 and 2. A decrease in force was accompanied by an increase or no change in the size of the aequorin signal. However, if we increased isoprenaline concentrations another tenfold to 5×10^{-4} M (requiring pre-treatment with 10^{-5} M-phentolamine to prevent α-receptor effects) somewhat different effects were observed (Fig. 8). Phentolamine alone at 10^{-5} M greatly diminished the amplitude of the spontaneous contractions of this muscle, so these results must be interpreted cautiously. The subsequent addition of 33 mM-potassium in the presence of 10^{-5} M-phentolamine produced a typical increase in tone and light levels. When the same muscle was then pre-treated with 10^{-4} M-isoprenaline in the presence of this high concentration of phentolamine, the size of the potassium-induced contraction was decreased compared to the control and the size of the light signal was also

decreased in comparison to the control. Thus, it appears that isoprenaline is capable of decreasing cytoplasmic $[Ca^{2+}]$ in this preparation, but only at very high concentrations.

DISCUSSION

The main finding of this study is that not all relaxations of vascular smooth muscle are associated with decreases in $[Ca^{2+}]_i$ (as detected by aequorin). We have found that isoprenaline, except in very high concentrations, causes an uncoupling of cytoplasmic calcium levels from force production in this smooth muscle. We have also found that two other agents which are associated with increases in cyclic AMP levels in the cell, forskolin and papaverine, caused a similar uncoupling of $[Ca^{2+}]_i$ from force production. In contrast, both extracellular calcium depletion and nitroprusside are associated with parallel decreases in force and $[Ca^{2+}]_i$. Others have demonstrated in skinned muscle preparations that cyclic AMP can shift the calcium–tension curve to the right (Itoh *et al.* 1982; Rüegg *et al.* 1983). We have now demonstrated that agents reported to increase cyclic AMP levels in cells can cause a decrease in the force–$[Ca^{2+}]_i$ ratio in intact smooth muscle cells. These results are also consistent with other reports using a measure of phosphorylase levels as an indication of $[Ca^{2+}]_i$, where phosphorylase levels were found to increase in the presence of isoprenaline while force decreased (Paul & Hellstrand, 1984).

Our present findings must be reconciled with reports of others indicating that in some tissues isoprenaline can decrease the rate of calcium influx (Meisheri & Van Breemen, 1982), increase the rate of calcium uptake into microsomes (Baudouin-Legros & Meyer, 1973; Webb & Bhalla, 1976), or hyperpolarize the cell membrane and decrease electrical spike activity (Somlyo *et al.* 1970; von Loh, 1971; Yamaguchi *et al.* 1982; Prehn & Bevan 1983). All of these effects would be expected to cause a decrease in cytoplasmic $[Ca^{2+}]$. Since we were looking at changes in the response to potassium depolarization we may have missed any effect of a hyperpolarization of the membrane potential under resting conditions. At very high concentrations of isoprenaline (10^{-4} M or above) we were able to detect a slight decrease in cytoplasmic Ca^{2+} responses. This effect may be caused by the increase in calcium efflux reported by others who also have used very high isoprenaline concentrations. The actual dose–response relations of these agents may vary with the different tissues, but our data are consistent with the idea that isoprenaline may have more than one effect which leads to relaxation of the smooth muscle cells; although, the predominant effect in this tissue is an uncoupling of calcium from force production. Multiple effects of isoprenaline would not be unexpected since both β_1- and β_2-receptors may be stimulated. It is intriguing that we were unable to see any sign of a decrease in cytoplasmic calcium levels with the two other agents we tried, forskolin and papaverine, since both of these agents have been reported to increase cyclic AMP levels in the cell. These results suggest that the effects of high concentrations of isoprenaline in this tissue may be unrelated to effects on cyclic AMP levels.

The finding that isoprenaline, forskolin, and papaverine can actually increase cytoplasmic calcium levels was surprising. However, in cardiac muscle, numerous investigators have demonstrated that isoprenaline and cyclic nucleotides can increase the influx of calcium into the cardiac muscle cell (Tsien, 1977). A decrease in the

sensitivity of the contractile apparatus to calcium has also been reported for cardiac muscle (Mope, McClellan & Winegrad, 1980), although the net effect of isoprenaline is to produce a positive inotropic effect on the cardiac muscle cell. Apparently, in cardiac cells, the increase in cytoplasmic Ca^{2+} is sufficiently large to overcome the decrease in the sensitivity of the contractile apparatus. In smooth muscle we may be seeing the two effects in different proportions. In other words, the decrease in the sensitivity of the contractile apparatus to calcium in the portal vein preparation appears to be sufficient to overcome the increase in cytoplasmic calcium levels we observed. In cardiac tissues, the decrease in the sensitivity of the contractile apparatus occurs through a modification of the troponin complex (Stull & Buss, 1977). Although troponin does not appear to exist in smooth muscle, a similar mechanism could be possible, either through an actin-regulated system or a myosin-based regulation of the contractile filaments. Others have suggested that a modification of myosin regulation may occur through a cyclic AMP-mediated phosphorylation of the enzyme myosin light chain kinase (Adelstein *et al.* 1978; Silver & DiSalvo, 1979). Our data are consistent with such an interpretation; however, we emphasize that this is not the only possible interpretation of the data.

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