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EFFECTS OF ISOPROTERENOL ON ACTIVE FORCE AND Ca^{2+}·CALMODULIN-SENSITIVE PHOSPHODIESTERASE ACTIVITY IN PORCINE CORONARY ARTERY*

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Abstract—Relaxation of vascular smooth muscle following beta-adrenergic stimulation may result from reduction of the cytoplasmic Ca^{2+} concentration, reduction in the sensitivity of the contractile apparatus to Ca^{2+}, or both. To help resolve these possibilities, we measured the extent of activation of Ca^{2+}·calmodulin-sensitive phosphodiesterase in intact porcine coronary artery strips as a functional indicator of the cytoplasmic Ca^{2+} concentration. Both calmodulin-stimulated phosphodiesterase activity and active force increased during K^+ stimulation of coronary artery strips. Relaxation of K^+-contracted artery strips following stimulus withdrawal was accompanied by rapid inactivation of Ca^{2+}·calmodulin-sensitive phosphodiesterase. The temporal relationship between isoproterenol-induced relaxation and inactivation of Ca^{2+}·calmodulin-sensitive phosphodiesterase was studied in both histamine- and K^+-contracted tissues. Stimulation of strips with 10 μM histamine or with 44 mM K^+ led to comparable increases both in active force and in calmodulin-stimulated phosphodiesterase activity. Thereafter, sustained contraction elicited by histamine was accompanied by a decrease in calmodulin-stimulated phosphodiesterase activity. Isoproterenol rapidly relaxed histamine-contracted strips and accelerated the decrease in phosphodiesterase activity. In contrast, sustained contraction in response to K^+ was accompanied by a sustained elevation of calmodulin-stimulated phosphodiesterase activity. Isoproterenol treatment of K^+-contracted tissues led to relaxation that was slow and incomplete, and it had very little effect on calmodulin-stimulated phosphodiesterase activity. We conclude that reduction of the cytoplasmic Ca^{2+} concentration is important for rapid relaxation of the coronary artery following beta-adrenergic stimulation. We cannot disallow the possibility that a decrease in the sensitivity of the contractile apparatus to Ca^{2+} is involved to some degree.

It has long been thought that the relaxation of smooth muscle that frequently follows beta-adrenergic stimulation is mediated by an increased rate of cyclic AMP§ production and activation of cyclic AMP-dependent protein kinase. But the subsequent intracellular events leading to inhibition of the contractile apparatus are yet to be demonstrated convincingly. Since muscle contraction results from an increase in the cytoplasmic free Ca^{2+} concentration, hypotheses advanced to explain smooth muscle relaxation by any agent usually fall into two categories: either (1) the cytoplasmic Ca^{2+} concentration is reduced, or (2) the sensitivity of the contractile apparatus to activating Ca^{2+} is reduced. The evidence bearing on the former hypothesis has been summarized recently by Hardman [1]. Regarding the latter possibility, purified myosin light chain kinases from various smooth muscles display decreased sensitivity to activation by Ca^{2+}·calmodulin when phosphorylated by cyclic AMP-dependent protein kinase (see, for example, Refs. 2 and 3). Since phosphorylation of myosin, catalyzed by myosin light chain kinase, is believed to be the primary mechanism by which Ca^{2+} elicits smooth muscle contraction [4], such a change in the activation properties of myosin light chain kinase could conceivably result in relaxation of smooth muscle without a reduction in the cytoplasmic Ca^{2+} concentration. Studies addressing this hypothesis have been reviewed by Kamm and Stull [5]. What additional effects cyclic AMP might exert to modify the sensitivity of the contractile proteins to Ca^{2+} are not known.

We have investigated recently the possibility that a decrease in the cytoplasmic Ca^{2+} concentration might explain the relaxation of porcine coronary artery strips in response to the beta-adrenergic agonist isoproterenol. Saitoh *et al.* [6] have developed a method for estimating the extent of activation of Ca^{2+}·calmodulin-sensitive phosphodiesterase (EC 3.1.4.17) in intact smooth muscle. Their findings strongly suggest that changes in calmodulin-stimulated phosphodiesterase activity reflect changes in the cytoplasmic Ca^{2+} concentration in intact coronary artery. They demonstrated that treatment of histamine-contracted coronary arteries with isoproterenol led to a decrease in phosphodiesterase

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§ Abbreviations: cyclic AMP, adenosine cyclic 3′,5′-phosphate; cyclic GMP, guanosine cyclic 3′,5′-phosphate; EGTA, ethylene glycol bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; PSS, physiological salt solution; and PDE, phosphodiesterase.

activation as well as relaxation. We have further studied these effects of isoproterenol in order to address two questions: (1) when contracting coronary artery strips are stimulated by a beta-adrenergic agonist, does the inactivation of Ca^{2+}·calmodulin-sensitive phosphodiesterase occur at least as rapidly as relaxation of the tissue, and (2) when coronary arteries are stimulated to contract in such a way that relaxation by isoproterenol is slow and incomplete, is the decrease in calmodulin-stimulated phosphodiesterase activity, likewise, slow and incomplete?

Our findings suggest that a decrease in the cytoplasmic Ca^{2+} concentration is the major mechanism for relaxation of coronary artery strips by isoproterenol, but they do not rule out the possibility that decreased sensitivity of the contractile apparatus to Ca^{2+} plays a minor role.

METHODS

Materials. Cyclic GMP, EGTA, trifluoperazine hydrochloride, and (+)-isoproterenol hydrochloride were purchased from the Sigma Chemical Co. Histamine dihydrochloride was purchased from Schwarz/Mann, Inc. Tritiated cyclic GMP was obtained from the New England Nuclear Corp. and further purified by the method of Hardman and Sutherland [7]. Calmodulin was purified from porcine testes by the method of Jamieson and Vanaman [8], slightly modified.

Composition of the physiological salt solution (PSS) used for contraction studies was as follows (in mM): 127 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 16 mM $NaHCO_3$, 2.5 $CaCl_2$, 10 glucose, and 1 pyruvate. The pH of buffer aerated with 95% O_2, 5% CO_2 was 7.4 at 37°. For cold storage of tissues, the bicarbonate concentration was adjusted to maintain pH 7.4. In experiments where buffers with high K^+ concentrations were used, the Na^+ concentrations were lowered in equimolar amounts.

Coronary artery strip preparations. Porcine hearts were carried on ice from the slaughterhouse to the laboratory. The right coronary artery was dissected from each heart. A glass rod was inserted into the arterial lumen, and the vessel was cleaned of loose fat. The arteries were cut into helical strips measuring approximately 4 × 30 mm and stored at 4° in aerated PSS for 16–40 hr before use.

Contraction studies. Each artery strip was suspended, using silk suture, between a mounting hook and a Statham UC-2 isometric force transducer. A jacketed muscle bath, containing 40 ml of continuously aerated PSS at 37°, was positioned around the tissue. Approximately 5 g passive tension was applied to each strip, which was then lengthened isotonically for 1–2 min. The tissues were allowed to equilibrate to a stable passive force of approximately 3 g. The strips were contracted to peak force with PSS containing 133 mM K^+ and then allowed to relax in low-K^+ PSS. This process was repeated until consistent responses to high K^+ were achieved.

Tissues were treated with histamine or isoproterenol by rapidly injecting into the bath 0.4 ml of 1 mM drug dissolved in PSS containing K^+ in appropriate concentration, using a tuberculin syringe fitted with a 20 gauge needle. After experimental treatment, the bath was lowered, and the tissues were frozen by immersion in dichlorodifluoromethane at its freezing point [9]. The frozen strips were stored at −75°.

Measurement of Ca^{2+}·calmodulin-stimulated phosphodiesterase activity. The following procedure is a modification of that described by Saitoh *et al.* [6] for estimating the extent of activation of Ca^{2+}·calmodulin-sensitive phosphodiesterase in frozen intact tissue. The technique involves extraction and assay of enzyme activity at a low temperature and in the presence of trifluoperazine to minimize changes in the degree of association between Ca^{2+}·calmodulin and the phosphodiesterase. Frozen strips (100 ± 20 mg) were powdered as described by Saitoh *et al.* [6]. The powder, having been cooled to liquid nitrogen temperature, was added to 22.5 vol. (ml per g tissue) of 10 mM Tris·HCl, pH 7.5, 3 mM magnesium acetate, and 125 μM trifluoperazine at 0°. The mixture was homogenized immediately with a Polytron PCU-2 homogenizer (Brinkmann Instruments) for 5 sec at speed setting 8. Aliquots (175 μl) of the homogenate were added immediately to 25 μl of one of the following: (1) 125 μM trifluoperazine, (2) 100 μM calmodulin, or (3) 4 mM EGTA (see below). The first aliquots (i.e. less calmodulin or EGTA) were assayed 2 min after homogenization for cyclic GMP phosphodiesterase activity. The calmodulin- and the EGTA-containing aliquots were warmed to 30° for 5 min and then cooled to −3° before assay. This was done to achieve maximum activation by excess calmodulin, or inactivation by EGTA, of Ca^{2+}·calmodulin-sensitive phosphodiesterase. Cyclic GMP phosphodiesterase activity measurements were made, using 1 μM substrate, by the method of Keravis *et al.* [10], except that reactions were carried out for 3 min at −3°.

Quantitation of Ca^{2+}·calmodulin-stimulated phosphodiesterase activity. This procedure yielded measurements of phosphodiesterase activity under three conditions: (1) without added calmodulin, (2) with added calmodulin in an amount sufficient to stimulate the enzyme fully even in the presence of trifluoperazine, and (3) in the presence of EGTA, to estimate Ca^{2+}-independent activity. The activities measured in the first two instances were corrected by subtraction of the activity measured in the presence of EGTA. The percent maximal calmodulin-stimulated PDE activity was then computed as:

$$\frac{\text{Activity } \textit{without} \text{ added calmodulin}}{\text{Activity } \textit{with} \text{ added calmodulin}} \times 100$$

The numerator in this fraction is an estimate of the calmodulin-stimulated activity in the sample tissue at the time of freezing, while the denominator represents the total amount of activity that is sensitive to calmodulin.

Miscellaneous procedures. Protein determinations were made by the method of Bradford [11], using commercially obtained reagent (Bio-Rad Laboratories). Cyclic AMP-dependent protein kinase activity ratios were measured by the procedure of

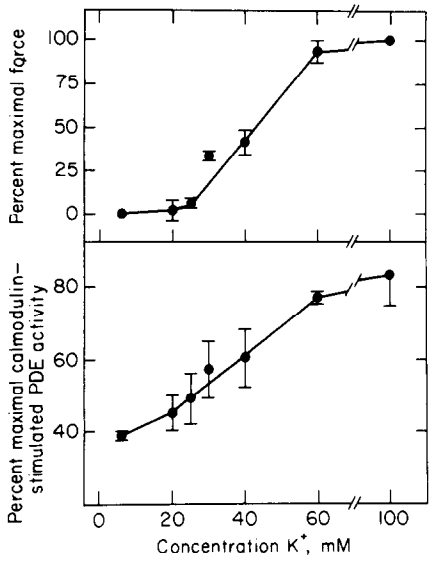

Fig. 1. Concentration-dependent effect of K^+ on percent maximal active force (upper panel) and percent maximal Ca^{2+}·calmodulin-stimulated cyclic GMP phosphodiesterase activity (lower panel) in porcine coronary artery strips. Strips were frozen after reaching maximal active force in response to an increase in K^+ concentration (5–7 min). Percent maximal calmodulin-stimulated phosphodiesterase activity was computed as described under Methods. Error bars indicate ± 1 SEM for three or four strips.

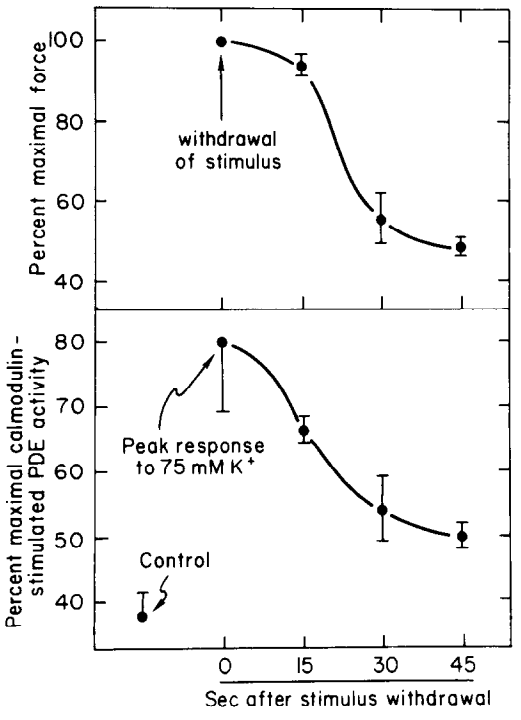

Fig. 2. Time–course of relaxation (upper panel) and inactivation of Ca^{2+}·calmodulin-stimulated cyclic GMP phosphodiesterase (lower panel) following withdrawal of a high-K^+ stimulus. Coronary artery strips were stimulated to contract by raising the K^+ concentration in the bath to 75 mM. At zero time, the medium was replaced with the usual incubation buffer containing 5.9 mM K^+. Strips were frozen, at the times indicated, for measurement of phosphodiesterase activation (lower panel). Other strips were frozen without treatment or at the point of maximum force response to 75 mM K^+ in order to obtain "control" and "peak response" values. Error bars indicate ± 1 SEM for three or four strips.

Corbin [12]. Statistical analysis was performed using two-factor analysis of variance and Student's *t*-test for comparison of unpaired data.

RESULTS

Stimulation of coronary arteries with K^+ at various concentrations. To determine how graded changes in the cytoplasmic Ca^{2+} concentration would affect activation of coronary artery Ca^{2+}·calmodulin-sensitive phosphodiesterase, we stimulated artery strips to contract in the presence of 20–100 mM K^+. In unstimulated tissue, the Ca^{2+}·calmodulin-sensitive phosphodiesterase appeared to be partially activated (to 40% of the maximal extent that the enzyme can be stimulated by calmodulin). As pointed out by Saitoh *et al.* [6], it is unclear whether this represents the extent of activation of the enzyme in unstimulated tissue or further association of the enzyme with Ca^{2+}·calmodulin during homogenization. Potassium elicited concentration-dependent increases in phosphodiesterase activity and active force between 20 and 60 mM (Fig. 1). Furthermore, half-maximal changes in both parameters occurred at about 40 mM K^+. Thus, under the tissue treatment and assay conditions used here, active force production and calmodulin-stimulated phosphodiesterase activity appeared to have similar dependencies upon the cytoplasmic Ca^{2+} concentration.

Inactivation of Ca^{2+}·calmodulin-sensitive phosphodiesterase following withdrawal of K^+ stimulus. Coronary artery strips were contracted fully in a buffer containing 75 mM K^+ and then allowed to relax in low (5.9 mM) K^+ buffer for various periods of time up to 45 sec. Contraction of the tissues was accompanied by an increase in the percent maximal calmodulin-stimulated phosphodiesterase activity from 38 to 80. The decrease in active force and the inactivation of Ca^{2+}·calmodulin-sensitive phosphodiesterase following withdrawal of the high-K^+ stimulus are shown in Fig. 2. While these data do not indicate how rapidly the cytoplasmic Ca^{2+} concentration returned to its control level, they do demonstrate similarity between the time–courses for phosphodiesterase inactivation and tissue relaxation following a decrease in cytoplasmic Ca^{2+}.

Effects of isoproterenol on histamine-contracted coronary artery strips. Artery strips were fully contracted approximately 4 min after the addition of 10 μM histamine. Thereafter, active force declined slowly—by 9% of the maximal histamine-induced response in 2 min (Fig. 3, upper panel). Tissues treated with isoproterenol at the point of peak contraction began to relax in approximately 9 sec, and they had relaxed half-maximally by about 50 sec.

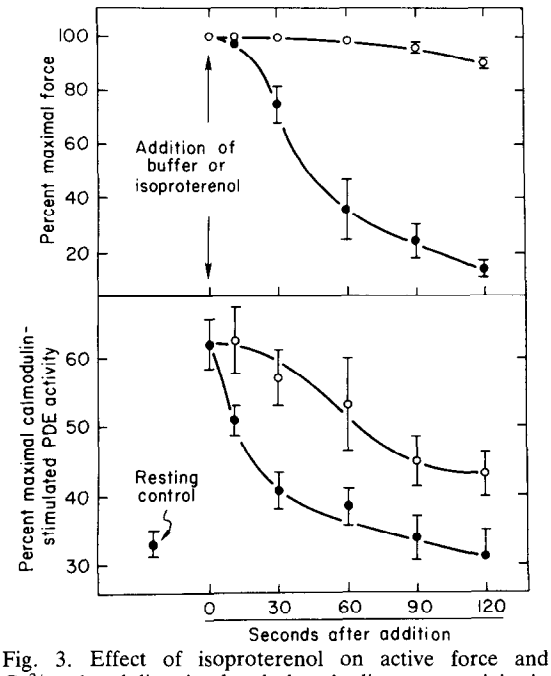

Fig. 3. Effect of isoproterenol on active force and Ca^{2+}·calmodulin-stimulated phosphodiesterase activity in coronary artery strips contracted with histamine. Coronary artery strips were allowed to contract fully in response to 10 μM histamine before isoproterenol at a final concentration of 10 μM (closed circles), or PSS (open circles) in an equivalent volume, was added to the tissue bath. The strips were frozen at the times shown for analysis of phosphodiesterase stimulation. Error bars indicate ± 1 SEM for six to seventeen strips and are omitted in cases of very small SEM. At each of the time points examined, the mean percent maximal calmodulin-stimulated phosphodiesterase activity following isoproterenol treatment differed statistically from that value obtained following buffer treatment ($P < 0.05$).

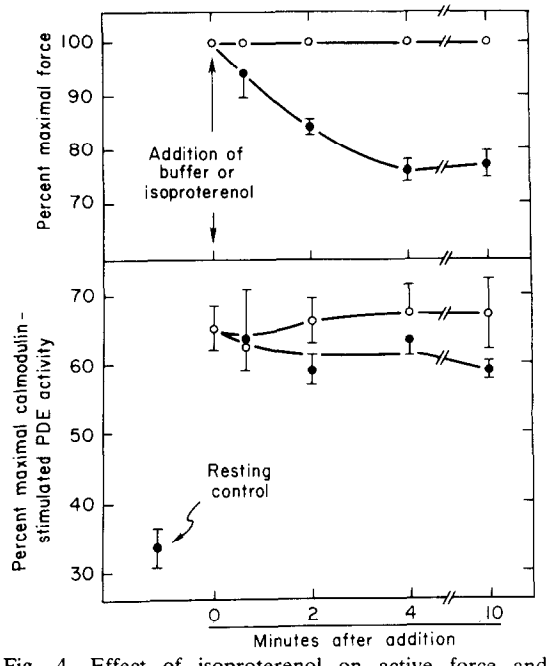

Fig. 4. Effect of isoproterenol on active force and Ca^{2+}·calmodulin-stimulated phosphodiesterase activity in coronary artery strips contracted by increased K^+. Coronary artery strips were contracted fully in response to 44 mM K^+ before isoproterenol at a final concentration of 10 μM (closed circles), or PSS (open circles) in an equivalent volume, was added to the tissue bath. The strips were frozen at the times shown for measurement of phosphodiesterase stimulation. Error bars indicate ± 1 SEM for six to eleven strips and are omitted in cases of very small SEM.

Changes in calmodulin-stimulated phosphodiesterase activity that accompanied these force responses are shown in Fig. 3 (lower panel). Histamine treatment increased the extent of phosphodiesterase activation from 33 to 62%. In the continued presence of histamine, that value fell faster than did active force, returning halfway to the resting-tissue control value in 60–90 sec. Isoproterenol treatment sharply accelerated the decrease in phosphodiesterase activity, which returned halfway to the resting-tissue control value in 10–30 sec. As in the case of the untreated (i.e. no isoproterenol) tissues, the first observed relaxation of isoproterenol-treated strips occurred when the calmodulin-stimulated phosphodiesterase activity had declined about one-third of the way toward resting tissue levels. Isoproterenol treatment did not alter the phosphodiesterase activities measured in the presence of excess calmodulin or EGTA, nor did it alter calmodulin-stimulated phosphodiesterase activities in histamine- or K^+-contracted strips when added to the homogenizing buffer (data not shown); any such effects of isoproterenol would have distorted our estimates of phosphodiesterase activation in the intact tissue.

Effects of isoproterenol on K^+*-contracted coronary artery strips.* When coronary artery strips were stimulated with 44 mM K^+, they produced the same maximal active force that they had produced in response to 10 μM histamine (approximately 80% of the response elicited by 133 mM K^+) in 5–7 min. In contrast to the histamine-contracted strips, K^+-contracted strips exhibited no decline in active force for at least 10 min (Fig. 4, upper panel). When K^+-contracted strips were treated with isoproterenol, the tissues responded much more slowly than had the histamine-contracted tissues, relaxing to about three-fourths of maximal force in 4 min, with no further relaxation between 4 and 10 min.

During stimulation by 44 mM K^+, the percent maximal calmodulin-stimulated phosphodiesterase activity increased by an amount comparable to that seen following treatment with 10 μM histamine, but phosphodiesterase activation did not decrease with sustained K^+ stimulation (Fig. 4, lower panel). And, in keeping with the slow rate of relaxation following treatment with isoproterenol, Ca^{2+}·calmodulin-sensitive phosphodiesterase activation declined only slightly. Two-factor analysis of variance indicated an overall effect in lowering phosphodiesterase activation; Student's *t*-test indicated a significant dif-

ference between buffer- and isoproterenol-treated tissues at 2 min ($P < 0.05$).

To test the possibility that isoproterenol was less effective in K^+-treated tissues because of a diminished ability to stimulate cyclic AMP-dependent protein kinase activity, we measured the effect of 10 μM isoproterenol on cyclic AMP-dependent protein kinase activity ratios in tissues that were contracted with either 10 μM histamine or 44 mM K^+. Within 30 sec, isoproterenol increased the cyclic AMP-dependent protein kinase activity ratio from 0.28 ± 0.02 to 0.34 ± 0.02 in histamine-contracted strips, and from 0.25 ± 0.01 to 0.35 ± 0.01 in K^+-contracted strips ($N = 5$ for each treatment), indicating that 44 mM K^+ stimulation was not an impediment to activation of the cyclic AMP-dependent protein kinase system.

DISCUSSION

Many attempts have been made to determine whether agents that increase intracellular cyclic AMP concentrations in smooth muscle produce relaxation of that tissue by reducing the cytoplasmic free Ca^{2+} concentration. The techniques employed include $^{45}Ca^{2+}$ influx–efflux measurements in both intact smooth muscle cells and isolated smooth muscle membrane vesicles and studies of smooth muscle cells loaded with the Ca^{2+}-sensitive photoprotein aequorin (see, for example, Refs. 13–15). Those approaches have yielded results that were inconsistent and, in some cases, difficult to extend to the situation in intact, contracting smooth muscle. We believe that measurements of the activity of a Ca^{2+}-regulated, cytoplasmic enzyme, Ca^{2+}·calmodulin-sensitive phosphodiesterase, may be usefully applied to this problem for a number of reasons: (1) The technique is simple. It does not necessitate disruption, disaggregation, injection, or hyperpermeabilization of smooth muscle cells prior to the experimental treatment. (2) It is easily applied to contracting smooth muscle strips. (3) It appears to detect changes in the cytoplasmic Ca^{2+} concentration that affect active force development, i.e. activation of Ca^{2+}·calmodulin-sensitive phosphodiesterase and activation of the contractile apparatus exhibited similar Ca^{2+} sensitivities (Fig. 1). (4) Inactivation of Ca^{2+}·calmodulin-sensitive phosphodiesterase occurred as rapidly as inactivation of the contractile apparatus (Fig. 2) and should, therefore, permit comparison of the time courses of cytoplasmic Ca^{2+} reduction and relaxation.

Our measurement of the increases in steady-state active force and in calmodulin-sensitive phosphodiesterase activity elicited by K^+ (Fig. 1) support the generally-held belief that the magnitude of force developed by smooth muscle depends upon the intracellular Ca^{2+} concentration. However, during the sustained phase of contraction, the intracellular Ca^{2+} concentration apparently remained constant or decreased, depending on the nature of the stimulus. Over the period of time during which active force was constant in response to histamine stimulation, the cytoplasmic Ca^{2+} concentration appeared to fall considerably. That these results are not attributable merely to slow inactivation of the contractile apparatus relative to Ca^{2+}·calmodulin-sensitive phosphodiesterase is evident from a comparison with the experiment illustrated in Fig. 2; where the Ca^{2+} concentration was reduced more rapidly, relaxation followed quickly. Our data are consistent with the notion that receptor-mediated stimulation of smooth muscle results in a transient increase in the cytoplasmic Ca^{2+} concentration, after which a high level of active force may be maintained at a much lower Ca^{2+} concentration. Morgan and Morgan [15], measuring intracellular Ca^{2+} directly, have previously observed similar effects in vascular smooth muscle cells stimulated with phenylephrine or angiotensin. Our data are also consistent with the observed temporal relationships between active force and other Ca^{2+}-dependent processes, such as myosin light chain phosphorylation and stimulation of phosphorylase kinase activity [16, 17].

Once maximal active force had been achieved in response to 44 mM K^+, phosphodiesterase activity remained unchanged for at least 10 min. We conclude from these data that the average cytoplasmic free Ca^{2+} concentration did not decline during the period of sustained force in the presence of K^+. This conclusion is also supported by the work of others who have assessed changes in intracellular Ca^{2+} levels or Ca^{2+}-dependent processes during K^+ stimulation of smooth muscle [18, 19].

When studying the time course of isoproterenol effects in histamine-contracted tissues, we concentrated on the 2 min after steady-state active force had been achieved. During this period, active force was constant within 10% of its maximal value. Isoproterenol significantly accelerated the inactivation of Ca^{2+}·calmodulin-sensitive phosphodiesterase, compared to the decrease in buffer-treated tissues (Fig. 3). The time–course of this effect was consistent with the hypothesis that beta-adrenergic stimulation of coronary smooth muscle produces or accelerates relaxation by increasing the rate of removal of Ca^{2+} from the cytoplasm.

While the rapid removal of cytoplasmic Ca^{2+} in response to isoproterenol may indicate that this effect is *involved* in relaxation of coronary smooth muscle, it does not establish that it is *necessary* in order for relaxation to occur. We therefore studied the effect of isoproterenol treatment of K^+-contracted tissues. We suspected that isoproterenol would have little effect on the cytoplasmic Ca^{2+} concentration in this case because it produces slow and incomplete relaxation. Indeed, treatment of K^+-contracted tissues with isoproterenol produced very little change in the calmodulin-stimulated phosphodiesterase activity. This indicates that reduction of the cytoplasmic Ca^{2+} concentration may be essential for rapid relaxation. We cannot exclude the possibility that some effect on the contractile proteins occurred that led to a reduction in Ca^{2+} sensitivity, since some degree of relaxation was observed with very little apparent change in the Ca^{2+} concentration.

For the purpose of this study, it is essential that beta-adrenergic stimulation of the porcine coronary

artery does not alter the extent of calmodulin-stimulated phosphodiesterase activity apart from its effect on the cytoplasmic Ca^{2+} concentration. Sharma and Wang [20] have reported recently that Ca^{2+}·calmodulin-sensitive phosphodiesterase purified from bovine brain is slowly phosphorylated by cyclic AMP-dependent protein kinase catalytic subunit and that the affinity of the enzyme for calmodulin is consequently reduced. Phosphorylation of activated (i.e. bound to Ca^{2+}·calmodulin) phosphodiesterase did not occur. We do not believe that such an effect explains the results shown in Fig. 3. Had isoproterenol treatment led to a decrease in the sensitivity of the phosphodiesterase to activation by Ca^{2+}·calmodulin, this effect would have been evident as a decrease in phosphodiesterase activity following isoproterenol treatment of artery strips contracted with 44 mM K^+ (Fig. 4). In fact, isoproterenol treatment did not result in rapid inactivation of Ca^{2+}·calmodulin-sensitive phosphodiesterase, even to the extent that would be expected to accompany the slow relaxation of the tissue. Furthermore, the isoproterenol-induced increases in the activity of cyclic AMP-dependent protein kinase were indistinguishable in histamine- and K^+-contracted strips. We conclude, therefore, that isoproterenol treatment of the coronary artery strips did not result in a decrease in sensitivity of the phosphodiesterase to Ca^{2+}·calmodulin.

In summary, we have measured the extent of activation of Ca^{2+}·calmodulin-sensitive phosphodiesterase in intact coronary artery strips as a functional indicator of the cytoplasmic Ca^{2+} concentration. Rapid relaxation of histamine-contracted tissues by isoproterenol was accompanied by an accelerated rate of decrease in calmodulin-stimulated phosphodiesterase activity, whereas slow and incomplete relaxation of K^+-contracted strips by isoproterenol was accompanied by only a slight effect on phosphodiesterase activation. We conclude that reduction in the cytoplasmic Ca^{2+} concentration is essential for rapid relaxation of coronary smooth muscle by isoproterenol and is a major, but perhaps not the only, mechanism by which beta-adrenergic agents inhibit smooth muscle contraction.

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