Biochemical Pharmacology, Vol. 24, pp. 9–12. Pergamon Press, 1975. Printed in Great Britain.

RELATIONSHIPS BETWEEN Ca^{2+} UPTAKE BY A MICROSOMAL FRACTION OF GUINEA-PIG *TAENIA CAECUM* AND ITS RELAXATION

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(*Received* 2 *April* 1974; *accepted* 31 *May* 1974)

Abstract—A microsomal fraction isolated from guinea-pig *taenia caecum* can accumulate calcium ions in the presence of ATP and $MgCl_2$. This calcium-accumulating ability depends on the ATP concentration in the reaction mixture, and its pH optimum is near 7·8. Procaine and quinidine inhibit Ca^{2+} accumulation by fraction and suppress the relaxation of this tissue induced by papaverine, isoprenaline and phenylephrine. These results suggest that Ca^{2+} accumulation by the microsomal fraction isolated from guinea-pig *taenia caecum* plays an important role during the relaxation of this smooth muscle. Cyclic adenosine 3′,5′-monophosphate (cAMP) does not accelerate Ca^{2+} accumulation by the microsomal fraction. The failure of cAMP to accelerate Ca^{2+} uptake by this fraction implies that some unknown steps or factors may exist between the intracellular increment of cAMP and the following relaxation of *taenia caecum*.

Calcium ions regulate the contraction and relaxation of smooth muscle induced by drugs [1–4]. It has been suggested that the basic mechanisms for the relaxation of smooth muscle are qualitatively the same as those for skeletal muscle; that is, relaxation is probably due to a removal of calcium from the contractile proteins.

Recently Gabella [5] and Devine *et al.* [6] have discovered a sarcoplasmic reticulum of smooth muscle similar to that of skeletal muscle. The microsomal fraction obtained from several smooth muscle tissues has been reported to be able to take up calcium ions in the presence of ATP and $MgCl_2$ [7–9]. The present study describes the ability of a microsomal fraction isolated from guinea-pig *taenia caecum* to take up calcium and the relationship between relaxation and Ca^{2+} uptake by this fraction, using agents which inhibit Ca^{2+} uptake.

Since it is clear that cyclic adenosine 3′,5′-monophosphate (cAMP) is involved in the regulation of smooth muscle tone [10–13], the effect of cAMP on Ca^{2+} uptake by this fraction also has been studied.

METHODS

About 10 guinea-pigs were sacrificed, at one time, by a blow on the head; the *taenia* was immediately removed from the *caecum* and washed with ice-cold 0·25 M sucrose solution. Approximately 2 g of the *taeniae* was cut into small pieces with a pair of scissors and placed in a test tube containing 10 vol of 0·25 M sucrose solution. The suspension was homogenized three times, employing a Polytron (PT-10, Brinkman Instruments), with a rheostat setting of 9 for 5 sec. The entire procedure was carried out in crushed ice. The resulting homogenate was centrifuged at 1000 *g* for 15 min. The supernatant fluid was centrifuged at 10,000 *g* for 15 min and the pellet obtained was sometimes used as a crude mitochondrial fraction; then the new supernatant was centrifuged again at 105,000 *g* for 60 min. The pellet obtained was rehomogenized before use with a glass homogenizer with Teflon pestle.

Ca^{2+} uptake was studied at 32° in 2·5 ml of Tris–HCl buffer (pH 7·4) containing 20 μM $CaCl_2$, 10 μCi ^{45}Ca, 30 mM KCl, 3 mM $MgCl_2$, 3 mM ATP, 5 mM sodium oxalate, in final concentrations, and the microsomal fraction. In some experiments, 5 mM sodium oxalate was omitted. The reaction was started by adding ATP and $CaCl_2$ to the reaction mixture, and stopped by filtering an aliquot on a Millipore filter (HA, 0·45 μm) which had previously been rinsed in 0·25 M KCl, according to the procedure of Palmer and Posey [14]. The Millipore filter was dried and dissolved in 2 ml methylcellosolve. This was dissolved in 15 ml toluene scintillator, which contained 4 g 2,5-diphenyloxazole, 0·2 g 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene, 300 ml methanol and 700 ml toluene. The radioactivity was counted in a Packard Tri-Carb liquid scintillation counter (model 3203) maintained at 6°. Ca^{2+} uptake was estimated from radioactivity on the filter. The drugs were applied 3 min before starting the reaction. Protein was determined by the method of Lowry *et al.* [15], using bovine albumin as a standard. The amount of protein contained in the incubation fluid was adjusted to 200 μg/ml. All solutions were prepared by using double-distilled water.

To examine the effects of procaine and quinidine on the relaxation induced by papaverine, isoprenaline and

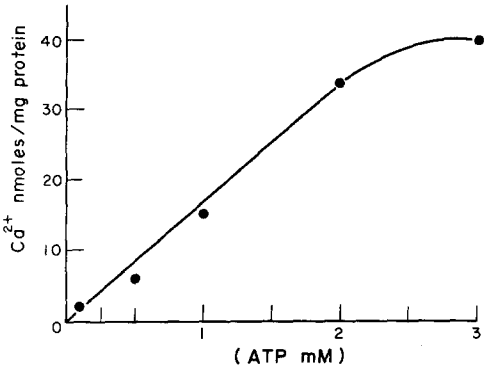

Fig. 1. Uptake of ^{45}Ca as a function of ATP concentration in the reaction mixture. Microsomes were incubated for 10 min in the presence of various ATP concentrations at 32°.

phenylephrine, the *taenia caecum* was incubated in Locke–Ringer solution containing 9·0 g NaCl, 0·4 g KCl, 0·2 g $CaCl_2$, 0·2 g $MgCl_2$, 0·5 g $NaHCO_3$ and 0·5 g glucose in a liter, gassed with air and kept at 32°. Movements of the smooth muscle were recorded on a smoked drum isotonically.

The following compounds were used: ATP (Sigma), isoprenaline hydrochloride (Kaken Kagaku), phenylephrine hydrochloride (Kowa Chemical Industries), ruthenium red (Wako Pure Chemical Industries), quinidine sulfate (Tokyo Kasei Kogyo), procaine hydrochloride (Wako Pure Chemical Industries) and cAMP (Kyowa Hakko).

RESULTS

Effect of procaine and quinidine on Ca^{2+} uptake. Figure 1 illustrates the dependency of Ca^{2+} uptake on the ATP concentration in the reaction mixture. Incubation time was 10 min, which was the time necessary to take up Ca^{2+} submaximally at a given concentration of Ca^{2+}. Ca^{2+} uptake by the microsomal fraction increases linearly up to 2 mM ATP and reaches a maximum at 3 mM ATP. Also Ca^{2+} uptake by the microsomes was dependent upon temperature, and its pH optimum for Ca^{2+} uptake was 7·4 (not shown).

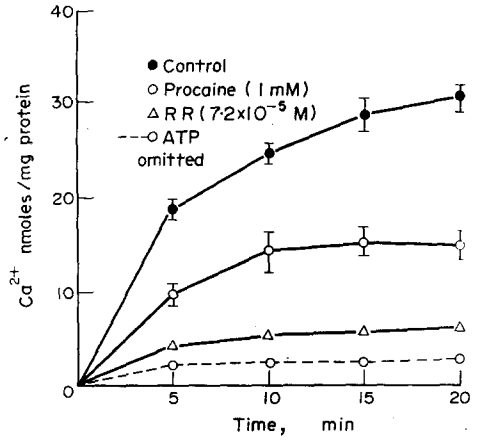

Fig. 2. Effects of 1 mM procaine (—○—), 7·2 × 10^{-5} M ruthenium red (—△—) and ATP omitted (---○---) on Ca^{2+} uptake. Procaine inhibits Ca^{2+} uptake significantly at any incubation time ($P < 0·01$) and ruthenium red inhibits almost completely. Vertical bars indicate the standard error of the mean. The number of observations at each point is between 5 and 7, except in the case of ATP omitted (N = 2).

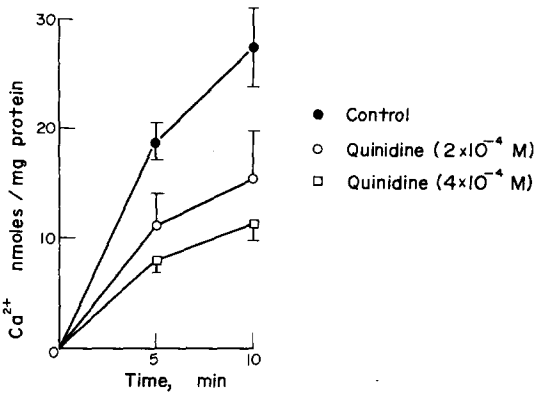

Fig. 3. Effects of 2 × 10^{-4} M quinidine (—○—) and 4 × 10^{-4} M quinidine (—□—) on Ca^{2+} uptake by the microsomal fraction. At each point, Ca^{2+} uptake is significantly inhibited ($P = 0·01$) compared with the control value. Vertical bars indicate the standard error of the mean (N = 6 or 7).

Procaine (1 mM) can suppress Ca^{2+} uptake by this fraction significantly ($P < 0·01$) and ruthenium red, a specific Ca^{2+} ATPase inhibitor, also inhibits Ca^{2+} uptake almost completely, as shown in Fig. 2.

Besides procaine, quinidine (Fig. 3) can also suppress significantly ($P < 0·01$) microsomal Ca^{2+} uptake in the presence of ATP and $MgCl_2$.

Effect of procaine and quinidine on the relaxation of taenia caecum *induced by drugs.* In order to determine

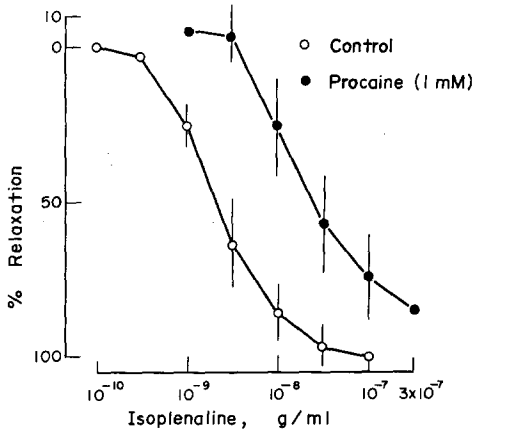

Fig. 4. Effect of procaine (1 mM) on the relaxation induced by isoprenaline. Procaine shifts the dose–response curve of isoprenaline to higher concentrations. Vertical bars indicate the standard error of the mean (N = 6).

whether or not there is a correlation between the inhibition of Ca^{2+} uptake observed in the previous experiments and the relaxation of smooth muscle, the following studies were performed.

Figure 4 presents the effect of procaine on the relaxation of isolated *taenia caecum* induced by isoprenaline. In the presence of procaine (1 mM), the dose–response curve of isoprenaline is shifted to its higher concentration. Because concentrations of procaine higher than 1 mM sometimes induce contraction, 1 mM procaine was used.

Since the α-adrenergic stimulant, phenylephrine, can also relax the guinea-pig *taenia caecum*, the effect of procaine on phenylephrine-induced relaxation was also examined. Procaine (1 mM) shifts the dose–response curve of phenylephrine to higher concentrations. Papaverine is a potent smooth muscle relaxant and is said to have a direct action. This means that papaverine induces its effect through neither α-adrenoceptors nor β-adrenoceptors. The mechanisms of action of papaverine have been considered to be mediated by cAMP through inhibition of phosphodiesterase, which hydrolyzes cAMP [16–18]. Procaine (1 mM) and 10^{-4} M and 2×10^{-4} M quinidine (Fig. 5) can inhibit the relaxation induced by papaverine and shift the dose–response curve to the right.

From these results, it can be said that drugs which can suppress Ca^{2+} uptake by the microsomal fraction of guinea-pig *taenia caecum* can also nonspecifically inhibit relaxation of this tissue induced by three kinds of relaxants. Therefore, it is apparent that Ca^{2+} uptake by this microsomal fracion may play an essential role during relaxation of this tissue.

Effects of cAMP on Ca^{2+} uptake by microsomes. Since there is evidence that cAMP levels are elevated during the relaxation of smooth muscle by isoprenaline and papaverine [10, 13], the following experiments were performed to determine whether or not cAMP could increase Ca^{2+} uptake. As shown in Fig. 6,

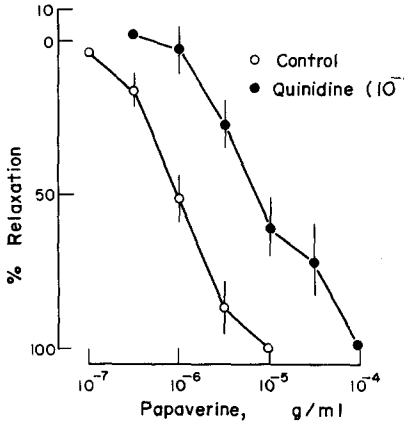

Fig. 5. Effect of quinidine (10^{-4}M) on the relaxation induced by papaverine. Quinidine shifts the dose–response curve of papaverine to higher concentrations. Vertical bars indicate the standard error of the mean (N = 7).

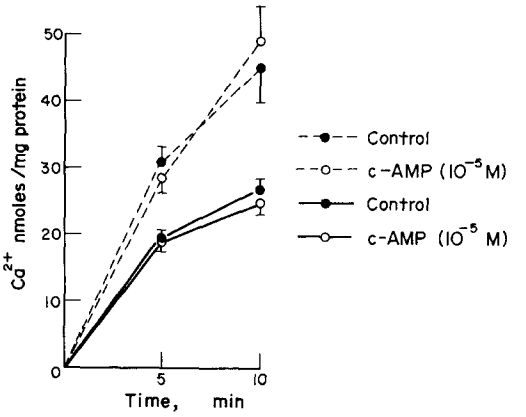

Fig. 6. Effects of 10^{-5}M cAMP (open circles) on Ca^{2+} uptake by the microsome in the presence (broken lines) and absence (solid lines) of 5 mM sodium oxalate. Vertical bars indicate the standard error of mean (N = 7).

10^{-5}M cAMP failed to potentiate Ca^{2+} uptake both in the presence and in the absence of oxalate, which increases Ca^{2+} uptake by precipitating Ca^{2+} in the vesicle.

DISCUSSION

Recently Devine *et al.* [6] and Gabella [5] have found the intracellular membrane structures in smooth muscle to be similar to those of skeletal muscle. The microsomal fractions from several kinds of smooth muscle have the ability to accumulate calcium ions in the presence of ATP[7–9]. Studies to determine whether Ca^{2+} uptake by microsomal fractions is done by plasma membrane, sarcoplasmic reticulum or by both, are in progress, using several smooth muscle tissues. There is, however, no study concerned with the relationship between relaxation of smooth muscle and Ca^{2+} uptake by the microsomal fraction. The present study has demonstrated that Ca^{2+} uptake by this fraction might play a role during relaxation of *taenia caecum*, because drugs which suppress Ca^{2+} uptake also inhibit relaxation induced by isoprenaline, phenylephrine and papaverine. Recently, Needleman *et al.* [19] postulated in their report on relaxation of rabbit aortic strips that the common vasodilator intermediate site, which contains sulfhydryl groups, is involved in various drug-induced vasodilations. The intermediate site they proposed may be close to the microsomal fraction that can sequester calcium ions, because in our study (unpublished data) *N*-ethylmaleimide, one of the sulfhydryl-reactive compounds, inhibits Ca^{2+} uptake completely.

Ruthenium red, which has been reported to be a specific Ca^{2+}-ATPase inhibitor[20], suppressed Ca^{2+} uptake almost completely (Fig. 2). As has already been reported, it can competitively inhibit only the action of isoprenaline and not those of phenylephrine and papaverine [21]. We concluded, therefore, that ruthenium

red cannot enter the cell, as reported by Luft [22], and can inhibit Ca^{2+} uptake only in subcellular experiments.

Several authors have observed [10, 13] elevation of cAMP levels associated with the initiation of relaxation induced by isoprenaline and papaverine. There has been controversy, however, as to whether cAMP can activate Ca^{2+} uptake by the microsomal fraction. Andersson [13], using the microsomal fraction from rabbit colonic muscle, found that the calcium-accumulating capacity of the microsome was potentiated by cAMP. On the other hand, Katz and Repke [23] pointed out the requirement of protein kinase for potentiation by cAMP in their study of the microsomal fraction from cat heart. In our study, cAMP failed to increase Ca^{2+} uptake. The discrepancy between these results suggested that there might be some unknown steps or factors which link the increment of cAMP in the cell to potentiation of Ca^{2+} uptake by the microsomal fraction. Though Katz and Repke [23] used soluble protein kinase, it has been reported more recently that isolated membranes from some tissues contain endogenous protein kinase [24, 25]. Phosphorylation of membrane-bound protein by protein kinase may participate in regulation of membrane ion permeability and binding.

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