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EFFECTS OF PAPAVERINE ON THE RELATIVE DISTRIBUTION OF ^{45}Ca IONS IN SUBCELLULAR FRACTIONS OF HOG BILIARY MUSCLES

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Abstract—The mechanisms by which papaverine (Pap) relaxes smooth muscles of hog bile ducts were investigated with respect to the effects of Pap on the distribution of Ca ions in intracellular organelles. Muscles loaded with ^{45}Ca ions in 139 mM KCl–Tyrode's solution were incubated with Pap, [^{3}H]Pap or theophylline (ThP) in Tyrode's solution. The Pap and Ca contents of various subcellular fractions were measured. Pap (40 μM) had no effect on Ca influx into the muscle in normal and 139 mM KCl–Tyrode's solutions. Pap was distributed to a greater extent in the cytosol fraction (F_4) than in microsome and mitochondria-enriched fractions (F_3) and in the nuclei-enriched fraction (F_2). Pap selectively accumulated in the plasma membrane-enriched fraction (PM-F) and was negligible in the sarcoplasmic reticulum-enriched fraction (SR-F) and the mitochondria-enriched fraction (Mit-F). The Ca release of PM-F was greater and more rapid than the release from SR-F or Mit-F. The increase in the content of Ca in PM-F induced by Pap was enhanced by oxalate, suggesting that the increase is based on the increase in Ca uptake, not on the reduced efflux. Pap increased dose-dependently the Ca content of PM-F and appeared to decrease the Ca contents of SR-F and Mit-F. ThP and cyclic AMP (4 μM) had no effect on the Ca uptake of PM-F and no influence on the effects of Pap. These results suggest that Pap relaxation of hog biliary smooth muscle results from the interaction of the drug with the plasma membrane causing cyclic AMP-independent sequestration of Ca.

Papaverine (Pap) is a non-specific smooth muscle relaxant, and the molecular mechanism of this action is not yet known despite numerous investigations. Thus there is no concensus regarding the mechanism of Pap-induced relaxation of smooth muscle.

Since the first finding in 1970 by Kukovetz and Pöch [1, 2], Pap has been increasingly described as inhibiting phosphodiesterase (PDE) activity of smooth muscle, causing a rise of cyclic AMP level. Recently, the competitive inhibition of Pap on cyclic AMP-PDE of smooth muscle was confirmed and it was suggested that the veratrylpyridine moiety in the Pap molecule was required [3]. On the other hand, since Pap antagonizes contractions produced by a number of stimulant substances acting at different receptors, it would appear that either Pap reduces Ca entry or that it antagonizes the effect of Ca at intracellular sites [4–7]. Pap has been reported to increase ^{45}Ca efflux and ^{45}Ca influx in polarized smooth muscle but to decrease ^{45}Ca influx in response to rising external Ca in high K solution [8–11]. However, the interpretation of these results on Ca fluxes is described as uncertain in the review of Bolton [12].

All of the evidence on Ca action was based on the inhibition by Pap of increasing Ca influx induced by stimulants or by raising external Ca concentrations. Pap normally relaxes smooth muscle in addition to reducing the contractile response to stimulant agents. Both of these responses induced by Pap seem to suggest the possibility of differences in the mechanisms of action with respect to Ca ions. The normal relaxation may occur by a mechanism by which Pap alters the distribution of Ca in the subcellular fractions without influencing the flux of external Ca ions. This mechanism is cyclic AMP-independent. Because Pap-relaxed biliary muscle increases Ca uptake by the sarcoplasmic reticulum fraction even in the presence of a saturating concentration of cyclic AMP [13], the possibility of a cyclic AMP-independent mechanism associated with intracellular calcium in relaxation induced by Pap is suggested. To obtain further data on this suggestion, the present studies were concerned with an investigation of the effects of Pap on the intracellular ^{45}Ca in hog biliary muscle.

MATERIALS AND METHODS

^{45}Ca loading and treatment of drugs in biliary muscle. Fatty tissues, connective tissues and blood vessels were removed from the hog terminal bile duct (0.8 g) following which the tissue was preincubated in Tyrode's solution containing 5 mM ATP for 60 min at 37°. 95% O_2–5% CO_2 was bubbled continuously in the organ bath. The ducts were then incubated in 139 mM KCl or normal Tyrode's solution containing $^{45}CaCl_2$ (0.2 μCi/ml) under the same conditions. In experiments to measure Ca influx, the ducts were incubated in these solutions with or without Pap (40 μM) for 10 min. The Ca-loaded bile ducts were blotted dry with polyethylene filters and incubated in Tyrode's solution with Pap or [^{3}H]Pap

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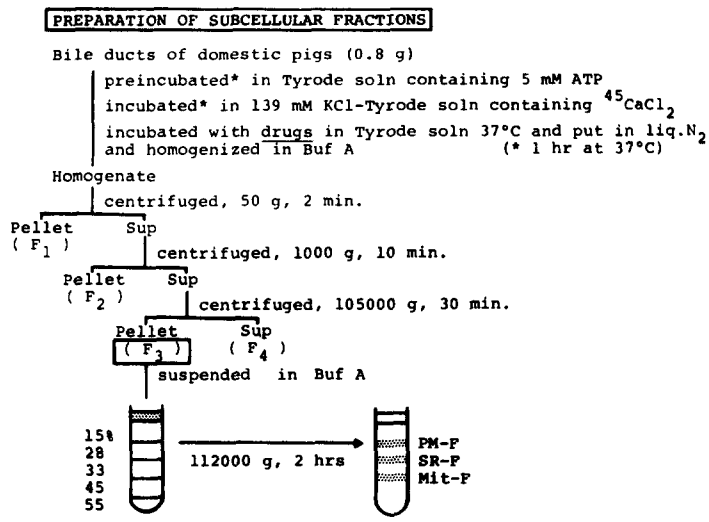

Fig. 1. Outline of the procedures used.

(1 μCi/ml) at 37°. Theophylline (ThP) was added 10 min before treating with Pap. The reaction was stopped by immersing the tissues in liquid N_2.

Preparation of subcellular fractions of bile ducts. Procedure I: Subcellular fractions of bile ducts were prepared by the procedure of Janis *et al.* [14] with some modifications. The Ca-loaded muscles were minced and homogenized with 13 vols of 40 mM histidine–HCl (pH 7.0) containing 250 mM sucrose. Figure 1 shows the outline of the procedure used. After the homogenate was centrifuged at 50 *g* for 2 min, the supernatant was centrifuged at 1000 *g* for 10 min to obtain fraction 1 (F_1), which was enriched with cell debris, and fraction 2 (F_2), which was nuclei-enriched. This supernatant was centrifuged at 105,000 *g* for 30 min, giving fraction 3 (F_3), which contained plasma membrane, sarcoplasmic reticulum and mitochondria, and supernatant fraction (F_4). F_3 (0.25 ml) was suspended in the same buffer and carefully layered on the sucrose gradient. The gradient was composed of (from the bottom of the polyallomer tube upwards) 55% (w/w) sucrose (0.5 ml) and 45, 33, 28, and 15% (w/w) sucrose (1 ml), respectively. The density gradient preparation was centrifuged at 112,000 *g* for 120 min in a RPS 50 rotor (Hitachi) to obtain the plasma membrane-enriched fraction (PM-F), the sarcoplasmic reticulum-enriched fraction (SR-F) and the mitochondria-enriched fraction (Mit-F). All of these procedures were performed at 2–4°.

Procedure II: PM-F, SR-F and Mit-F were prepared by the procedure of Katz and Repke [15] with some modifications. The bile ducts were minced and homogenized with 2.5 vols of ice-cold 5 mM Tris–oxalate, 0.3 M sucrose and 5 mM histidine (pH 7.4). After the homogenate was centrifuged at 1000 *g* for 15 min, the supernatant was centrifuged at 10,000 *g* for 20 min. This pellet (Mit-F) was suspended with 1 mM Tris–oxalate, 0.3 M sucrose and 5 mM histidine (pH 7.4) and the supernatant was centrifuged at 105,000 *g* for 90 min to obtain the microsomal fraction. This fraction was suspended in 1 mM Tris–oxalate, 0.3 M sucrose and 5 mM histidine (pH 7.4) and layered on sucrose gradient consisting of 35% (w/w) sucrose (2 ml) and 20% (w/w) sucrose (2 ml). The density gradient preparation was centrifuged at 50,000 *g* for 120 min in a RPS 40 rotor (Hitachi). PM-F was obtained in 20–35% sucrose and SR-F was in 35% sucrose.

Activity of marker enzyme for subfraction. The activity of 5'-nucleotidase, used as a marker enzyme for the plasma membrane, was determined by the

Table 1. Comparison of specific activities of marker enzymes in subcellular fractions of hog biliary muscles

Fractions	5'-nucleotidase[a]	NADPH-cytochrome *c* reductase[b]	Succinate-cytochrome *c* reductase[b]
Plasma membrane-enriched fraction	2.68 ± 0.43	3.08 ± 1.11	0.11 ± 0.10
Sarcoplasmic reticulum-enriched fraction	1.57 ± 0.36	8.18 ± 1.16	0.97 ± 0.10
Mitochondria-enriched fraction	0.32 ± 0.09	3.39 ± 0.71	18.36 ± 1.54

Each value represents the mean ± S.E. in three different preparations.
[a] unit: μmole Pi/mg protein per 15 min.
[b] unit: nmole cytochrome reduced/mg protein per min.

procedure of Song and Bodansky [16]. The assay medium (0.5 ml) contained 100 mM Tris–HCl buffer (pH 7.5), 10 mM $MgSO_4$, 5 mM 5′-AMP and subfractions (7.5–48.0 μg protein). The reaction was incubated at 37° for 15 min and stopped by the addition of ice-cold 20% trichloroacetic acid (0.5 ml). Released inorganic phosphate from 5′-AMP was determined by the method of Martin and Dorty [17] using NaH_2PO_4 as a standard. The activities of NADPH-cytochrome *c* reductase, a marker enzyme for endoplasmic reticulum [18], and succinate-cytochrome *c* reductase, a marker enzyme for mitochondria, were determined by the procedure of Scottocasa *et al.* [19]. The assay mediums (1.5 ml) contained 0.1 mM NADPH or 3 mM succinate, 0.033 mM cytochrome *c*, 0.3 mM KCN, 50 mM phosphate buffer (pH 7.0) and subfractions (14.9–96.0 μg protein). Table 1 shows the specific activities of these enzymes in the subcellular fractions of the muscles.

Assay for Ca *uptake.* Ca uptake assay was performed by filtration through a millipore filter (0.45 μm), as previously described [13]. The final composition of the reaction medium (0.5 ml) was 40 mM histidine–HCl buffer (pH 6.8), 120 mM KCl, 2.5 mM Tris–oxalate (pH 6.8), 5 mM ATP, 5 mM $MgCl_2$, subcellular fractions (11.2–25.1 μg protein) and Ca-EGTA buffer (Ca^{2+} = 1 μM; EGTA 391 μM, $CaCl_2$ 125 μM containing 0.08 μCi/ml $^{45}CaCl_2$) with or without a saturable concentration of cyclic AMP (4 μM). The effects of Pap were investigated in two ways. First, the effects of Pap on tissue levels of Ca were investigated following incubation of tissues for 30 min at 37°. Secondly, Ca uptake in tissue subfractions was investigated with Pap being added 10 min before assay of the uptake.

Detection of bound [^{3}H]*Pap in PM-F.* Bound [^{3}H]Pap in PM-F obtained from the muscles incubated with 120 μM [^{3}H]Pap for 30 min was detected by filtration through a Sephadex G-25 column (1 × 36 cm) equilibrated with 10 mM phosphate buffer (pH 7.4).

Measurement of radioactivity. Radioactivity of ^{45}Ca or [^{3}H]Pap in the subfractions was measured in Bray's solution by a liquid scintillation spectrometer.

Determination of protein concentration. Protein concentration of the subfractions was measured by the procedure of Lowry *et al.* [20] using bovine serum albumin as a standard.

Materials. Papaverine hydrochloride 3′,4′-methoxy-^{3}H (209 mCi/mmole) was prepared by the Laboratory of Kanebo Ltd. The other compounds used were $^{45}CaCl_2$ (34.4 mCi/mg Ca) (New England Nuclear), papaverine hydrochloride, theophylline, succinic acid monosodium salt (Nakarai), adenosine 5′-monophosphoric acid, cytochrome *c* (Type III) (Sigma), NADPH and cyclic AMP (Kohjin).

RESULTS

^{45}Ca *loading in biliary muscle in the presence and absence of papaverine*

In Tyrode's solution with high KCl (139 mM), the ^{45}Ca contents of F_4, F_3, F_2 and F_1 in hog biliary muscles were measured for 1 hr at 37°. The ^{45}Ca contents in F_3 were significantly increased by changing the KCl concentrations (from 2.7 to 139 mM) as well as in F_1 (Fig. 2). Those in F_2 or F_4 were not significantly different between both KCl concentrations.

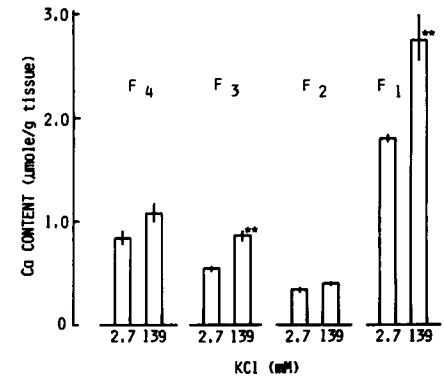

Fig. 2. Effect of 139 mM KCl on the Ca content of fractions enriched in cytosol (F_4), fractions enriched in microsome and mitochondria (F_3), fractions enriched in nuclei (F_2) and fractions enriched in cell debris (F_1) isolated from hog terminal bile ducts, with means ± S.E. (n = 3). The muscles were incubated in normal or 139 mM KCl–Tyrode's solution with $^{45}CaCl_2$ (0.1 μCi/ml) for 1 hr at 37°. ** indicates significantly different values from those in normal Tyrode's solution at $P < 0.01$. Note that the increase in Ca content in F_3 by 139 mM KCl was larger than other fractions except F_1.

The ^{45}Ca contents of the above fractions were measured to examine the effect of Pap on biliary muscle incubated in normal and 139 mM KCl–Tyrode's solution for 10 min. Pap (40 μM) had no significant effect on the ^{45}Ca contents in any of the fractions, even with high KCl as shown in Fig. 3. This indicates that Pap did not inhibit Ca influx in hog biliary muscle under these conditions.

Distribution of [^{3}H]*papaverine in the intracellular organelle in hog biliary muscle*

The time course of distribution of [^{3}H]Pap was investigated. Of the fractions obtained after applying 120 μM [^{3}H]Pap to hog biliary muscle, F_4 contained

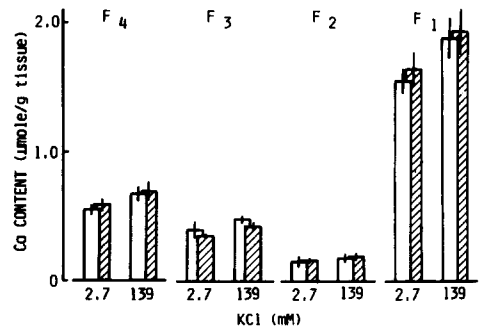

Fig. 3. Effect of papaverine (Pap) on the Ca content in F_4, F_3, F_2 and F_1 of biliary muscles incubated in normal and 139 mM KCl-Tyrode's solutions, with means ± S.E. (n = 3). The muscles were incubated with (hatched columns) or without (open columns) Pap (40 μM) in the solutions for 10 min at 37°. Note that Pap did not inhibit Ca influx in hog biliary muscle under these conditions.

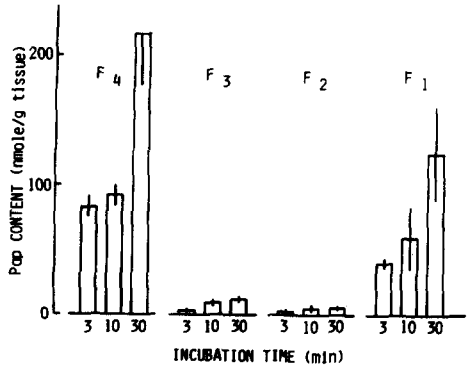

Fig. 4. Time course of [^{3}H]Pap distribution in F_4, F_3, F_2 and F_1 of biliary muscles, with means ± S.E. (n = 3–4). These fractions were isolated from the muscles incubated with [^{3}H]Pap (120 μM) in Tyrode's solution for various time at 37°.

a greater amount of [^{3}H]Pap than F_3, F_2 and F_1, as shown in Fig. 4. In F_3 also, which plays an important role in muscle relaxation, the amount of [^{3}H]Pap increases with time.

The three subfractions of F_3, PM-F enriched in plasma membranes, SR-F enriched in sarcoplasmic reticulum, and Mit-F enriched in mitochondria, obtained by centrifugation on a sucrose density gradient, were investigated further in the same way. Figure 5 shows the time–course curve of [^{3}H]Pap uptake in these fractions. [^{3}H]Pap is shown to be distributed predominantly in PM-F and negligibly in SR-F and Mit-F. PM-F in the muscles incubated with [^{3}H]Pap for 30 min was applied to a Sephadex G-25 column to detect the binding of [^{3}H]Pap to proteins in PM-F.

Figure 6 shows two peaks of [^{3}H]Pap, the first peak (fractions 5–14) and the second (fractions 53–65). The first peak is [^{3}H]Pap bound to PM-F, decreasing from 0.949 to 0.092 nmole/mg protein by gel filtration, and the second peak is free [^{3}H]Pap.

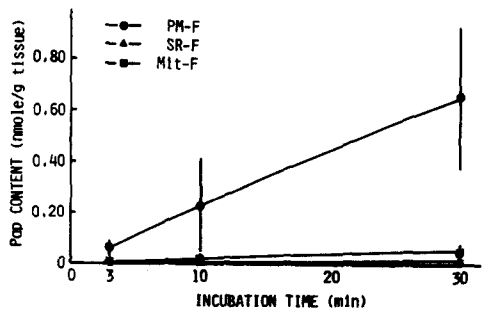

Fig. 5. Time course of [^{3}H]Pap distribution in PM-F enriched in plasma membrane, SR-F enriched in sarcoplasmic reticulum and Mit-F enriched in mitochondria, with means ± S.E. (n = 1–4). Three subfractions were obtained by procedure I from F_3 of the muscles incubated with [^{3}H]Pap (120 μM) in Tyrode's solution for various times. Note that [^{3}H]Pap was dominantly distributed in PM-F, and negligibly in SR-F and Mit-F.

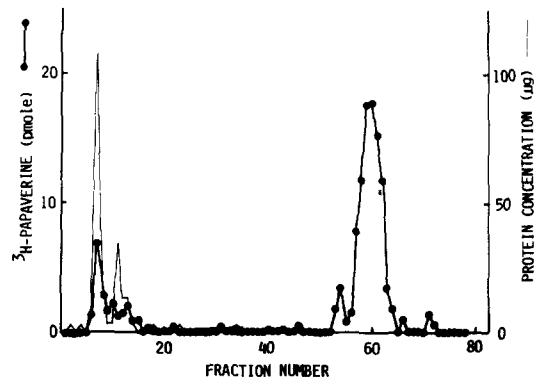

Fig. 6. Gel chromatography of PM-F in [^{3}H]Pap-treated muscles. PM-F was obtained by procedure I from the muscles incubated with [^{3}H]Pap (120 μM) in Tyrode's solution for 30 min. The fraction (328 μg protein) was applied to Sephadex G-25 column (1 × 36 cm) equilibrated with 10 mM phosphate buffer (pH 7.4). The eluate was collected (2.1 ml) from each test tube. Note that [^{3}H]Pap bound with proteins in PM-F (fractions 5–14) was detected.

Effects of papaverine on the distribution of ^{45}Ca ions to intracellular organelle fractions

The effect of Pap on the time course for the ^{45}Ca contents of PM-F, SR-F and Mit-F in ^{45}Ca-loaded muscles is shown in Fig. 7. The ^{45}Ca contents of the fractions at the initial time were 0.159 ± 0.015 μmole/g in PM-F, 0.062 ± 0.018 μmole/g in SR-F and 0.065 ± 0.022 μmole/g in Mit-F. In the course of the incubation time, the loaded ^{45}Ca decreased to

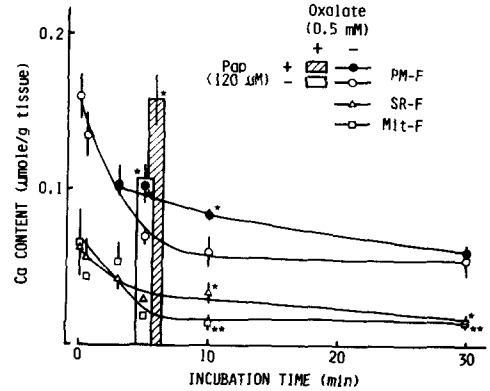

Fig. 7. Time–course curves of the ^{45}Ca content in PM-F, SR-F and Mit-F in ^{45}Ca-loaded muscles and effects of Pap on the ^{45}Ca content in PM-F, with means ± S.E. (n = 1–4). These fractions were obtained by procedure I from F_3 in biliary muscles incubated with or without Pap (120 μM) in Tyrode's solution in the presence or absence of 0.5 mM oxalate for various times. * and ** indicate significantly different values from those of PM-F in the absence of Pap with or without oxalate at the corresponding incubation time at $P < 0.05$ and 0.01, respectively. Note that PM-F contained a great deal more ^{45}Ca than SR-F and Mit- F, and that the promotion of oxalate resulted in it containing ^{45}Ca ions more significantly in the presence than in the absence of Pap, suggesting that Pap increases Ca uptake of PM-F from cytosol.

a greater extent from PM-F than from SR-F and Mit-F, and after 10 min of incubation time, PM-F retained significantly higher ^{45}Ca ions than SR-F and Mit- F.

PM-F in the muscle incubated with 120 μM Pap for 5 and 10 min contained ^{45}Ca more significantly than the control ($P < 0.05$). This difference did not appear after 30 min. The increase in the ^{45}Ca content of PM-F induced by Pap was greatly accelerated by 0.5 mM oxalate. This potentiation caused by oxalate resulted in PM-F containing a significantly higher content of ^{45}Ca in the presence rather than in the absence of Pap.

To obtain further data on the effect of Pap, Pap-induced dose–effect relations were investigated to examine the distribution of ^{45}Ca in intracellular organelle fractions obtained from hog biliary muscle. A 10 min incubation time in Pap was used. The ^{45}Ca contents in F_4, F_3 and F_2 were not significantly altered even in very high concentrations up to 400 μM of Pap, as shown in Fig. 8. This indicates that Pap does not significantly influence the distribution of extracellularly applied Ca. Nevertheless, in the three subfractions of F_3, the ^{45}Ca contents in PM-F, SR-F and Mit-F were remarkably changed by Pap as shown in Fig. 9. Pap dose-dependently increased the ^{45}Ca content in PM-F, and appeared to decrease it in SR-F and Mit-F.

Influence of theophylline upon increase of ^{45}Ca content induced by Pap

The effect of Pap described above was investigated to determine whether it was influenced by ThP acting as a cyclic AMP-phosphodiesterase (PDE) inhibitor. No significant effect of ThP applied before fractionation was observed on both ^{45}Ca content and the effect of Pap in F_3, as shown in Fig. 10. In addition, ThP had no effect of Pap on ^{45}Ca content in PM-F which was shown to be increased by Pap even in the presence of ThP.

Influence of cyclic AMP upon ^{45}Ca uptake induced by Pap

The effect of Pap on PM-F was investigated directly by applying a saturable concentration of cyclic AMP (4 μM) after fractionation. As shown in Fig. 11, Pap, treated on the tissue level for 30 min, dose-dependently increased ^{45}Ca uptake into PM-F. Cyclic AMP, however, had no effect on ^{45}Ca uptake and no influence on the effect of Pap, indicating that Pap increases Ca uptake into PM-F by a cyclic AMP-independent mechanism. The result induced by Pap was compared with the effect on $^{45}Ca^{2+}$ uptake by Pap which was directly applied to subfractions. Pap increased ^{45}Ca uptake in PM-F at a concentration of 3.6 μM, although higher concentrations of Pap had no effect on the uptake (Fig. 12). The addition of cyclic AMP (4 μM) produced a dose-dependent increase on the effect of Pap on ^{45}Ca uptake into PM-F (Fig. 12). In the case of SR-F, Pap increased

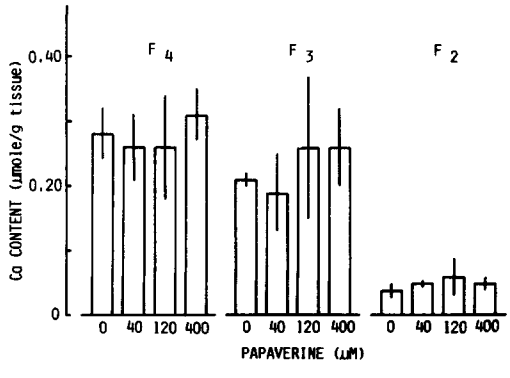

Fig. 8. Dose–effect relation of Pap on ^{45}Ca distribution to intracellular fractions obtained from ^{45}Ca-loaded muscles, with means ± S.E. ($n = 3$). ^{45}Ca-loaded muscles were incubated with Pap in Tyrode's solution for 10 min.

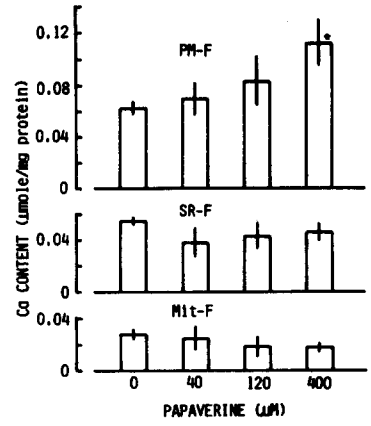

Fig. 9. Dose–effect relation of Pap on ^{45}Ca distribution in PM-F, SR-F and Mit-F, with means ± S.E. ($n = 3$). These fractions were obtained by procedure I from ^{45}Ca-loaded muscles incubated with Pap for 10 min. * indicates significantly different values from those in the absence of Pap at $P < 0.05$. Note that Pap dose-dependently increased the ^{45}Ca content in PM-F and appeared to decrease the content in SR-F and Mit-F.

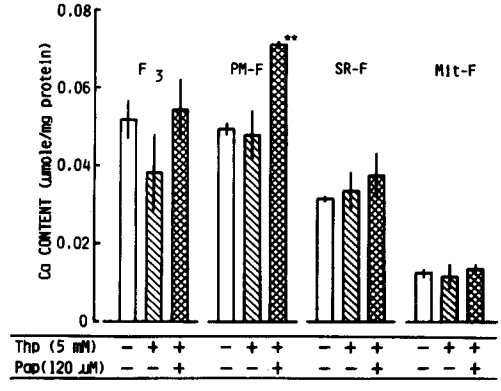

Fig. 10. Effects of Pap in the presence of theophylline (ThP) on Ca distribution in F_3, PM-F, SR-F and Mit-F, with means ± S.E. ($n = 3$). These fractions were obtained by procedure I from ^{45}Ca-loaded muscles incubated with Pap (120 μM) for 10 min after preincubation with ThP (5 mM) for 10 min. ** indicates significantly different values from those in the absence of Pap and ThP at $P < 0.01$. Note that ThP had no effect on the ^{45}Ca content in these fractions, whereas the ^{45}Ca content in PM-F was increased by Pap even in the presence of ThP.

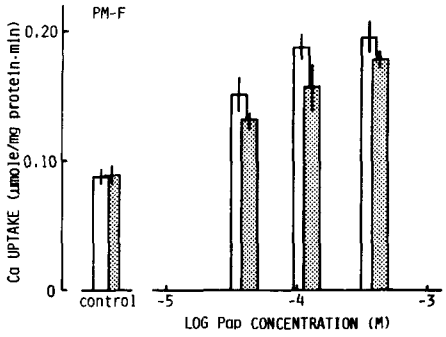

Fig. 11. Effects of Pap in the presence of cyclic AMP on Ca uptake into PM-F, with means ± S.E. (n = 3–8). PM-F was prepared by procedure I from the muscles incubated with Pap for 30 min. ^{45}Ca uptake of PM-F was assayed in the presence (shaded columns) or absence (open columns) of a saturable concentration of cyclic AMP (4 μM). Note that cyclic AMP had no effect of ^{45}Ca uptake and no influence on the effect of Pap.

the ^{45}Ca uptake both in the presence and absence of cyclic AMP (4 μM). The Ca uptake induced by Pap in the absence of cyclic AMP was greater than that in the presence of cyclic AMP, because cyclic AMP increased the ^{45}Ca uptake into SR-F by itself (Fig. 12). But in the case of Mit-F, Pap had no effect on ^{45}Ca uptake significantly in the presence or absence of cyclic AMP (Fig. 12).

DISCUSSION

The mechanism by which Pap relaxes smooth muscle was investigated from the standpoint of determining whether Pap altered the distribution of Ca ions in fractions highly enriched with plasma membranes, sarcoplasmic reticulum and mitochondria in hog biliary muscle. Ca accumulation by the microsomal fraction isolated from guinea-pig taenia caecum was suggested to play an important role during the relaxation of smooth muscle [21]. However, the results were obtained under experimental conditions where Ca ions or tested drugs were directly applied to tissue subfractions. In the present studies, the effects of all agents were observed under the same conditions as those causing the relaxation of biliary tissue. Therefore this procedure is useful for the purpose, despite the fact that the fractions containing the formed elements are not completely purified.

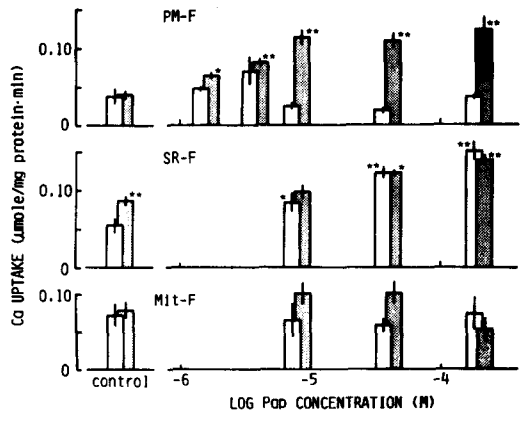

Fig. 12. Dose–effect relation of papaverine on ^{45}Ca uptake to PM-F, SR-F and Mit-F in the presence and absence of cyclic AMP, with means ± S.E. (n = 3–8). These fractions, prepared by procedure II, were applied with Pap in the presence (shaded columns) or absence (open columns) of a saturable concentration of cyclic AMP (4 μM). * and ** indicate significantly different values from those of the corresponding control at $P < 0.05$ and 0.01, respectively.

The intracellular Ca was exchanged with ^{45}Ca ions externally applied in normal concentration, following which the Ca contents were significantly increased in the presence of high KCl (139 mM). Pap resulted in no influence on the intracellular ^{45}Ca contents at least at a concentration (40 μM) tested in biliary muscle. These results were in agreement with observations using guinea-pig taenia coli [9]. On the other hand, Pap increasingly altered the binding of ^{45}Ca ions in PM-F enriched in plasma membrane, while reducing slightly the content in SR-F enriched sarcoplasmic reticulum. These observations were supported also by experiments on Ca uptake by the isolated microsomal fraction [22]. The result that Pap acts predominantly on PM-F also corresponded to the data that the distribution of [^{3}H]Pap applied in intact biliary muscle showed that the drug concentrates in PM-F. Although it is difficult to extrapolate from *in vitro* data for a reversibly acting compound such as Pap, an examination of the time–course curve (Fig. 5) shows that [^{3}H]Pap is specifically taken up and bound by PM-F, suggesting that this is an important site of action of the drug. The increase in ^{45}Ca content induced by Pap was to depend upon enhanced uptake by PM-F rather than inhibition of Ca efflux. This conclusion is based on the effect of Pap on Ca uptake from cytosol which was increased in the presence of oxalate more significantly than the control as described by Wuytack *et al.* [23]. The influence of cyclic AMP, on the other hand, had been reported to stimulate the binding of Ca in a microsomal fraction of smooth muscle [24], but other workers could not confirm this finding [21]. In SR-F, however, Ca uptake was significantly increased by cyclic AMP [13]. In the present studies, Ca uptake by PM-F was examined using ThP of phosphodiesterase inhibitor and a saturable concentration of cyclic AMP. The influence of ThP and cyclic AMP on Pap-induced Ca uptake in PM-F was not demonstrated, indicating that the process is independent of cyclic AMP. In the mechanism by which ThP also relaxes smooth muscle, there is this suggestion of an association with adenosine [25] more than with Ca ions, in addition to the inhibitory action on cyclic nucleotide PDE.

From the present results, Pap is demonstrated to accelerate Ca uptake by PM-F independent of cyclic AMP and without the influence of external Ca concentration. This means there is an alteration of the distribution of Ca in the intracellular organelles, suggesting action on the intracellular sequestration of activator Ca. This suggestion is supported by other workers as well [26–28]. The mechanism by which Pap lowers normal tone of the biliary muscle seems

to involve an accumulation of Ca from sarcoplasmic reticulum to plasma membrane, perhaps, to a Ca store in the inner membrane as suggested previously [29] by disturbing the equilibrium state of Ca levels in the cytosol. This mechanism may be different from the one by which Pap reverses the increased tone produced by muscle stimulants.

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