iprindole on responses to ACh was abolished in the presence of cocaine and vice versa.

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Increased ^{45}Ca-efflux from smooth muscle microsomes by a rise in an extramicrosomal Ca ion concentration, and the effect of thymol

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It is well accepted that myoplasmic Ca ion concentration regulates muscle contractility. In skeletal muscle, in the contraction process, Ca ion is supplied exclusively from an intracellular Ca source in the sarcoplasmic reticulum, whereas, in smooth muscle, both intracellular and extracellular Ca sources have been proposed to contribute to contraction (Bolton 1979). At present, it is unclear where the intracellular Ca source (Ca store) is located in smooth muscle cells and how Ca ions are released from this store. Using guinea-pig mesentric artery chemically skinned with saponin, which makes the cell membrane, but not the sarcoplasmic reticulum, selectively permeable to solute molecules (Endo 1977), Itoh et al (1981) recently demonstrated the presence of Ca-induced release of Ca preloaded by ATP-dependent processes, and that its nature appears to resemble that observed in skeletal muscle. We now report that increased efflux of ^{45}Ca preloaded in microsomes from guinea-pig taenia caecum is caused by a rise in extramicrosomal Ca ion concentration. Moreover, to assess this phenomenon pharmacologically, the effect of thymol on Ca-induced stimulation of ^{45}Ca-efflux was also examined.

Methods and results

Taeniae caeci from guinea-pigs of either sex (300–500 g) were minced and disrupted in a buffered sucrose solution (0·3 M sucrose, 1 mM dithiothreitol, 0·1 mM phenylmethylsulphonyl fluoride, 10 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethane-sulphonic acid, pH 7·5) in a Potter-type homogenizer by 6-strokes, followed by 3-bursts of 5 s with a Polytron PT-10 at setting no. 7. Large particles and mitochondria were removed by centrifugation, first at 2500 *g* for 10 min and then 15 000*g* for 20 min. The supernatant was centrifuged at 100 000*g* for 1 h. The resulting pellet was subjected to treatment with 0·6 M KCl for 2·5 h and resedimented. Microsomal concentration was determined by the method of Lowry et al (1951), using bovine serum albumin as a standard. ^{45}Ca-efflux was carried out as follows. At first, microsomes were preloaded with ^{45}Ca in 0·5 ml of a solution which contained: 30 mM Tris/maleate (pH 7·0), 100 mM KCl, 5 mM ATP, 5 mM $MgCl_2$, 5 mM NaN_3, 10 mM creatine phosphate, 100 μg ml^{-1} creatine kinase, 4 μCi ml^{-1} ^{45}Ca(16·8 Ci mg^{-1}), 0·1 mM $CaCl_2$, 0·045 mM ethyleneglycol-bis-(β-aminoethylether)-*NN'*-tetra-acetic acid (EGTA), 0·5 mg ml^{-1} microsomes. The concentration of free Ca ion in the reaction medium was calculated to be 10 μM from the apparent affinity constant ($1·26 \times 10^6$ M^{-1}) between EGTA and Ca at pH 7·0 (Ogawa 1968). After preloading microsomes with ^{45}Ca by incubation at 32 °C for 10 min, ^{45}Ca-efflux was carried out by diluting the uptake medium 10-fold with a buffer to give final concentrations of 30 mM Tris/maleate (pH 7·0), 100 mM KCl, 5 mM ATP, 5 mM $MgCl_2$, 5 mM NaN_3, 10 mM creatine phosphate, 100 μg ml^{-1} creatine kinase, 2 mM $CaCl_2$, and 2·13 (or 7·29) mM EGTA (pCa = 5 (or 6·5)). Since, by this method, $^{45}Ca/^{40}Ca$ was decreased to 1/200 after dilution, reuptake of ^{45}Ca during efflux was almost negligible.

^{45}Ca content in microsomes was trapped on a Millipore filter (HAWP) by a vacuum filtration, and counted as described by Takayanagi et al (1979). When necessary, thymol in ethanol (1:1 w/v) was added in a diluting buffer, where the final concentrations of thymol and ethanol were 0·5 mM and 0·0075%, respectively. ^{45}Ca-efflux was not influenced by this concentration of ethanol as vehicle (data not shown).

Microsomes obtained as described above took up Ca

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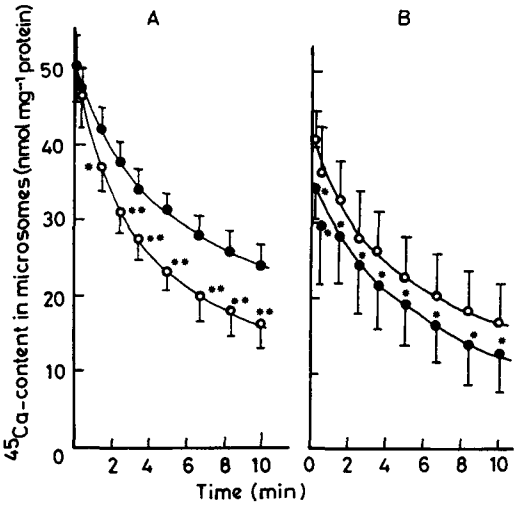

FIG. 1. A. ^{45}Ca-efflux at pCa = 5·0 (○) and 6·5 (●). After microsomes were preloaded with ^{45}Ca at pCa = 5 and 32 C for 10 min, ^{45}Ca-efflux was carried out by diluting with the buffer containing no ^{45}Ca as in the text. 0-time on the abscissa indicates the point when uptake media were diluted to start ^{45}Ca-efflux. Number of experiments was 5. Since levels of ^{45}Ca-uptake were varied from preparation to preparation, statistical significance was analysed using paired *t*-test,'. *, ** significantly different from the corresponding values at pCa = 6·5 at $P < 0.05$ and 0·01, respectively.

B. ^{45}Ca-efflux at pCa = 6·5 in the presence (●) and absence (○) of 0·5 mM thymol. Number of experiments was 4. Since levels of ^{45}Ca-uptake were varied from preparation to preparation, statistical significance was analysed using paired *t*-test. * Significantly different from the corresponding control group at $P < 0.01$.

in the presence of MgATP, and approached a nearly steady-state level at 10 min after a start of reaction at pCa = 5·0 to 7·5 (data not shown). Consequently, after being preloaded with ^{45}Ca for 10 min, microsomes were diluted at pCa = 5.0 and 6·5 as described above, to trace the efflux of ^{45}Ca from microsomes. Fig. 1A shows that ^{45}Ca-efflux from microsomes was faster at pCa = 5 than 6·5. Many physiological studies using skinned muscle preparations have claimed the presence of Ca-induced Ca-release, that is, some quantity of Ca could trigger the substantial release of Ca from sarcoplasmic reticulum (Endo 1977). It was recently reported that much the same mechanism could also operate in smooth muscle (Itoh et al 1981).

Some experiments using a skinned fibre of skeletal muscle have indicated that thymol is a potent stimulator of Ca-induced Ca-release (Ogawa 1970). Therefore, we next examined the effect of thymol on ^{45}Ca-efflux. Fig. 1B indicated that 0·5 mM thymol stimulates the rate of efflux of ^{45}Ca.

These results suggest that Ca-induced stimulation of ^{45}Ca-efflux might be an in-vitro reflexion of Ca-induced Ca release which is observed in skinned muscle preparations.

Discussion

Subcellular fractionation studies in skeletal muscle have indicated that vesicles from both plasma membrane and sarcoplasmic reticulum can take up Ca ATP-dependently (Wibo et al 1981). Since the microsomes used in this study contained ouabain-sensitive (Na,K)-ATPase and NADPH-cytochrome c reductase activities (18- and 11-fold enrichment compared with muscle homogenate, respectively) (unpublished data, Hisayama & Takayanagi), it is uncertain where a component involved in Ca-induced stimulation of ^{45}Ca-efflux is distributed in a smooth muscle cell. Magaribuchi et al (1973) pointed out the possibility that thymol might release Ca from a component closely resembling plasma membrane from the additional effect of thymol on membrane properties, whereas its effect is confined to that on sarcoplasmic reticulum in skeletal muscle (Ogawa 1970).

Our results suggest the presence of a possible mechanism for smooth muscle contraction as an amplifying system triggered by processes, accompanying change of Ca distribution, which follows the first stimulus (e.g. drug-receptor interaction).

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