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Relaxation of intestinal smooth muscle and calcium movements

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The effects of papaverine, isoprenaline and N^6,2′-*O*-dibutyryl 3′,5′-cyclic adenosine monophosphate (DiBu.C-AMP) on calcium movements in the taenia from the guinea-pig caecum have been investigated and compared with the effects of phenylephrine. Papaverine and DiBu.C-AMP antagonized the contraction of KCl-depolarized muscle induced by $CaCl_2$ and increased ^{45}Ca-efflux from the taenia. Papaverine also significantly depressed ^{45}Ca-uptake by this preparation. These findings suggest that papaverine impairs the availability of calcium to the contractile system and that there may be a correlation between relaxation and increase of ^{45}Ca-efflux. The action of DiBu.C-AMP resembles that of papaverine but its potency is less.

The action of β-adrenoceptor stimulants is thought to be related to their ability to increase the level of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) in the smooth muscle cells (Polacek & Daniel, 1971; Polacek, Bolan & Daniel, 1971). Some authors (Triner, Vulliemoz & Nahas, 1970; Pöch, Juan & Kukovetz, 1969; Takagi, Takayanagi & Tsuchida, 1972; Takayanagi, Uchida & others, 1972) have reported that papaverine may also relax smooth muscle through the accumulation of the endogenous cyclic AMP in the cells. Moreover, mechanical and electrical responses of the taenia from the guinea-pig caecum to papaverine and to isoprenaline are mimicked by exogenously applied N^6,2′-*O*-dibutyryl-3′,5′-cyclic adenosine monophosphate (DiBu.C-AMP) (Takagi, Takayanagi & Tomiyama, 1971a,b). The response to phenylephrine, an α-adrenoceptor stimulant, is not concerned with the intracellular cyclic AMP (Wilkenfeld & Levy, 1969; Takagi & others, 1972; Takayanagi & others, 1972).

We have examined the effects of isoprenaline, papaverine and DiBu.C-AMP on ^{45}Ca-efflux from the guinea-pig taenia and on ^{45}Ca-uptake by the KCl-depolarized taenia of the guinea-pig to assess possible mechanisms for relaxation of the intestinal smooth muscle by these drugs, compared with phenylephrine. Furthermore, influence of these drugs on the extracellular space of smooth muscle was investigated by the use of [^{14}C]sorbitol.

METHODS

A male guinea-pig, 300 to 400 g, was killed by a blow on the head and the taenia isolated from the caecum and suspended in a 30 ml organ bath filled with calcium-free KCl-Locke Ringer solution (g litre^{-1}: KCl 11·8, $MgCl_2$ 0·2, $NaHCO_3$ 0·5, glucose 0·5). This solution was gassed with air and kept at 32°. After an initial incubation (lasting about 30 min), $CaCl_2$ was added cumulatively and tension induced by $CaCl_2$ was recorded isotonically. The drugs were added to the bath 3 min before addition of $CaCl_2$.

To examine ^{45}Ca uptake by the muscle, the isolated taenia was attached to a glass hook and was rinsed in calcium free KCl-Locke Ringer solution bubbled with air and kept at 32°. At 3, 6 and 9 min after application of KCl-Locke Ringer solution

containing DiBu.C-AMP (10^{-4} g ml^{-1}), 0·5 mM Ca^{2+} and ^{45}Ca (3μ Ci ml^{-1}), the muscle was removed from the radioactive solution, blotted with a filter-paper and quickly weighed. In the experiments with papaverine (10^{-5} g ml^{-1}), KCl-Locke Ringer solution containing 1 mM Ca^{2+} and ^{45}Ca (3μ Ci ml^{-1}) was used as bath fluid. To determine ^{45}Ca radioactivity in the muscles, they were digested by HNO_3 according to Pfeffer, Weinstein & others (1971) and counted by liquid scintillation counter (Packard, Type 3214).

To measure alterations of ^{45}Ca efflux, the muscles were attached to a glass hook with nylon thread and were allowed to equilibrate for 1 h in ^{45}Ca Locke Ringer solution (^{45}Ca, 30μCi ml^{-1}) and then at intervals of 10 or 5 min quickly transferred to a series of test tubes containing 3 ml of inactive Locke Ringer solution at 27° bubbled with air. One ml of solution was removed from each test-tube and poured into vials which contained 10 ml of Bray's cocktail. Then radioactivity was measured by liquid scintillation counter.

To detect any change of extracellular space of this tissue induced by drugs, [^{14}C]sorbitol was used. After 10 min incubation with [^{14}C]sorbitol and one of the drugs, the radioactivity in muscles was measured according to Bozler (1967).

RESULTS

In experiments with the depolarized taenia, papaverine (3×10^{-6} g ml^{-1}) or DiBu. C-AMP (10^{-4} g ml^{-1}) shifted the dose-response curve of $CaCl_2$ towards higher concentrations (Fig. 1). The effect of papaverine on the dose-response curve of $CaCl_2$

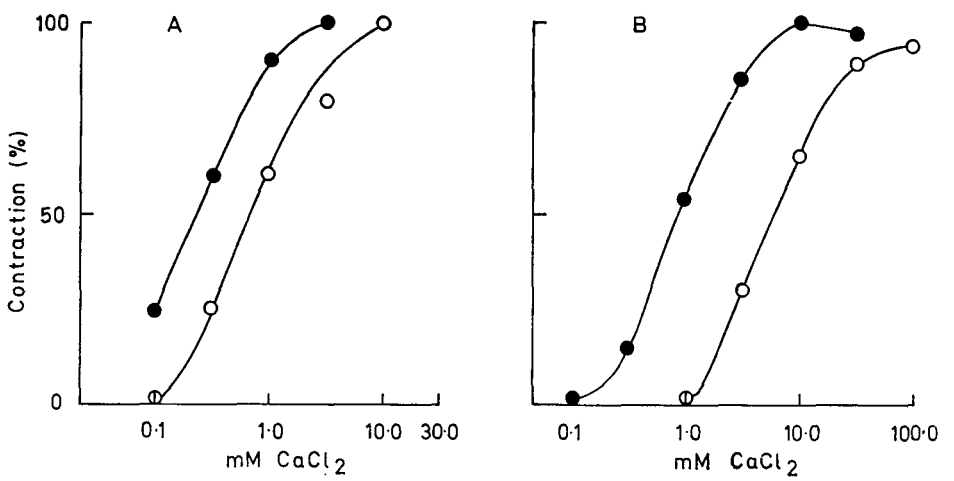

FIG. 1. Effect of papaverine and dibutyryl C-AMP on the concentration action curve of $CaCl_2$ tested on the KCl-depolarized taenia of the guinea-pig. ●—● controls. ○—○ with DiBu C-AMP (A) or papaverine (B).

is similar to that reported by Ferrari & Carpendo (1968) and by Simonis, Ariëns & Van den Broke (1971). In this, papaverine is apparently more potent than DiBu. C-AMP. To ascertain whether these drugs suppress the uptake of calcium by the taenia, ^{45}Ca-uptake was measured in KCl-depolarized muscles. As shown in Fig. 2A, papaverine (10^{-5} g ml^{-1}) inhibits ^{45}Ca-uptake by the taenia and especially after 9 min incubation in ^{45}Ca-solution, ^{45}Ca-uptake was decreased significantly ($P < 0{\cdot}01$). In the experiment with DiBu.C-AMP (10^{-4} g ml^{-1}), however, ^{45}Ca uptake was only

slightly but not significantly depressed as shown in Fig. 2B. Because of the lower concentration of calcium in bath fluid (0·5 mM), the amount of calcium uptake by the muscles was smaller with DiBu.C-AMP (Fig. 2A and B). The effect of some

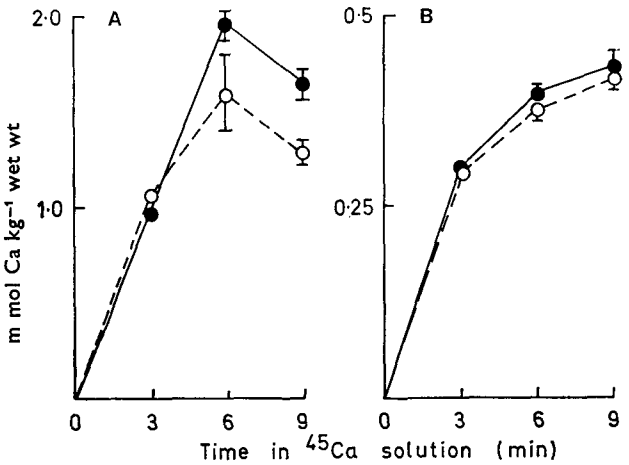

FIG. 2. The uptake of ^{45}Ca by the taenia in the presence or absence of (A) papaverine or (B) DiBuC-AMP. ●—● control. A ○—○ papaverine (10^{-5} g ml^{-1}) B ○—○ DiBu.C-AMP (10^{-4} g ml^{-1}). Vertical bars indicate the standard errors. The uptake of ^{45}Ca in the presence of papaverine was significantly different from the control at 6 and 9 min ($P < 0{\cdot}01$).

relaxants on ^{45}Ca-efflux were compared. Isoprenaline (10^{-7} g ml^{-1}), papaverine (3×10^{-6} g ml^{-1}) and DiBu.C-AMP (10^{-4} g ml^{-1}) increased the loss of ^{45}Ca from muscle (Fig. 3A). Moreover, phenylephrine (3×10^{-6} g ml^{-1}), whose action is not

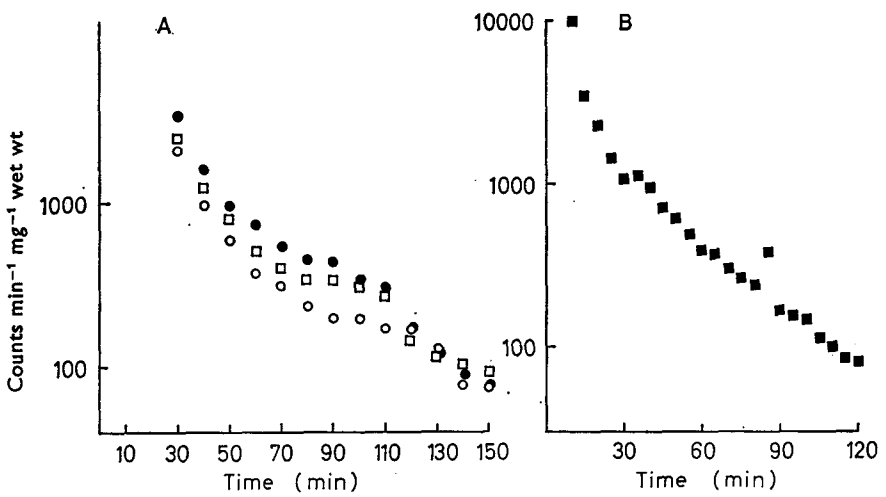

FIG. 3A. Effect of isoprenaline (10^{-7} g ml^{-1}, ●), papaverine (3×10^{-6} g ml^{-1}, ○), DiBu.C-AMP (10^{-4} g ml^{-1}, □), on ^{45}Ca-efflux from the taenia caecum. The abscissa: the time (min) after transfer from ^{45}Ca-solution, the logarithmic ordinate: counts min^{-1} mg^{-1} wet weight. Drugs present from 90 min to 110 min.

B. Effect of phenylephrine (3×10^{-6} g ml^{-1}) on ^{45}Ca-efflux. Phenylephrine present from 75 to 90 min.

considered to involve intracellular cyclic AMP, increased radioactive calcium leakage from the taenia (Fig. 3B). However, only the action of papaverine persisted after its removal from Locke Ringer solution. Perhaps this may be because papaverine is not easily washed out. None of the drugs used influenced the extracellular space of guinea-pig taenia.

DISCUSSION

We have previously reported (Takagi & others, 1971a,b) that both papaverine and DiBu.C-AMP do not affect the tension of glycerinated muscle induced by ATP-Mg and Ca^{2+} and that these drugs stop spontaneous spike discharge. We have considered from these facts that both papaverine and dibutyryl cyclic AMP do not inhibit the function of the contractile element but suppress the membrane activity of the smooth muscle. Therefore, we examined the actions of isoprenaline, papaverine and DiBu.C-AMP to see if they were due to a change of calcium movements. Since these drugs did not influence the extracellular space of the taenia, it appears that the effects of the drugs on calcium movements observed were not the result of change of the extracellular space of the preparation.

Papaverine inhibited the contraction of the KCl-depolarized muscle induced by $CaCl_2$ and also decreased ^{45}Ca-uptake by the depolarized taenia. However, DiBu. C-AMP depressed only the contraction of the KCl-depolarized taenia induced by $CaCl_2$ but not ^{45}Ca-influx. Since the α- and β-adrenoceptor stimulants have been found to relax tension of the taenia induced by KCl only minimally (Takagi, Takayanagi & Ohta, 1969), the action of isoprenaline seems to be induced through the polarized membrane. However, papaverine and DiBu.C-AMP can inhibit the supply of calcium ions to the contractile element even in the KCl-depolarized muscle.

Isoprenaline, papaverine and DiBu.C-AMP much increased the loss of labelled calcium ions from the taenia which was incubated in ^{45}Ca-Locke Ringer solution for 1 h. Phenylephrine, whose action is not concerned with endogenous cyclic AMP, also accelerated the ^{45}Ca-efflux from the taenia. This effect of the drugs may accelerate the decrease of the intracellular calcium concentration. Therefore, acceleration of loss of calcium ions from the taenia may be one of the possible mechanisms for the relaxation of smooth muscle induced by the drugs.

REFERENCES

BOZLER, E. (1967). *J. gen. Physiol.*, **50**, 1459–1465.

FERRARI, M. & CARPENEDO, F. (1968). *J. Pharm. Pharmac.*, **20**, 317–318.

PFEFFER, M., WEINSTEIN, S., GAYLORD, J. & INDINDOLI, L. (1971). *Analyt. Biochem.*, **39**, 46–53.

PÖCH, G., JUAN, H. & KUKOVETZ, W. R. (1969). *Arch. exp. Path. Pharmak.*, **264**, 293–294.

POLACEK, I. & DANIEL, E. E. (1971). *Canad. J. Physiol. Pharmac.*, **49**, 988–998.

POLACEK, I., BOLAN, J. & DANIEL, E. E. (1971). *Ibid.*, **49**, 999–1004.

SIMONIS, A. M., ARIËNS, E. J. & VAN DEN BROKE, J. J. (1971). *J. Pharm. Pharmac.*, **23**, 107–110.

TAKAGI, K., TAKAYANAGI, I. & OHTA, K. (1969). *Jap. J. Pharmac.*, **19**, 604–612.

TAKAGI, K., TAKAYANAGI, I. & TOMIYAMA, A. (1971a). *Ibid.*, **21**, 271–273.

TAKAGI, K., TAKAYANAGI, I. & TOMIYAMA, A. (1971b). *Ibid.*, **21**, 477–483.

TAKAGI, K., TAKAYANAGI, I. & TSUCHIDA, Y. (1972). *Ibid.*, **22**, 403–409.

TAKAYANAGI, I., UCHIDA, M., INATOMI, N., TOMIYAMA, A. & TAKAGI, K. (1972). *Ibid.* In the press.

TRINER, L., VULLIEMOZ, Y. & NAHAS, G. G. (1970). *Biochem. Biophys. Res. Commun.*, **40**, 64–69.

WILKENFELD, B. E. & LEVY, B. (1969). *J. Pharmac. exp. Ther.*, **169**, 61–67.