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Antagonism between calcium ions and some myolytic agents on depolarized guinea-pig taenia coli

SIR,—Our previous investigations on the mechanism of action of papaverine and certain derivatives demonstrated that these drugs exert an inhibitory effect on oxidative phosphorylation and that an antagonism occurs between calcium ions and spasmolytic agents, both in polarized and in KCl- or K_2SO_4-depolarized smooth muscle preparations. According to these results, it was suggested that the mechanism of action of papaverine-like drugs could be ascribed to an impairment of the energy supply to the contractile system and to an interference with the essential function of calcium ions in muscular contraction (Santi, Contessa & Ferrari, 1963; Santi, Ferrari & Contessa, 1964; Ferrari, 1964; Ferrari & Gaspa, 1965).

Further investigations demonstrated that the inhibition of oxidative phosphorylation was elicited only by papaverine and other oxy-alkyl-benzylisoquinoline derivatives, whereas the interference with calcium ions was shared by all the spasmolytic agents tested (Tòth, Ferrari & others, 1966). In view of the importance of the latter property as a general mechanism of action of spasmolytic drugs, we have attempted to elucidate whether calcium ions and some myolytic agents behave as competitive or non-competitive antagonists.

The investigations were made with guinea-pig taenia coli depolarized by immersion in calcium free, potassium rich solution, at 35°; contractions were triggered by addition of $CaCl_2$ at concentrations ranging from 0·25 to 100 mM; higher concentrations elicited auto-inhibitory effects. The experiments were made according to Rossum (1963), following the conventional dose-response method and the cumulative dose-response method. In the conventional dose-response experiments, after each contraction, the preparations were bathed in calcium-free Tyrode medium for 5 min and then washed briefly with K_2SO_4-Ringer at room temperature (Ferrari & Gaspa, 1965) to obtain rapid relaxation; finally, K_2SO_4-Ringer was substituted after 3 min by KNO_3-Ringer (Urakawa, Karaki, Ikeda, 1967) to which $CaCl_2$ was added. In this medium $CaCl_2$ induces rapid well-maintained contractions and precipitation of calcium salts is avoided. As antagonists, we employed three myolytic agents with different mechanisms of action: papaverine (hydrochloride) ($1·4$ and $3·2 \times 10^{-5}$M), eupaverin (sulphate) ($1·4$ and $3·2 \times 10^{-5}$M), KCN (2×10^{-4} and 10^{-3}M). These drugs were added to the bath 3 min before $CaCl_2$. In the cumulative dose-response experiments five $CaCl_2$ doses were applied at 3 min intervals in a geometric sequence of increasing doses, to a final $CaCl_2$ concentration giving the maximal response (8–12 mM). Spasmolytic drugs were added 3 min before initiating the cumulative dose-response curve, at the lowest indicated concentrations.

The results obtained both with the conventional dose-response method and with cumulative dose-response procedure demonstrate that all the drugs tested

exert myolytic activity against $CaCl_2$-induced contraction of depolarized guinea-pig taenia coli; however, their effects are fully counteracted by increasing $CaCl_2$ concentration and the dose-response curves in the presence of the inhibitors show a parallel shift compared with the controls. These facts could suggest that within the range of concentrations of the present experiments the drugs tested behave as competitive antagonists of calcium ions. The auto-inhibitory effects occurring at $CaCl_2$ concentrations exceeding 100 mM did not allow us to test the myolytic agents at higher dose levels or after a longer incubation period; such experiments could elucidate whether these drugs act by a pure competitive antagonism or have a dual mechanism, as seems likely especially with cyanide and papaverine, which exert metabolic inhibitory effects.

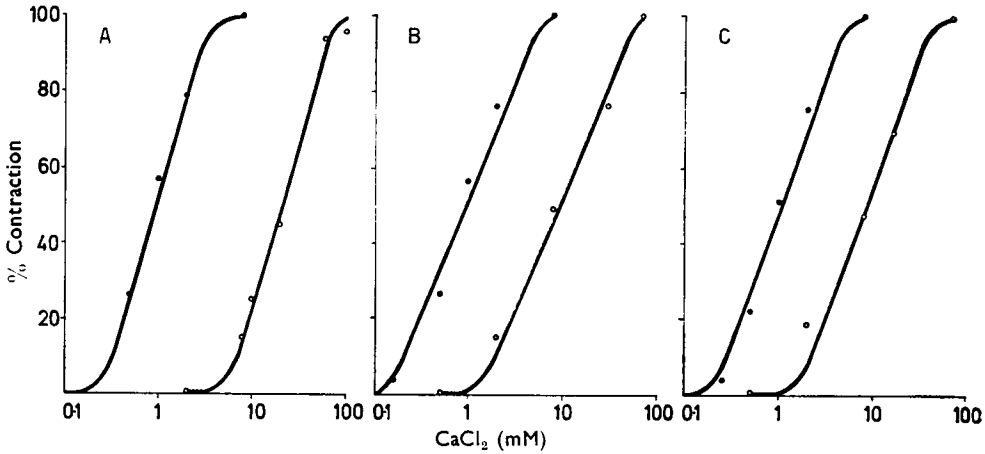

FIG. 1. Guinea-pig taenia coli suspended in KNO_3-Ringer at 35°: effects of myolytic agents on the cumulative log concentration-response curve for calcium ions. ● = controls; ○ = $1{\cdot}4 \times 10^{-5}$ M papaverine (in A), $1{\cdot}4 \times 10^{-5}$ M eupaverin (in B), 2×10^{-4} M KCN (in C). Each point represents the mean of six experiments.

Furthermore, independently of a dual action, a parallel displacement of the dose-response curve at low concentrations of the antagonists may indicate the presence of a reserve in receptors for the agonist (Rossum, 1963; Bowman, Rand & West, 1968)

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