The effect of papaverine on $^{45}Ca^{2+}$ uptake in partly depolarized taenia coli of the guinea-pig

The effect of papaverine on calcium uptake in isolated smooth muscles has been previously studied. In non-depolarized ileum and taenia coli of the guinea-pig, papaverine did not decrease the uptake of calcium but did increase its release (Banerjee & Lewis, 1963; Hattingberg, Kuschinsky & Rahn, 1966). In the ileum preparation depolarized by total replacement of sodium by potassium, papaverine decreased the resting calcium uptake (Banerjee & Lewis, 1964). The relaxation induced by papaverine in depolarized intestinal smooth muscle was ascribed to the impaired utilization of either the membrane bound calcium (Imai & Takeda, 1967) or the external calcium (Ferrari & Carpenedo, 1968). In our previous paper the relaxant action of papaverine in partly depolarized taenia coli was described (Kadlec & Bauer, 1971). Papaverine blocked only the contractions evoked by the addition of external calcium but not those elicited by the addition of acetylcholine. The present aim was to study the effect of papaverine on the uptake of $^{45}Ca^{2+}$ in partly depolarized guinea-pig taenia coli stimulated by the addition of external calcium.

Male guinea-pigs were stunned, exsanguinated and the taenia was quickly dissected. A strip 20 mm long weighing 8 to 16 mg was mounted on a steel rod under an initial tension of 5 g. In some experiments isometric tension changes were also recorded. The preparations were first left for 30 min to equilibrate at room temperature in Krebs solution of the following composition (mM): NaCl 120, KCl 5·9; $CaCl_2$ 2·5; $NaHCO_3$ 15·4; $MgCl_2$ 1·2; and glucose 11·5. The preparations were then partially depolarized by immersion in a solution where 80 mM NaCl was replaced by KCl and the divalent cations were omitted. Papaverine was added to the preparations 10 min before the addition of 8 mM $CaCl_2$. $^{45}Ca^{2+}$ as a trace element was added, either at the same time, or after a 5 min delay. $^{45}Ca^{2+}$ was obtained from the Radiochemical Centre, Amersham, U.K. and the concentration used for uptake measurement was 1 μCi ml^{-1}. After exposure to $^{45}Ca^{2+}$ from 2 to 15 min the strips were rinsed in the non-radioactive depolarizing solution either once for 30 s or three times within 4 min, gently blotted between filter papers, weighed and ashed in 0·1 ml concentrated nitric acid. The radioactivity of the samples was measured on a Tricarb liquid scintillation counter. Calculation of $^{45}Ca^{2+}$ uptake was based on 1 mg wet tissue weight. For statistical evaluation of the results by *t*-test, average results from 5 to 10 preparations were used.

In the first group of experiments, strips of taenia coli from the guinea-pig were exposed to a solution containing both 8 mM $CaCl_2$ and $^{45}Ca^{2+}$ for different periods and after incubation the preparations were washed only once (Fig. 1). The uptake of $^{45}Ca^{2+}$ took place in two phases and only the second phase which started after 4 min was decreased by papaverine (2×10^{-4}M). When the preparations were rinsed three times after the exposure to $^{45}Ca^{2+}$, the decrease of uptake by papaverine was more prominent after 15 min exposure to $^{45}Ca^{2+}$. In the second group of experiments $^{45}Ca^{2+}$ was added 5 min after the introduction of 8 mM $CaCl_2$, and the preparations were rinsed once. A steady increase in the uptake of radioactive calcium from 2 to 15 min was registered and it was lowered by papaverine (4×10^{-5} M). In the experiments where the isometric tension was recorded, the preparations were fully relaxed before the calcium ions were added. The introduction of 8 mM $CaCl_2$ resulted in a persistent contraction. While papaverine in a dose higher than 10^{-4} M completely blocked the contractions, lower doses only partially antagonized the contractions.

In a taenia coli preparation depolarized by 30 mV (Kadlec & Šeferna, 1972), papaverine even in high concentration did not decrease the uptake of $^{45}Ca^{2+}$ in the muscle during the first 4 min after the introduction of 8 mM $CaCl_2$. The tissue radio-

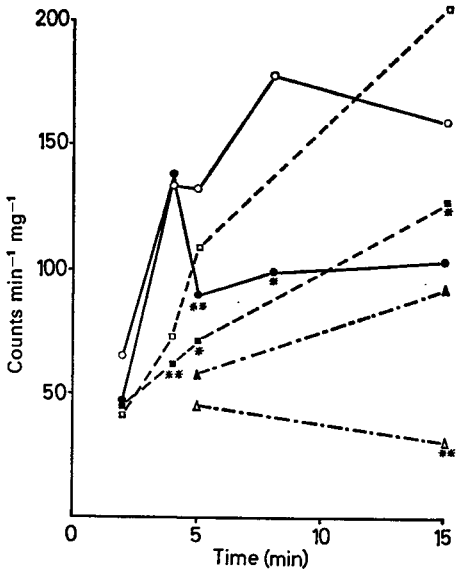

FIG. 1. The effects of papaverine on $^{45}Ca^{2+}$ uptake by strips of partly depolarized taenia coli from the guinea-pig. The ordinate represents radioactivity in counts min^{-1} mg^{-1} of strips of muscle exposed to $^{45}Ca^{2+}$ (1 μCi ml^{-1}). The abcissa represents the time of exposure to $^{45}Ca^{2+}$. All open symbols represent results in the absence of papaverine, closed circles or triangles represent results in the presence of papaverine 2×10^{-4}M, and closed squares represent results in the presence of papaverine 4×10^{-5}M. In some experiments $^{45}Ca^{2+}$ was added together with 8 mM $CaCl_2$ and the preparations rinsed once (O———O) or three times (△ - . - . - △) after incubation. In other experiments $^{45}Ca^{2+}$ was added 5 min after the preparations had been exposed to 8 mM $CaCl_2$, and the preparations rinsed once after incubation (□ - - - - □). Each point represents the mean of 5 to 10 measurements and the results which differ significantly ($P < 0.1$ or $P < 0.025$) are denoted by one or two asterisks respectively.

activity in the present experiments, on the other hand, was significantly decreased between 4 and 5 min after papaverine treatment and this suggests that a slower component of calcium exchange is involved. (A "tightly bound fraction" has been proposed by Karaki, Ikeda & Urakawa, 1969.) The hypothesis that papaverine addition affected mainly the slower component of calcium exchange was tested by repeated washing of the preparations for 4 min. The results showed that this fraction of smooth muscle calcium was significantly decreased by papaverine administration. Moreover, the addition of $^{45}Ca^{2+}$ at a time when no mechanical changes occurred and when non-radioactive calcium should be equilibrated within extracellular fluid (Lüllmann & Siegfriedt, 1968) manifested a steady increase of $^{45}Ca^{2+}$ uptake which was inhibited by a five times smaller concentration of papaverine than in previous experiments.

The present results support the view that papaverine probably blocked the slower components of $^{45}Ca^{2+}$ uptake. This is less obvious in partially depolarized than in fully depolarized smooth muscles. The precise mechanism of action of papaverine is not clear; papaverine however may compete for some receptor site with calcium (Tashiro & Tomita, 1970) which is provided from extracellular fluid in depolarized smooth muscle.

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The effect of cannabidiol on maximal electroshock seizures in rats

The natural marihuana compounds, cannabidiol, cannabinol, Δ^9- and Δ^8-tetrahydrocannabinol, raise the threshold for hippocampal seizures obtained by electrical stimulation (Izquierdo, Orsingher & Berardi, 1973). Cannabidiol is the most potent of these compounds and apparently acts by a mechanism similar to that proposed for diphenylhydantoin (Nasello, Montini & Astrada, 1972), namely, an interference with hippocampal K^+ release upon afferent bombardment (Izquierdo & others, 1973). Hippocampal seizures are known to result from the extracellular accumulation of this released K^+ (Izquierdo & Nasello, 1970, 1972; Izquierdo, Nasello & Marichich, 1970; Izquierdo, 1972). Cannabidiol and diphenylhydantoin have several other common actions, such as inhibition of hippocampal facilitation and post-tetanic potentiation, of the RNA concentration increase caused by afferent stimulation, and of acquisition of avoidance conditioned responses (Izquierdo & Nasello, 1973). Like diphenylhydantoin (Swinyard, Brown & Goodman, 1952), cannabidiol has little effect against leptazol seizures in mice, where significant antagonism may only be observed with doses 40 to 100 times higher than those needed to inhibit hippocampal seizures (Carlini, Leite & others, 1973). Since diphenylhydantoin is known to be a potent antagonist of maximal electroshock seizures (Swinyard & others, 1952), we have examined the effect of cannabidiol on this test.

Adult female albino rats (160 to 250 g) were submitted to maximal electroshock convulsions by currents passed between both eyes as recommended by Swinyard & others (1952). Twelve animals received 0·2 ml per 100 g of 0·9% NaCl (i.p.) 1 h before electroshock; full-fledged convulsions were obtained in all rats, with a late hindlimb tonic extensor phase lasting 12·6 $\pm$ 1·1 s. Cannabidiol (1·5, 3, 6 and 12 mg kg^{-1}) was thinly suspended in the saline solution with a few drops of Tween 80 (Izquierdo & others, 1973; Izquierdo & Nasello, 1973; Carlini & others, 1973), and each dose was given to groups of 12 rats. The time of peak effect was investigated with a 3 mg kg^{-1} dose and was found to be 1 h. This time was then chosen to study the effect of the other doses. The results are in Fig. 1. At the two higher doses there was some protection against all components of the convulsion, and the ED50 for inhibition of the tonic extensor phase was 3 mg kg^{-1}. This is a lower dose than that of diphenylhydantoin and of other anticonvulsant agents (Swinyard & others, 1952), and close to that previously found in this laboratory to raise hippo-