

Influence of drugs upon  $^{47}\text{Ca}^{2+}$  uptake in depolarised intestinal smooth muscle

SIR,—Acetylcholine (10  $\mu\text{g}/\text{ml}$ ) increases  $^{45}\text{Ca}^{2+}$  uptake by depolarised smooth muscle (Robertson, 1960), and carbachol (0.3  $\mu\text{g}/\text{ml}$ ) acts similarly in the depolarised guinea-pig taenia coli (Durbin & Jenkinson, 1961a). In frog skeletal muscle, contracture-producing drugs increase both uptake and release of  $^{47}\text{Ca}^{2+}$  (Ahmad & Lewis, 1962) and in non-depolarised smooth muscle acetylcholine, carbachol, 5-hydroxytryptamine and barium chloride increase  $^{47}\text{Ca}^{2+}$  uptake, but papaverine, adrenaline and histamine do not alter the inward flow of  $^{47}\text{Ca}^{2+}$  although papaverine decreases  $^{42}\text{K}^{+}$  influx (Banerjee & Lewis, 1963, 1964).

We have studied a group of drugs which stimulate or relax smooth muscle, using the longitudinal muscle of the guinea-pig ileum depolarised in potassium-rich Krebs solution (Durbin & Jenkinson, 1961a,b). In this preparation it is possible to observe effects upon  $^{47}\text{Ca}^{2+}$  uptake in conditions in which the marked mechanical changes, associated electrical changes and alterations in ion flux seen in normal Krebs solution are absent.

The technique used was based on that of Durbin & Jenkinson (1961a). 10 cm long sections of terminal ileum were removed from freshly killed guinea-pigs. Extraneous tissues were removed, the ileum gently pulled on to a glass rod and an incision made along the attachment of the mesentery. The longitudinal muscle layer was gently removed from the circular layer, the process being carried out under the surface of Krebs solution. For each series of experiments, 4 pieces each 2 cm long were taken from the same length of longitudinal muscle. Adjacent pieces served respectively as test and control preparations. Each strip was stretched with an 0.2 g weight and incubated for 30 min at 37° in non-radioactive Krebs solution. It was then kept for not less than 2 hr in potassium-rich Krebs solution at 37°. Each strip was now exposed for 2–3 min to radioactive potassium-rich Krebs solution. Drug or control solution was added and exposure continued for a further 7–8 min. The tissues were then washed for 3 periods of 30 sec with a stream of non-radioactive, potassium-rich Krebs solution and counted for 100 sec using a thallium-activated sodium iodide crystal scintillation counter (EKCO type N.597). 22 to 24 pairs of strips were used for each drug. The number of counts taken up per mg of tissue during the period of exposure was calculated and the difference between drug and control tested for significance by Student's "t" test. The results are shown in Table 1.

TABLE 1. UPTAKE OF  $^{47}\text{Ca}^{2+}$  BY ISOLATED DEPOLARISED STRIPS OF THE LONGITUDINAL MUSCLE OF THE GUINEA-PIG ILEUM IN RESPONSE TO DRUGS

Drug	Dose ( $\mu\text{g}/\text{ml}$ )	No. of pairs	Mechanical Response	Effect on $^{47}\text{Ca}^{2+}$ uptake
Acetylcholine chloride .. ..	10	22	Contraction	Increased 0.05 < P < 0.10
Adrenaline hydrogen tartrate ..	10	23	No change	No change 0.60 < P < 0.70
Carbachol .. .. .	0.3	24	Contraction	Increased 0.01 < P < 0.02
Histamine acid phosphate ..	2	24	Small contraction	No change 0.10 < P < 0.20
5-Hydroxytryptamine creatinine sulphate .. .. .	50	24	Small contraction	Increased 0.05 < P < 0.10
Papaverine sulphate .. ..	10	24	Relaxation	Decreased P < 0.001

Carbachol and acetylcholine significantly increased  $^{47}\text{Ca}^{2+}$  uptake, thus confirming the results of Durbin & Jenkinson (1961a,b) and Robertson (1960) respectively. Histamine and adrenaline caused no significant change, but 5-hydroxytryptamine significantly increased  $^{47}\text{Ca}^{2+}$  uptake. The highly significant decrease in uptake induced by papaverine is of considerable interest because, at the dose level used, this drug relaxes depolarised smooth muscle. Papaverine does not, however, depress  $^{47}\text{Ca}^{2+}$  uptake in non-depolarised ileum (Banerjee & Lewis, 1963). Failure of adrenaline to alter  $^{47}\text{Ca}^{2+}$  uptake significantly may be linked with its failure to relax the depolarised tissue and points to adrenaline and papaverine exerting their effects by different mechanisms. Calcium is probably essential in greater amounts for maintained contraction than is required for relaxation and during relaxation calcium permeability is much reduced. This effect underlines the importance of calcium ions for the contraction of smooth muscle.

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