Influence of drugs on $^{47}Ca^{2+}$ release from depolarized intestinal smooth muscle

SIR,—Schatzmann (1963) noted that acetylcholine or excess potassium increased the rate of $^{45}Ca^{2+}$ exchange in the taenia coli. Durbin & Jenkinson (1961a) have shown that carbachol-stimulation of the depolarized taenia coli does not alter $^{45}Ca^{2+}$ efflux, and more recently von Hattingberg & Rahn (1964) have found that $^{47}Ca^{2+}$ release from the taenia coli was increased by papaverine.

Using strips of longitudinal muscle from the guinea-pig ileum, depolarized in potassium-rich Krebs solution, we have observed the effects upon $^{47}Ca^{2+}$ release of a group of drugs which act upon smooth muscle. The muscle was prepared according to Ambache (1954) and Weiss, Coalson & Hurwitz (1961). Four 2 cm long pieces from the same length of muscle were used for each series. Adjacent pieces served as test and control. Each strip was stretched by a 0·2 g weight, incubated for 30 min at 37° in Krebs solution and then for not less than 2 hr in radioactive, potassium-rich Krebs solution at the same temperature. After washing (30 sec) in a stream of non-radioactive, potassium-rich Krebs solution, each pair of strips was exposed for 8 consecutive 1 min periods to tubes containing 5 ml of non-radioactive potassium-rich Krebs solution; the 4th and 7th tubes of one series contained the drug, those of the parallel series, the control solution. With papaverine, (Table 1) the exposure period was lengthened to 10 min to allow relaxation to be more complete; with adrenaline (Table 1), this was to attempt to obtain a significant relaxation. Each solution was counted (100 sec; thallium-activated sodium iodide crystal scintillation counter; ECKO type N597) and the total counts released added to those remaining in the tissue at the end of the experiment. The % counts released during exposure to the drug were calculated and compared with the control. The difference between control and drug-treated muscles was tested for significance using Student's "*t*" test. The results are shown in Table 1. These confirm the findings of Durbin & Jenkinson (1961a) with respect to carbachol. Acetylcholine and histamine behaved similarly to carbachol. Adrenaline

TABLE 1. RELEASE OF $^{47}Ca^{2+}$ BY DEPOLARIZED STRIPS OF THE LONGITUDINAL MUSCLE OF THE GUINEA-PIG ILEUM

Drug	Dose (μg/ml)	No. of pairs	Effect on $^{47}Ca^{2+}$ release		Mechanical response
			4th min	7th min	
Acetylcholine chloride	10	24	No change $0{\cdot}30<P<0{\cdot}40$	No change $0{\cdot}70<P<0{\cdot}80$	Contraction
Adrenaline hydrogen tartrate ..	10	24	No change $0{\cdot}20<P<0{\cdot}30$	No change $0{\cdot}60<P<0{\cdot}70$	No change
,, ,,	100	12	Increased $0{\cdot}01<P<0{\cdot}02$	Increased $P<0{\cdot}001$	No change
,, ,,	100	12*	Increased $0{\cdot}02<P<0{\cdot}05$	Increased $P<0{\cdot}001$	No change
Carbachol chloride	0·3	20	No change $0{\cdot}40<P<0{\cdot}50$	No change $0{\cdot}70<P<0{\cdot}80$	Contraction
Histamine acid phosphate ..	2	24	No change $0{\cdot}90<P$	No change $0{\cdot}30<P<0{\cdot}40$	Small contraction
5-Hydroxytryptamine creatinine sulphate	50	24	Increased $P<0{\cdot}001$	No change $0{\cdot}20<P<0{\cdot}30$	Small contraction
Papaverine sulphate	10	24	Increased $P<0{\cdot}001$	No change $0{\cdot}40<P<0{\cdot}50$	Relaxation
,,	200	24*	Increased $P<0{\cdot}001$	Increased $0{\cdot}01<P<0{\cdot}02$	Relaxation

*Exposure time 10 min.
All other cases 1 min.

(10 μg/ml) had no significant effect on $^{47}Ca^{2+}$ release, but a large dose (100 μg/ml) increased $^{47}Ca^{2+}$ release significantly without causing any relaxation. Papaverine caused a marked and prolonged relaxation of the depolarized muscle with a significantly increased release of $^{47}Ca^{2+}$. At 4 min 5-hydroxytryptamine significantly increased $^{47}Ca^{2+}$ efflux but had no significant effect at 7 min. A similar pattern was shown by 10 μg/ml papaverine.

During an acetylcholine or carbachol-induced contraction of the depolarized muscle neither we nor Durbin & Jenkinson (1961a) could record a significant increase in calcium efflux, although an increased uptake can be shown (Robertson 1960; Durbin & Jenkinson, 1961a; Banerjee & Lewis, 1964). Schatzmann (1963) has however observed an increased release but no change in uptake of calcium, during stimulation of the non-depolarized taenia coli with acetylcholine or potassium.

Although acetylcholine, carbachol and histamine cause the depolarized muscle to contract, $^{47}Ca^{2+}$ efflux does not significantly increase. This may indicate a failure to mobilize the less freely-exchangeable calcium fractions despite the increase in calcium permeability indicated by increased $^{47}Ca^{2+}$ uptake (Robertson, 1960; Durbin & Jenkinson, 1961a; Banerjee & Lewis, 1964). However at 4 min, 5-hydroxytryptamine causes both a small contraction and a significantly increased $^{47}Ca^{2+}$ efflux, but at 7 min had no significant effect. It may therefore mobilize and cause the loss of one or more of the less readily exchangeable fractions of calcium, and at the same time increase calcium permeability as shown by the increased $^{47}Ca^{2+}$ uptake (Banerjee & Lewis, 1964).

If the presence in the muscle of calcium is essential for maintenance or development of tone—as is suggested by the observation that calcium is essential for contraction in depolarized muscle (Robertson, 1960; Durbin & Jenkinson, 1961b), then calcium may be involved in those reactions which yield the energy for the maintenance of smooth muscle tone. Interference with energy-yielding cellular reactions may thus cause loss of calcium because calcium cannot be utilized and this would be reflected in a decreased calcium uptake (Banerjee & Lewis, 1964) and increased calcium release (Table I). The actions of papaverine might be explained on this basis.

Although we could observe no muscle relaxation, the higher dose of adrenaline caused a significant increase in $^{47}Ca^{2+}$ release. The mechanism here is apparently different from that of papaverine. The calcium released may come mainly from a fraction not involved in maintaining muscle tone in depolarized muscle.

Experimental Pharmacology Division,
Institute of Physiology,
University of Glasgow
Glasgow, W.2

A. K. BANERJEE
J. J. LEWIS

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