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THE ACTION OF ISOPRENALINE IN THE DEPOLARIZED RAT UTERUS

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Although the relaxant and contractile effects of drugs in depolarized smooth muscle were described simultaneously (Evans, Schild & Thesleff, 1958) the former have been less investigated and probably less widely accepted. They conflict more with the accepted notion that the mechanical effects of drugs are associated with changes in membrane potential and they are also technically more troublesome to demonstrate. A contractile effect by acetylcholine in a depolarized smooth muscle could still be considered as a dissociation of the normally concurrent events of depolarization and contraction, but no corresponding argument could be applied to the relaxation of a depolarized muscle by adrenaline since in the normal muscle this relaxation is accompanied, if anything, by a hyperpolarization (Bülbring, Goodford & Setekleiv, 1966). Technically there has been some difficulty in demonstrating relaxant effects of adrenaline in the depolarized taenia coli (Axelsson, Holmberg & Högberg, 1965), although others have found this preparation to be relaxed by isoprenaline (Jenkinson & Morton, 1967).

In the present investigation the effects of isoprenaline were studied in the rat depolarized uterus preparation which is particularly sensitive to this drug (Edman & Schild, 1963). The main object was to examine the role of calcium in relation to the relaxant action of isoprenaline in the depolarized uterus. Some of this work has already been briefly reported (Schild, 1966, 1967).

METHODS

Female rats weighing 190–210 g were used. Their excised uterus horns were suspended in jacketed, temperature-controlled isolated organ baths of 6 ml. volume normally kept at 25° C. Isometric tension on the uterus was measured by a Swema (SG4/3) strain gauge transducer and recorded by an ink-writing Grass polygraph. The transducers were joined to screw stands to which a vernier-type sliding scale was also attached which enabled length changes of the uterus to be measured to the nearest 0.1 mm. An automatic assay apparatus was employed (Boura, Mongar & Schild, 1954) and two horns of the same uterus were usually investigated in parallel.

Experimental procedure

The uterus was immersed in Tyrode solution first and stimulated with increasing doses of acetylcholine (ACh) until maximal responses were produced; the maximal force exerted by a uterus horn was in the range of 3–6 g with 10^{-5} ACh. Immersion of the uterus in potassium Ringer caused a rapid near-maximal contraction followed at once by relaxation which levelled off

exponentially to a new base-line in the course of 5–15 min. If tension was relieved at this point the muscle was seen to have shortened considerably (as further discussed in the text).

The following procedure was used for studying drug effects in the depolarized preparation. Compared with Tyrode solution, the amplification of the tension record was increased about five-fold and the muscle was kept at a basic tension of 0.3–0.6 g (about 10% of maximal ACh tension). Tension had to be frequently readjusted, since the depolarized uterus undergoes slow changes in length in the course of prolonged experiments usually in the sense of shortening; it also undergoes length changes after each wash. When depolarization was induced with Ca-free KCl Ringer the muscle was as a rule stimulated intermittently by calcium before administering isoprenaline. Calcium causes a slow maintained contraction from which relaxant drug effects can be conveniently initiated. An adequate stimulant concentration is 5×10^{-5}M Ca^{++}, but this may be varied since the sensitivity to calcium tends to increase at first and decrease later in the course of depolarization. Changes of sensitivity to isoprenaline are discussed in the text.

Ringer solutions and chemicals

The standard depolarizing Ringer solution used was Ca-free KCl Ringer (145 mM KCl, 12 mM $KHCO_3$, 6 mM glucose). In some experiments K_2SO_4 Ringer (74 mM K_2SO_4, 59 mM sucrose, 12 mM $KHCO_3$, 6 mM glucose) was employed, which has a similar potassium content to KCl Ringer and is iso-osmotic with Tyrode solution (Wolf, 1966). The K_2SO_4 Ringer preserves the responsiveness of the uterus to isoprenaline rather better than KCl Ringer, but sulphate tends to precipitate when high doses of Ca^{++} or Ba^{++} are added to the bath. The term NaCl Ringer refers to a solution of similar composition to KCl Ringer except that potassium salts are replaced by equivalent sodium salts. The Ringer solutions were made up in glass distilled deionized water. It was calculated from measurements of the conductivity of deionized water including the upper limits of contamination of the analytical (BDH) reagents used that the calcium content of KCl Ringer was definitely less than 10^{-5} molar.

The chelating agents ethylenediaminetetraacetic acid (EDTA) and ethyleneglycol bis (2-amino-ethylether) tetraacetic acid (EGTA) were used; the stability constant of the EGTA-calcium complex is 0.3 log units higher than the corresponding EDTA complex, whereas the stability constant of the EGTA-magnesium complex is 3.5 log units less than the corresponding EDTA complex (Ringbom, 1963). Isoprenaline was used as isopropylnoradrenaline sulphate (Burroughs Wellcome) and dichloroisoprenaline as dichloroisopropylnoradrenaline hydrochloride (Aldrich).

RESULTS

The relaxant effect of isoprenaline in the depolarized rat uterus

The KCl-depolarized rat uterus is highly sensitive to isoprenaline. Figure 1 shows, in a uterus immersed in Ca-free KCl Ringer at 25° C, a contractile response to 5×10^{-6}M Ca^{++} followed by relaxation beyond the original base-line after a dose of 5×10^{-11}M isoprenaline (1.3×10^{-12} isoprenaline sulphate) was added to the bath. It further shows a threshold relaxant response with 1.25×10^{-11} isoprenaline.

The threshold concentrations of isoprenaline varied between 10^{-11}M and 10^{-9}M in different uteri. Adrenaline and noradrenaline also relaxed the depolarized rat uterus, but they were less active than isoprenaline. The mean activity ratios noradrenaline: adrenaline: isoprenaline as estimated from 2+2 assays were 1:8:32 (Fig. 2).

Loss and restoration of isoprenaline response

The isoprenaline sensitivity of the depolarized rat uterus in KCl Ringer changes with time; it declines at a varying rate but can be restored by a short period of immersion in sodium Ringer followed by renewed depolarization.

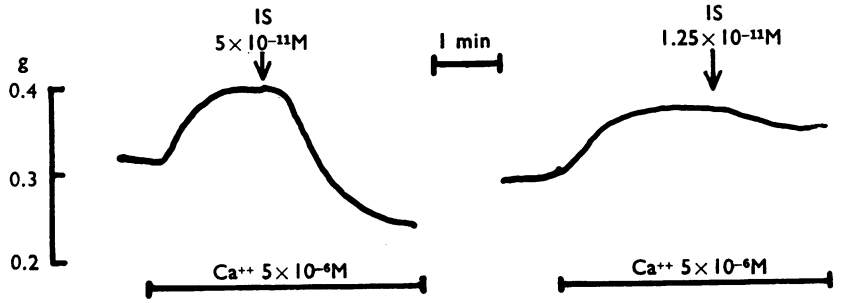

Fig. 1. Contractile effect of calcium chloride and relaxant effect of a superimposed dose of isoprenaline sulphate. Note high degree of sensitivity to both substances. Isometric tension of depolarized rat uterus in Ca-free KCl Ringer, 25° C, in this and subsequent figures. On all tracings IS=isoprenaline.

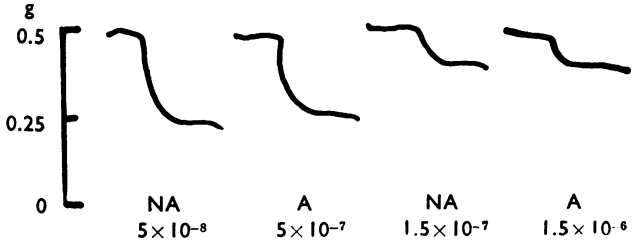

Fig. 2. Excerpt from 2+2 assay of adrenaline (A) and noradrenaline (NA). Preparation intermittently stimulated by 5×10^{-5} M Ca++.

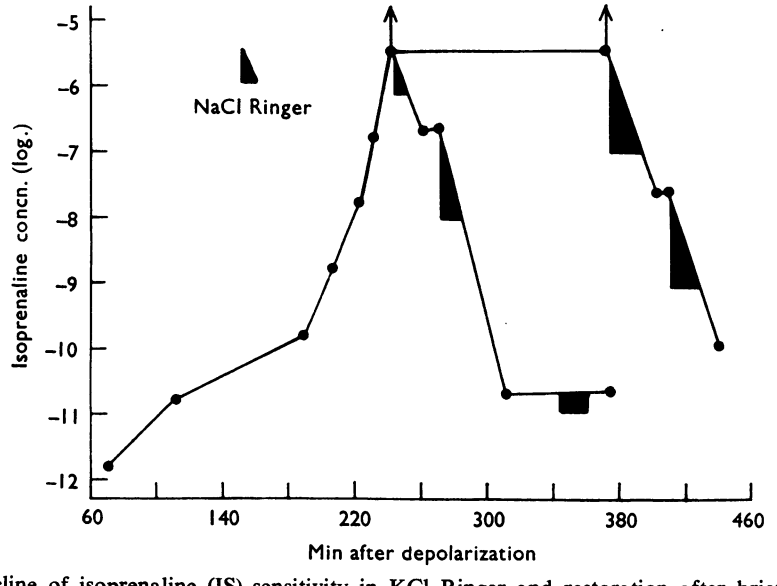

Fig. 3. Decline of isoprenaline (IS) sensitivity in KCl Ringer and restoration after brief immersion in NaCl Ringer followed by return to KCl Ringer (25° C). Two rat uterus horns in parallel stimulated intermittently by $CaCl_2$ followed by isoprenaline. Concentration of isoprenaline for a standard relaxation by interpolation from dose-response curves.

A typical example is shown in Fig. 3, which represents an experiment in which two horns of the same uterus were suspended in Ca-free KCl Ringer, 25° C. Intermittently calcium was administered followed by isoprenaline and the dose required to produce a standard relaxant effect was estimated by interpolation from dose-response curves. In the course of a 4-hr immersion in KCl-Ringer (of which the last three are recorded) the sensitivity to isoprenaline declined by a factor of $>10^6$. When one of the horns was immersed for 10 min in Ca-free NaCl Ringer and afterwards replaced in KCl Ringer its isoprenaline sensitivity was partly restored, and after a further 10 min in the sodium Ringer it reverted to near starting level. The second horn, which was kept in KCl Ringer, in the meantime retained its insensitivity but sensitivity was later restored after two brief periods of immersion in sodium Ringer. A number of experiments of this kind were carried out in the form of comparisons on the two horns of the same uterus. The main findings were as follows.

1. In KCl Ringer the isoprenaline sensitivity declined at different rates. Generally a period of decline from initial high sensitivity was followed by a period of relative or complete stability ; finally, as a rule after several hours' immersion in the depolarizing solution, the sensitivity declined rapidly and completely. Rate of decline in two horns of the same uterus was generally similar. Higher temperatures accelerated the decline. In a few experiments carried out in K_2SO_4 Ringer the decline was less.

2. Although the isoprenaline responses declined at a faster rate, other drug responses also declined including responses to calcium.

3. The decline was not in the nature of tachyphylaxis ; it occurred independently of frequency of administration.

4. The calcium-content of the solution did not seem to affect the decline of sensitivity to isoprenaline.

5. Bathing fluids capable of restoring the response of the uterus included Tyrode solution, NaCl Ringer with and without calcium and buffered isotonic sucrose solution. When the uterus was returned from the restoring solutions to the depolarizing solution it generally exhibited a typical transient depolarization-contraction indicating that it had been partly repolarized.

The theoretical implications of the decline and restoration of isoprenaline sensitivity, including a possible connection between resensitization and repolarization, were not followed up further. From the practical experimental point of view, the phenomenon provided a convenient means of restoring the sensitivity of the KCl-depolarized uterus, thus making it possible to perform prolonged experiments with successive assays on the same preparation (cf. Fig. 10).

Isoprenaline receptors in the depolarized uterus

On the principle that receptors can be identified by antagonists, an attempt was made to find out whether isoprenaline activates the same receptors in normal and depolarized muscle by measuring in both the pA_x values of a typical antagonist, the β-adrenergic receptor blocking agent dichloroisoprenaline (DCI). In the non-depolarized uterus immersed in de Jalon Ringer, isoprenaline was applied after stimulation by carbachol (Ash & Schild, 1966), in the KCl-depolarized uterus after stimulation by calcium.

The antagonism between DCI and isoprenaline in a stable depolarized preparation is shown in Fig. 4; two uterus horns were tested with 10^{-8} and 10^{-6} DCI. The action of the antagonist was assessed after an average contact period of 30 min. The parallel displacement of the log dose-response curves suggests a competitive antagonism.

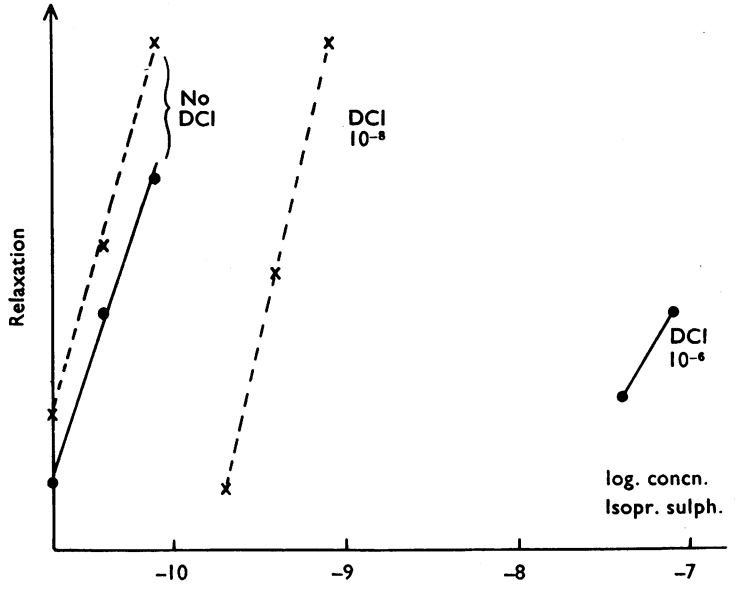

Fig. 4. Antagonism of relaxant effect of isoprenaline by dichloroisoprenaline (DCI). Two depolarized rat uterus horns in parallel.

In order to correlate measurements in normal and depolarized muscle the data for affinity of DCI for isoprenaline receptors in the normal uterus obtained by Ash & Schild (1966) (Fig. 5 of their paper) and those from Fig. 4 of the present investigation were jointly plotted after the manner of Arunlakshana & Schild (1958). The points lie on a common straight line giving a joint pA_2 value of 8.4 (Fig. 5). The slope of the regression line (approximately 1) is compatible with a simple competitive antagonism. The findings thus suggest that isoprenaline activates the same receptors in normal and depolarized uterus.

Antagonism of isoprenaline by chelating agents

The depolarized uterus is in a state of partial contraction even in the absence of calcium in the bath fluid (cf. Fig. 9) and it can be relaxed under these conditions by isoprenaline, but if a chelating agent is added to the bath the relaxant effect of isoprenaline becomes reversibly abolished.

Figure 6a illustrates the antagonism between EDTA and isoprenaline; it shows relaxation by isoprenaline of a uterus immersed in Ca-free KCl Ringer, failure of isoprenaline to cause relaxation after 1 mM EDTA has been added and restoration of the isoprenaline effect after EDTA is removed. Small but significant length changes of the muscle are caused by the EDTA. Following its administration the muscle relaxed, and in order to

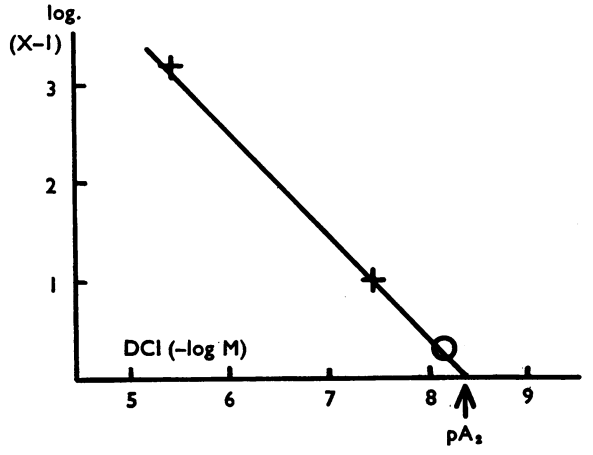

Fig. 5. Antagonism of IS by DCI. Combined results of experiments in non-depolarized (Ash *et al.*, 1966) and depolarized (Fig. 4) rat uterus. Dose ratio = ×. + = depolarized. ○ = polarized.

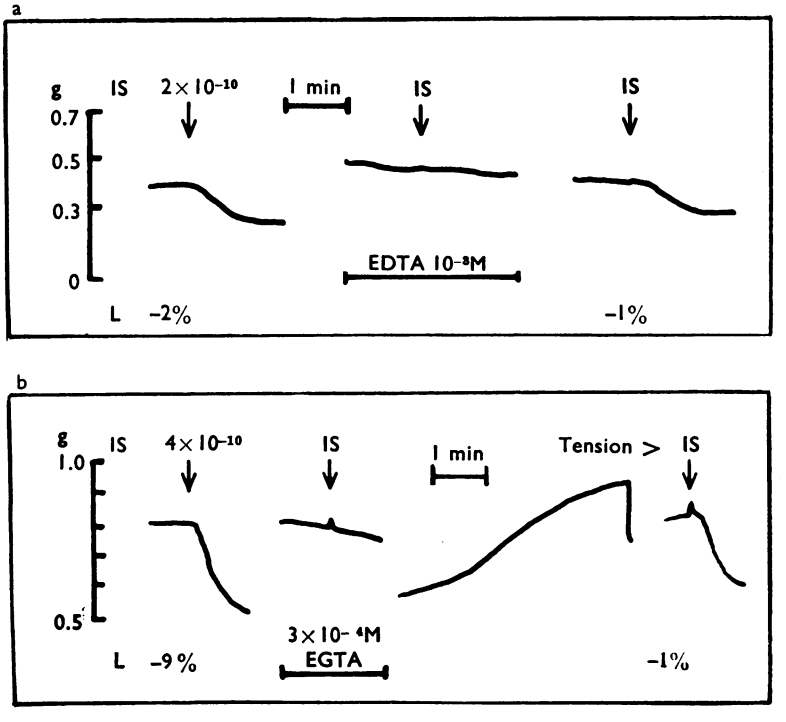

Fig. 6. Antagonism of IS by chelating agents. All effects in Ca-free KCl Ringer without prior stimulation by Ca. (a) Reversible antagonism by 10^{-3} M EDTA. An increase in length (L) of 2% after EDTA and a spontaneous shortening of 1% after its washout were compensated by respectively raising and lowering the transducer attachment point. (b) Similar effects with 3×10^{-4} M EGTA. The spontaneous shortening after EGTA washout is shown.

compensate for this and maintain the original tension the attachment point was raised by 0.8 mm (2% of the resting length of the uterus); following removal of EDTA the muscle shortened spontaneously, which was compensated by lowering the attachment point by 0.4 mm.

Several experiments were carried out with EDTA, all of which showed its antagonism of isoprenaline (Table 1). Another chelating agent, EGTA, was also tested which has affinity for calcium corresponding to EDTA but differs from it by a much lower affinity for magnesium. Table 1 shows that the two chelating agents antagonized isoprenaline in comparable concentrations, suggesting that their action is due to chelation of calcium rather than magnesium. Figure 6b illustrates the antagonism between EGTA and isoprenaline. In the presence of EGTA the relaxant effect of isoprenaline is completely abolished. Again length changes occur. Following the administration of EGTA the muscle lengthens by 9%. After the removal of EGTA the muscle contracts, although its isotonic length change is only 1%; the true shortening of the contractile element is probably masked to some extent by irreversible stretching.

TABLE 1

ANTAGONISTIC EFFECT OF CHELATING AGENTS AGAINST ISOPRENALINE RELAXATION

Numbers of experiments using different molar concentrations of EDTA and EGTA.

	M	5×10^{-3}	10^{-3}	3×10^{-4}	2×10^{-4}	10^{-4}	5×10^{-5}	10^{-5}
EGTA	−					1		4
	+		1		2	3	2	
	†		6	6		7		
	‡		3			2		
EDTA	−							
	+				1			
	†	1	9					
	‡	4	3					

− no effect, + reversible reduction, † reversible abolition, ‡ irreversible abolition.

It is of interest that, while $10^{-3}-10^{-4}$ EGTA antagonized isoprenaline, 10^{-5} M was ineffective (Table 1). This concentration of EGTA also failed to cause relaxation. Since contaminant calcium is below 10^{-5} M, this means that the chelating agents affect tissue calcium rather than contaminant calcium in Ringer solution.

Antagonism between EGTA and papaverine

This was investigated to find out whether chelating agents antagonize isoprenaline at the receptor level. It would then be expected that papaverine, which does not act through β-receptors (Ariëns, 1964), would not be antagonized.

It was found that papaverine relaxed the depolarized uterus and that its action was antagonized by EGTA (Fig. 7). When equiactive doses of papaverine and isoprenaline were used they seemed equally antagonized by EGTA; it thus would appear that EGTA acts on some mechanism which is common to both relaxant drugs, and probably not on receptors.

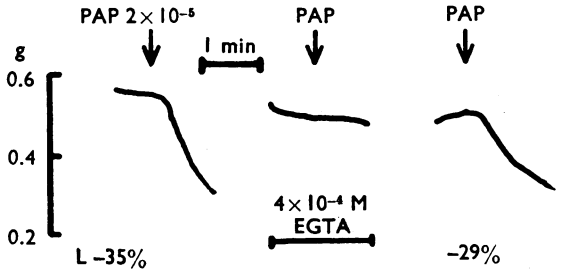

Fig. 7. Antagonism of papaverine by EGTA. Note extensive relaxation in this case by EGTA and spontaneous shortening after removal of EGTA. L=change in length of uterus compared to length in EGTA.

The mechanism of the antagonistic effect of EGTA

EGTA has a relaxant effect in the depolarized uterus which is graded and reversible. Figure 8 shows relaxation by 1 mM EGTA of a uterus suspended in Ca-free KCl Ringer, followed by spontaneous recovery to the original base line after EGTA is removed. Spontaneous shortening after the removal of EGTA is also shown in Figs. 6b and 7. Similar effects after EDTA are shown in Fig. 6a. The question arises whether the antagonism between chelating agents and inhibitory drugs is due simply to a prior relaxation of the contractile element or whether it also involves a more specific aspect connected with calcium-binding. Two approaches were used in the investigation of this problem: (1) the extent of relaxation by EGTA was measured, (2) the inhibitory effect of isoprenaline was tested after stimulation by calcium and by barium.

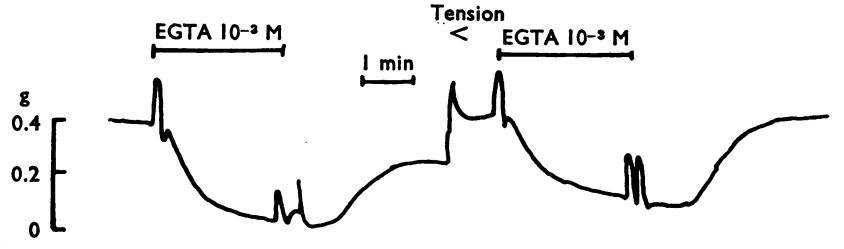

Fig. 8. Relaxation of uterus in Ca-free Ringer by 1 mM EGTA and spontaneous return to original baseline after washout.

Extent of relaxation by EGTA

Length-tension curves of isolated rat uterus were obtained in different media. Preparations were immersed consecutively in Ca-free KCl Ringer, 1 mM Ca-KCl Ringer, 1 mM EGTA-KCl Ringer, 1 mM EGTA-NaCl Ringer, 25° C, varying the sequences. After allowing 5–10 min for equilibration in a new medium, the muscle was stepwise stretched and relaxed, taking care to avoid excessive stretching; maximal tensions were kept below 10% of the maximal acetylcholine tension. Quick stretch was followed in depolarized muscle by a slow fall of tension and quick release by a slow rise, as happens in normal muscle (Hill, 1926). Measurements were made during the relaxation phase; a steady level of tension after quick release was generally reached within 1 min. Length changes were read concomitantly on a vernier scale. Absence of spontaneous activity

was considered a prerequisite for valid measurements. In none of the above mentioned test media did spontaneous activity in fact occur, but when similar measurements were attempted in Tyrode solution or Ca-NaCl Ringer spontaneous activity was induced by stretching and length-tension curves were unobtainable.

The " horizontally " averaged (cf. Ariens, 1964) results of four experiments are shown in Fig. 9. The uterus was most extended in EGTA-NaCl Ringer; this may constitute its normal relaxed length since it corresponded to its length (for the same tension) in Tyrode solution during spontaneous relaxation. Measurements in KCl-Ringer refer to steady-state conditions after the initial transient shortening which follows immediately upon depolarization has worn off. The KCl-depolarized uterus is seen to be shortened to a remarkable extent. During usual experimental procedures this is often masked by non-elastic stretching which in the present case was kept to a minimum. In Ca-KCl Ringer the uterus reached only 50–60% of its full length and even in Ca-free KCl Ringer it remained greatly shortened. By contrast in the presence of 1 mM EGTA the depolarized uterus relaxed to 90% of its length in 1 mM EGTA-NaCl Ringer.

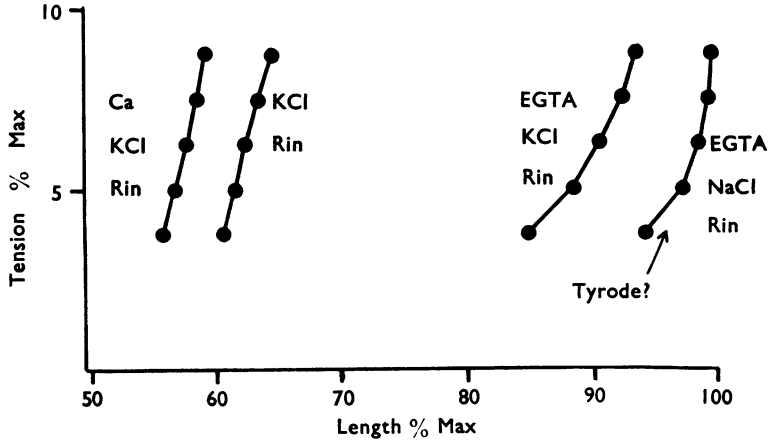

Fig. 9. Length-tension curves of rat uterus in various Ringer solutions. For details see text. Arrow indicates probable position of Tyrode.

The greater length of the muscle in the presence of EGTA agreed with findings (Fig. 8) of spontaneous shortening when a depolarized uterus was transferred from an EGTA-containing solution to a Ca-free solution without EGTA. The extent of spontaneous shortening under these conditions varied; it was seen mostly after short (5–10 min) exposures to chelating agent. The shortening may occur because the intracellular free calcium compartment becomes replenished from an internal store after the chelating agent is removed. Alternatively there may be enough contaminant calcium in the " Ca-free " solution to cause shortening. The greater length in EGTA-NaCl Ringer compared to EGTA-KCl Ringer also agreed with other findings which showed a lengthening of depolarized uterus even after this had been exposed to EGTA upon transference to sodium Ringer.

Differential effect of isoprenaline after Ca and Ba

A different attempt to provide evidence for a specific role of calcium in isoprenaline relaxation was based on Yukishada & Ebashi's (1961) finding that barium contracts depolarized smooth muscle in the absence of calcium. The plan was to administer either calcium or barium to depolarized uterus and to test the effect of isoprenaline subsequently. In order to remove mobile calcium stores as far as possible the uterus was exposed to EGTA beforehand for a sufficient period to abolish its isoprenaline response completely.

Two horns of the same uterus suspended in Ca-free KCl Ringer were exposed to 1 mM EGTA until they ceased to respond to isoprenaline. At this point 2 mM Ca^{++} was added to one bath and 2 mM Ba^{++} to the other, whereupon both horns shortened greatly. Shortening was allowed to proceed isotonically under maintenance of the original tension and after a steady level of contraction was reached isoprenaline was applied. The two preparations were then allowed to recover by immersion in Tyrode solution followed by renewed depolarization and the procedures were repeated in a cross-over arrangement.

Nine paired experiments of this type were carried out in one of which a four-fold cross-over plan was adopted. In each case there was a clear distinction between the two cations, isoprenaline producing a brisk relaxation after calcium and none after barium (Fig. 10). Preliminary treatment with EGTA was essential for the success of this type of experiment. If omitted, isoprenaline relaxed the uterus also after a barium-induced contraction, although its relaxant effect after barium was more sluggish and less pronounced than after calcium.

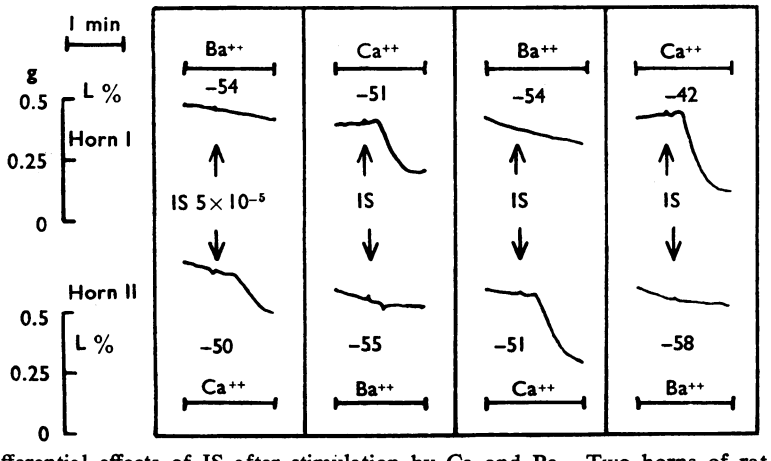

Fig. 10. Differential effects of IS after stimulation by Ca and Ba. Two horns of rat uterus were immersed in 1 mM EGTA-KCl Ringer until responses to iso ceased; then 2 mM $CaCl_2$ or 2 mM $BaCl_2$ added. After readjustment of original tension IS administered. Between panels preparation allowed to recover in Tyrode solution followed by renewed depolarization and repetition of procedure in reversed order. L=shortening as % of length with EGTA; the shortening was slightly greater with Ba than with Ca.

External calcium and isoprenaline relaxation

It has been suggested that the contractile effect of acetylcholine in depolarized smooth muscle might involve a calcium shift either by way of extracellular calcium entering the

cell (Robertson, 1960 ; Durbin & Jenkinson, 1961) or by the intracellular release of bound calcium (Edman & Schild, 1962 ; van Breemen & Daniel, 1966). It could be envisaged that the relaxant effect of isoprenaline in the depolarized muscle involves a calcium shift of the opposite sign and this might likewise take the form of either an elimination of calcium into the extracellular space or an internal calcium shift. In the former case, high concentrations of extracellular calcium relative to the presumably low free intracellular calcium would tend to oppose the extrusion of calcium and might thus interfere with isoprenaline relaxation. The effect of isoprenaline was, therefore, tested in the presence of a wide range of concentrations of external calcium.

Varying doses of calcium were followed by constant submaximal doses of isoprenaline after the calcium response had reached a plateau. The calcium doses were given in a random sequence in order to counteract tachyphylaxis and also the usual decline of isoprenaline responses. Figure 11 shows an experiment on two horns, each point representing their mean response. Calcium gave a graded S-shaped log. dose-response curve, while isoprenaline produced much the same relaxation independently of the external calcium concentration. That isoprenaline is capable of producing graded effects was shown by other experiments in which the dose of calcium was kept constant and that of isoprenaline varied when steeply graded dose response curves were obtained.

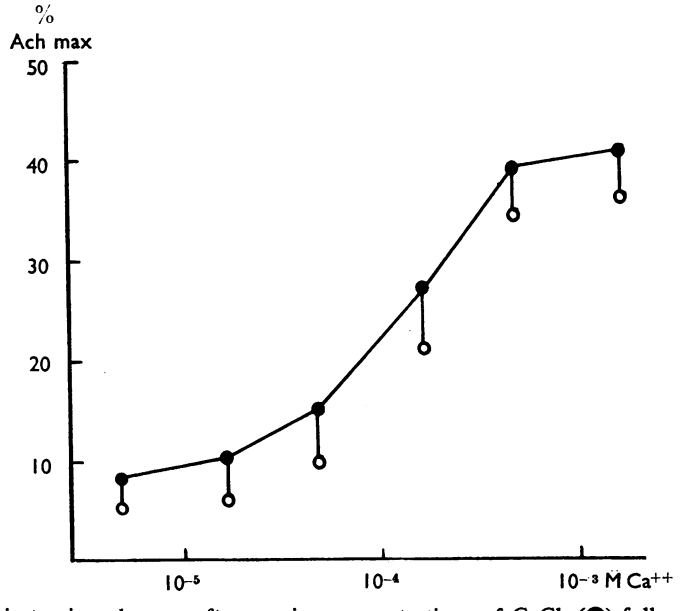

Fig. 11. Isometric tension changes after varying concentrations of $CaCl_2$ (●) followed by a constant dose of 3×10^{-10} IS (○).

DISCUSSION

Action of DCI

Isoprenaline relaxes the depolarized rat uterus in low concentrations which correspond to those affecting the normal uterus and its action is antagonized by DCI. The

quantitative agreement between the affinity of DCI for receptors in normal and depolarized uterus (Fig. 5) is evidence that isoprenaline acts on the depolarized uterus by way of its normal β-adrenergic receptors.

There is no reason to believe that the relaxation mechanism of depolarized muscle is fundamentally different from that of normal muscle and in this sense the depolarized uterus can be regarded as a simplified model of the non-depolarized muscle in which certain aspects of drug action can be studied "in isolation." In other respects, the depolarized smooth muscle is an obviously inadequate model since it excludes those electrical events which are intimately bound up with a polarized cell membrane and which are an integral aspect of normal drug action in smooth muscle.

Chelating agents

The chelating agents EDTA and EGTA produced two main effects in the depolarized rat uterus (1) a loss of tone followed by spontaneous recovery when the chelating agent was removed, (2) a reversible antagonism of the relaxant effects of isoprenaline and papaverine. Since EDTA and the more specific calcium-chelating agent EGTA had a similar action it may be inferred that both produce their effects by the chelation of calcium rather than magnesium. A likely assumption is that relaxation by chelating agents is due directly or indirectly to a reduced level of free intracellular calcium. The chelators may penetrate the cell (a fractional penetration of the unionized moiety would be adequate) or alternatively they may combine with an extracellular bound calcium fraction (as suggested by v. Breemen, Daniel & v. Breemen, 1966), which in turn is in equilibrium with an intracellular calcium compartment.

The antagonism between chelating agents and relaxant drugs is of special interest, since it suggests that calcium is somehow involved in drug-induced relaxation. Unlike the antagonism by DCI, the antagonism by chelating agents does not appear to be exerted at the drug-receptor level, since isoprenaline and papaverine are both affected. Experimentally, the relaxant and antagonistic effects of chelating agents seemed connected, but it was not possible to establish whether a causal link exists. If it were assumed that drug-induced relaxation as well as relaxation by chelating agents involves a reduction of free intracellular calcium, some connection between the two events would in any case have to be expected.

Effects of barium

The contractile effect of barium in the depolarized uterus resembles that of calcium except that on a molar basis calcium is the more active (Schild, 1966). The graded and repeatable character of the effect of barium suggests a direct action rather than an indirect action through the release of calcium. This agrees with the finding that barium remained fully active as a contractile agent after the uterus was exhaustively treated with EGTA. Barium may penetrate the smooth muscle cell and take the place of calcium in activating the contractile element.

An important discrepancy between the action of calcium and barium occurred in relation to isoprenaline. It was found that in a uterus previously treated with EGTA, calcium produced a contraction which could be relaxed by isoprenaline, while contraction

by barium could not be so relaxed. A hypothesis which might account for this discrepancy is discussed below.

A hypothetical mechanism for isoprenaline relaxation

It is postulated that a calcium-accumulating mechanism operates in the uterine smooth muscle cell which is functionally, though not necessarily morphologically, related to the relaxing factor of striated muscle. Isoprenaline, and possibly other smooth muscle inhibitors, activate this factor and cause it to accumulate calcium, thus lowering the concentration of sarcoplasmic free Ca^{++} below the threshold of 0.3–1.5 μM (Portzehl, Caldwell & Rüegg, 1964) and causing relaxation. The process is thus considered to entail an internal calcium shift of opposite sign to that postulated for acetylcholine-induced contractions of the depolarized uterus (Edman & Schild, 1962 ; v. Breemen *et al.*, 1966).

The observed effects of barium in the depolarized uterus have possible counterparts in the excitation-contraction coupling system of striated muscle. Thus it has been shown that their contractile element can be activated by barium since intracellular injections of barium in Maia muscle cause contraction (Caldwell & Walster, 1963), on the other hand it has been shown that the sarcoplasmic reticulum is unable to accumulate barium under conditions in which it accumulates calcium (Nagai, Takahashi & Takauji, 1965). By analogy, barium might be able to activate the contractile element in the uterus cell but its calcium-accumulating mechanism might be unable to accumulate barium, thus explaining the inability of isoprenaline to relax the barium-contracted uterus.

This conception entails a number of unverified assumptions. Thus it is not known whether a low-concentration compartment of free Ca^{++} exists in smooth muscle, although the finding (Schild, 1966) that a threshold contraction of the depolarized uterus can be induced by the addition of 2×10^{-6} M Ca^{++} to the bath suggests that it may exist. The main difficulty is the failure, so far, to isolate a calcium-accumulating factor from smooth muscle. Electron-microscopically, the occurrence of a sarcoplasmic reticulum in the smooth muscle of the uterus has been established (Mark, 1956 ; Schoenberg, 1958), but no appreciable relaxing factor properties for uterine muscle have been demonstrated (Hasselbach & Ledermair, 1958). This may possibly be due to lability of the factor as in heart muscle in which earlier work seemed to indicate a very low calcium-accumulating activity, but more recent work has shown activity of about one-third that in striated muscle (Inesi, Ebashi & Watanabe, 1964).

Effect of extracellular calcium on isoprenaline relaxation

An alternative possibility put forward by Hasselbach (1964) is that the smooth muscle cell may eliminate calcium by extrusion. In order to test this possibility the effect of isoprenaline was examined at a wide range of external calcium concentrations but the extent of relaxation was found to be essentially independent of external calcium. This does not necessarily eliminate the possibility of calcium extrusion which could operate against a high concentration gradient as in nerve (Hodgkin & Keynes, 1959) but it makes it less likely. Whichever mechanism of calcium accumulation is postulated for isoprenaline relaxation, it would probably be an active, energy requiring process as in the calcium accumulation by the relaxing factor which requires ATP (Hasselbach & Makinose, 1961 ; Ebashi & Lipmann, 1962).

SUMMARY

1. The β-adrenergic blocking agent DCI has similar affinities for receptors in normal and KCl-depolarized rat uterus, suggesting that isoprenaline acts on the depolarized muscle through its normal β-receptors.

2. The Ca-chelating agents EDTA and EGTA inhibit the relaxant effect of isoprenaline in the depolarized uterus. They also inhibit the relaxant effect of papaverine, suggesting that their inhibitory effect is not exerted on β-receptors. The chelating agents themselves relax the depolarized muscle.

3. If a depolarized uterus whose Ca stores have been depleted by EGTA is then stimulated by Ba it contracts but cannot be relaxed by isoprenaline, in contrast to a uterus stimulated by Ca, suggesting that Ca plays a special role in the relaxation.

4. Isoprenaline relaxation of depolarized uterus occurs independently of the external Ca concentration.

5. The hypothesis is discussed that isoprenaline may induce an intracellular Ca shift in the depolarized muscle of opposite sign to that which has been postulated for acetylcholine.

6. A gradual deterioration of the isoprenaline response of the uterus in KCl Ringer and a restoration by brief immersion in sodium Ringer is described.

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