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SOME PHARMACOLOGICAL PROPERTIES OF THE CIRCULAR AND LONGITUDINAL MUSCLE STRIPS FROM THE GUINEA-PIG ISOLATED ILEUM

BY

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A circular and a longitudinal muscle strip were prepared from adjacent parts of a guinea-pig ileum and a direct pharmacological comparison made under identical conditions. The longitudinal preparation was sensitive to acetylcholine, methacholine, carbachol, 5-hydroxytryptamine, histamine and nicotine, while the circular preparation was insensitive to 5-hydroxytryptamine, histamine and nicotine, and responded to the choline esters only in high concentrations. Incubation of the preparations with the anticholinesterase, mipafox (*NN*-diisopropylphosphodiamidic fluoride), sensitized both preparations to the action of acetylcholine; potentiation of the contraction of the longitudinal muscle was 16-times; that of the circular one 4,000-times. The longitudinal muscle was more sensitive than the circular muscle to acetylcholine whether both were treated with mipafox or not. Bradykinin and substance P both stimulated the longitudinal but not the circular muscle, an effect not modified after mipafox. Hyoscine antagonized the responses of the circular muscle strip, treated with mipafox, to acetylcholine and to histamine, but on the longitudinal muscle strip the response to histamine was not affected, the response to acetylcholine being competitively antagonized. Morphine, in the same concentrations on both circular and longitudinal muscle strips, antagonized the stimulant actions of nicotine and to a lesser extent of 5-hydroxytryptamine, but the responses to histamine on the longitudinal muscle strip were not antagonized by morphine which was in contrast to its action on the circular muscle strip. These observations showed that the main differences in the responses of the circular and longitudinal muscle of the guinea-pig ileum to drugs were in the intrinsic properties of the smooth muscle cells. In addition cholinesterase may protect the circular muscle cells. Finally the circular muscle strip preparation proved to be a useful tool to study the action of drugs on the nervous plexuses of the ileum of the guinea-pig.

The properties of the circular and longitudinal muscle layers of the intestine were found to differ by several workers; for the rabbit by Feldberg & Solandt (1942) and by Vogt (1943); for the dog by Bozler (1949); for the cat by Admiraal, Myers & Houten (1955); and for the guinea-pig by Kosterlitz & Robinson (1957). All these experiments were based on records of persistalsis recorded from a segment of intestine. Harry (1963) described a muscle strip preparation of the guinea-pig isolated ileum and showed that the circular muscle differed from the longitudinal muscle in its responses to drugs. In this paper the properties of circular and longitudinal muscle strips are compared directly.

METHODS

Two adjacent segments each 1 cm in length were taken from the isolated ileum of a guinea-pig 15 cm from the ileo-caecal junction. One segment was opened by a longitudinal incision through the wall along its mesenteric border, and the resultant rectangle of ileum was pinned out under Krebs solution with the mucosal surface upwards. A strip was produced by cutting the rectangle in the direction of the circular fibres. Some four to six cuts produced a strip of circular muscle of sufficient length (Harry, 1963). A cotton ligature was tied around each end of the strip and one end was attached to a glass holder and the other to a small metal hook. From the other segment a longitudinal muscle strip was prepared in the same manner as for the circular muscle strip, but the cuts were made along the length of the longitudinal fibres. The same number of cuts were similarly spaced in each of the preparations. The tissues were immersed in organ-baths containing 23 ml. of Krebs solution maintained at 37° C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The metal hooks were attached to isotonic levers with frontal writing points and with loads of 290 mg ; the responses were magnified six-times.

Drug injection cycle

Circular muscle. A drug was added to the organ-bath fluid from a syringe or pipette and left in contact with the tissue for 1 min, after which the fluid was changed. After a further minute the organ-bath fluid was changed once more, and after another minute the drug was added to the organ-bath fluid again, so making a 3 min drug injection cycle. To record the effects of an antagonist drug the tissue was first exposed to the antagonist for 30 min and each time the organ-bath fluid was changed the antagonist was replaced.

Longitudinal muscle. The drug injection cycle was the same as that for the circular muscle except that the time for drug contact was 30 sec instead of 1 min.

Drugs. The drugs used were: *NN*-diisopropylphosphodiamidic fluoride (mipafox), acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate, histamine phosphate, methacholine chloride, nicotine acid tartrate, carbachol chloride, hyoscine hydrobromide, morphine sulphate, pure synthetic bradykinin and a crude extract of substance P from horse intestine. All drugs, except bradykinin, were dissolved in Krebs solution, the lower concentrations made freshly each day from concentrated stock solutions in distilled water. The bradykinin was dissolved in sterile distilled water. Except where stated drug concentrations are expressed as μg of base/ml. of final organ-bath concentration, with the exception of mipafox, hyoscine and morphine which were expressed as the salts.

RESULTS

The effect of mipafox on the responses of a longitudinal and a circular muscle strip to acetylcholine, 5-hydroxytryptamine, histamine and nicotine

The circular muscle strip responded to acetylcholine, methacholine or carbachol only in high concentrations, but not to 5-hydroxytryptamine, histamine or nicotine (Fig. 1, upper record). In contrast the longitudinal muscle strip was sensitive to these compounds in the low concentrations usually seen with the Magnus longitudinal preparation (Fig. 1, lower record). Carbachol was more effective than methacholine which, in turn, was more effective than acetylcholine in stimulating the circular muscle strip ; the respective concentrations which produced equivalent contractions were 0.2, 10 and 100 μg/ml.

After incubation with mipafox (100 μg/ml.) for 90 min the circular muscle strip responded to 5-hydroxytryptamine and the response to acetylcholine was potentiated about 4,000-times (Harry, 1963).

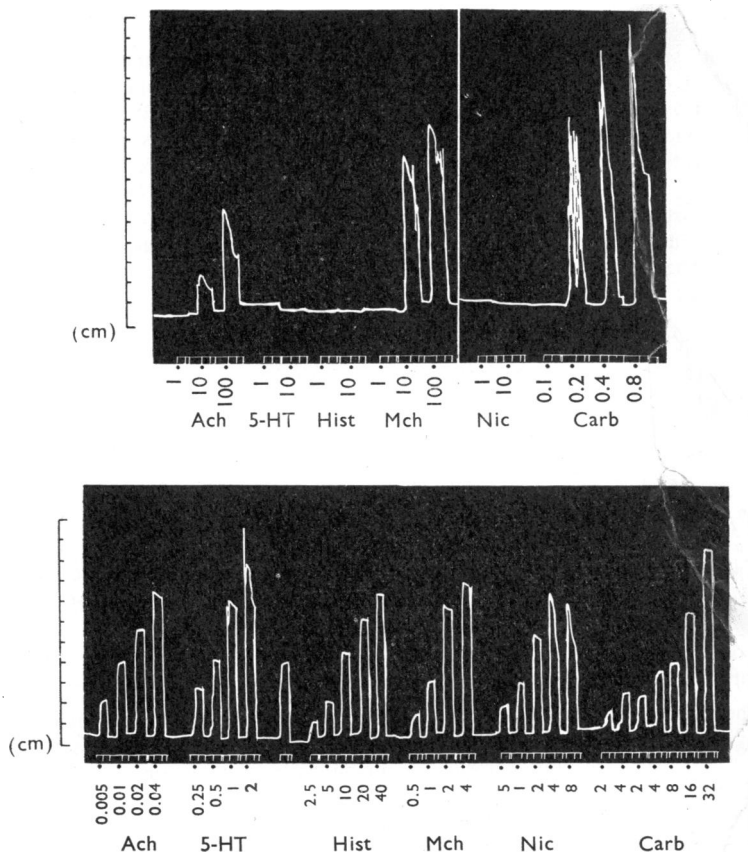

Fig. 1. The effects of acetylcholine (Ach), 5-hydroxytrytpamine (5-HT), histamine (Hist), nicotine (Nic), methacholine (Mch) and carbachol (Carb) on a circular and a longitudinal muscle strip. Upper record shows responses of the circular strip. Lower record shows responses of the longitudinal strip. Numbers refer to drug concentrations expressed as μg/ml. organ-bath concentration, with the exception of histamine, methacholine and carbachol, which are expressed as ng/ml. organ-bath concentration on the longitudinal strip. Time signal=30 sec.

The longitudinal muscle strip, after mipafox, gave dose/response lines which for acetylcholine, 5-hydroxytryptamine and nicotine were shifted to the left (potentiation), but that to histamine was shifted to the right (Fig. 2). The potentiation of the response of the longitudinal strip to acetylcholine was about 16-fold.

The effects of bradykinin and of substance P on the circular and longitudinal muscle strips

Both bradykinin (Day & Vane, 1963) and substance P (Pernow, 1953 ; Blair & Clarke, 1956) have strong smooth muscle stimulating actions. Neither pure bradykinin (5.0 μg/ml.), nor a crude extract of substance P (400 μg of crude extract/ml.) had any stimulating action on the circular muscle strip either before or after exposure to mipafox (100 μg/ml.) for 90 min (Fig. 3, uppermost and middle records). In

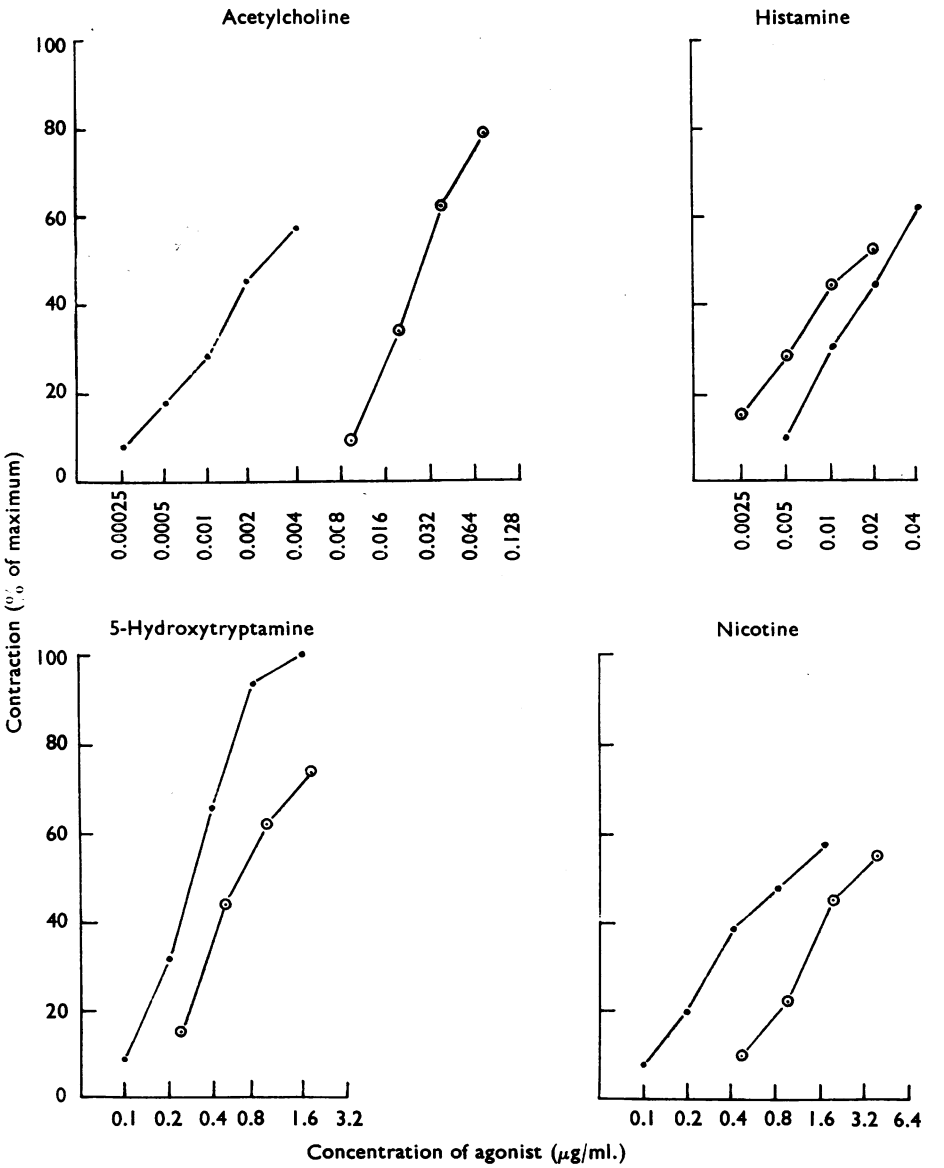

Fig. 2. The effects of mipafox on dose/response curves for acetylcholine, histamine, 5-hydroxytryptamine and nicotine from a longitudinal muscle strip. Abscissa, concentration of agonist (μg/ml.) on log scale; ordinate, contraction expressed as a percentage of maxima. ⊙——⊙ no mipafox; ●——● after exposure of the tissue to mipafox (100 μg/ml.) for 60 min.

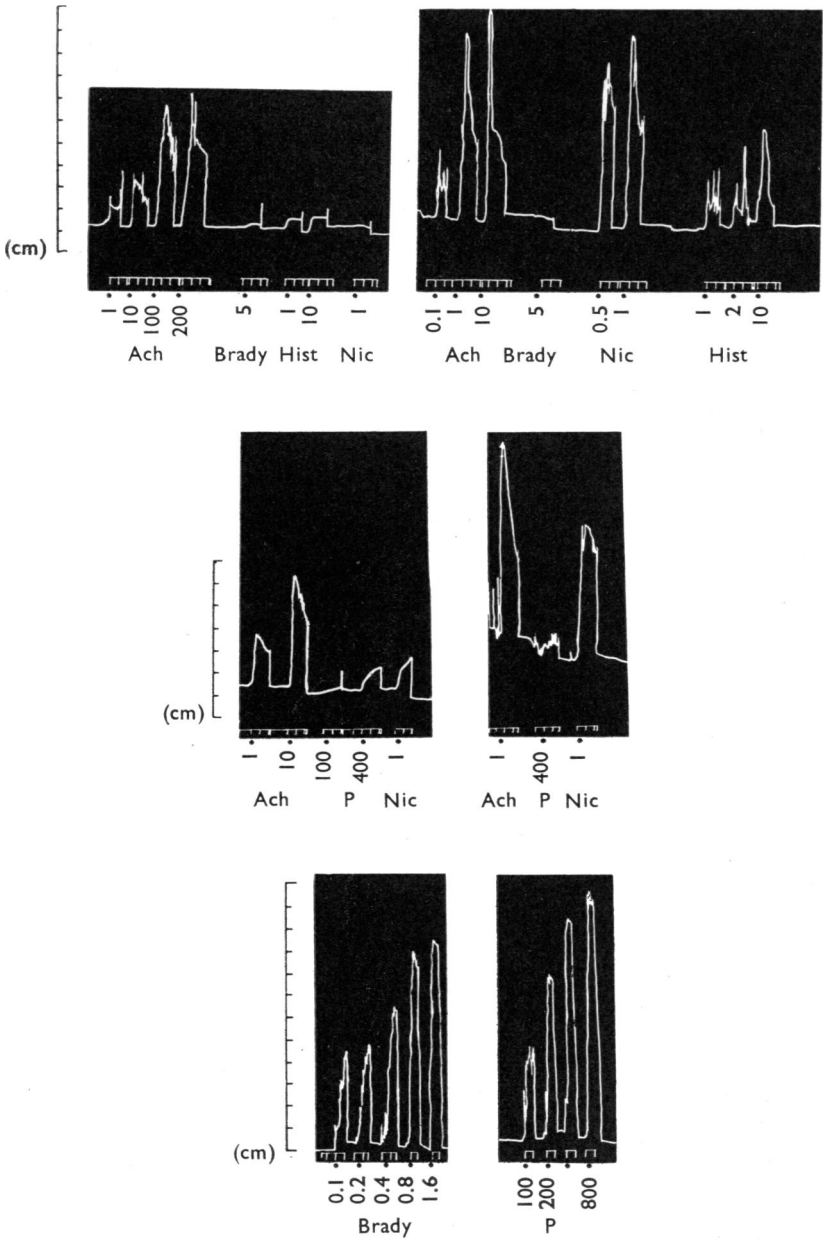

Fig. 3. The effects of bradykinin and substance P on circular and longitudinal muscle strips. All doses in μg/ml. Uppermost record shows the responses of a circular muscle strip to acetylcholine (Ach), bradykinin (Brady), histamine (Hist) and nicotine (Nic), before (left panel) and after (right panel) mipafox. Middle record shows the responses of another circular strip to acetylcholine, substance P (P) and nicotine before (left panel) and after (right panel) mipafox. Lowest record shows responses of a longitudinal strip to bradykinin and to substance P. Time signal=30 sec.

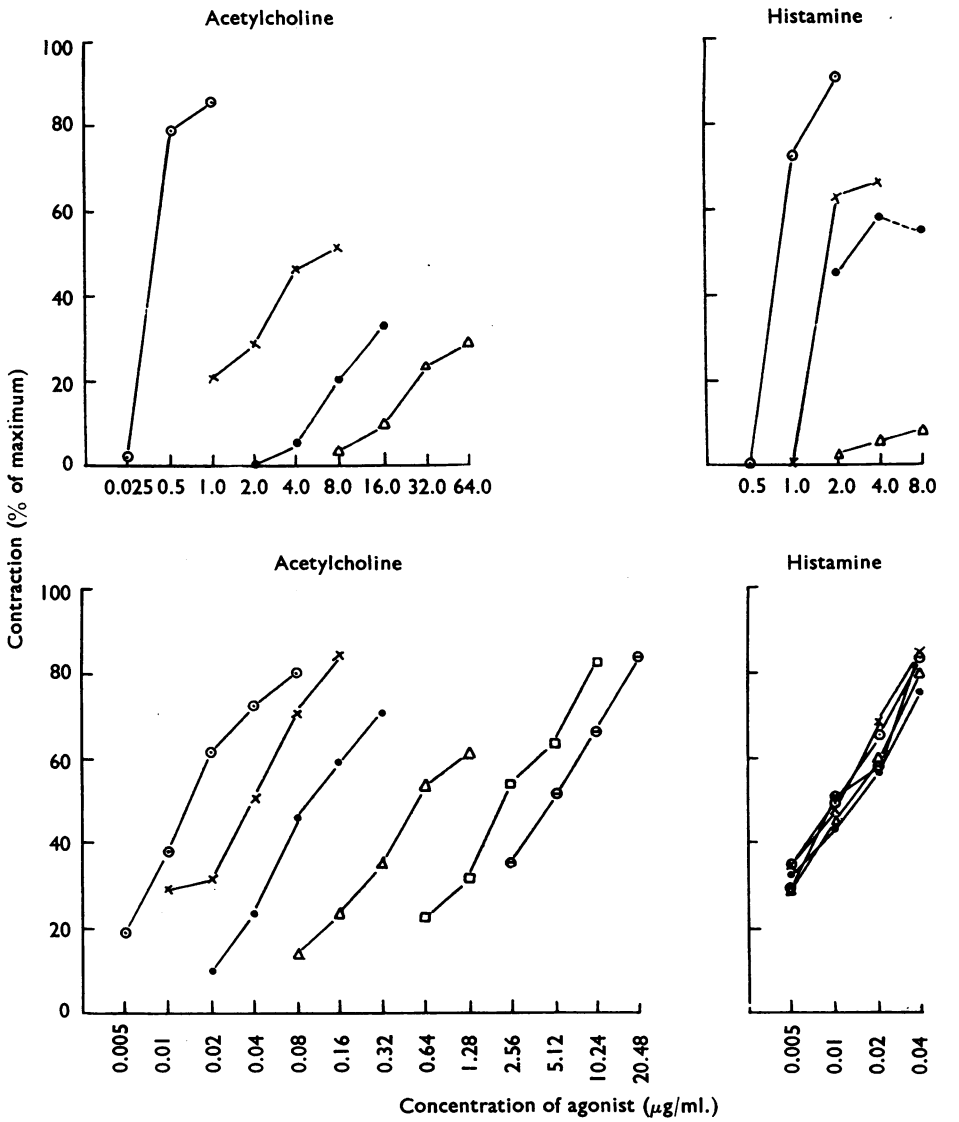

Fig. 4. The effects of increasing concentrations of hyoscine on dose/response curves to acetylcholine and histamine from a circular and a longitudinal muscle strip. Curves from the circular strip are shown in the upper graphs: ⊙ —— ⊙ no hyoscine; × —— × 5 ng/ml.; ● —— ● 10 ng/ml.; △ —— △ 50 ng/ml. of hyoscine in the organ-bath fluid. Curves from the longitudinal strip are shown in the lower graphs: ⊙ —— ⊙ no hyoscine; × —— × 1 ng/ml.; ● —— ● 5 ng/ml.; △ —— △ 10 ng/ml.; □ —— □ 50 ng/ml.; ⊖ —— ⊖ 100 ng/ml. of hyoscine in the organ-bath fluid. Abscissa, concentration of agonist (μg/ml.) on log scale; ordinate, contractions expressed as percentage of maxima.

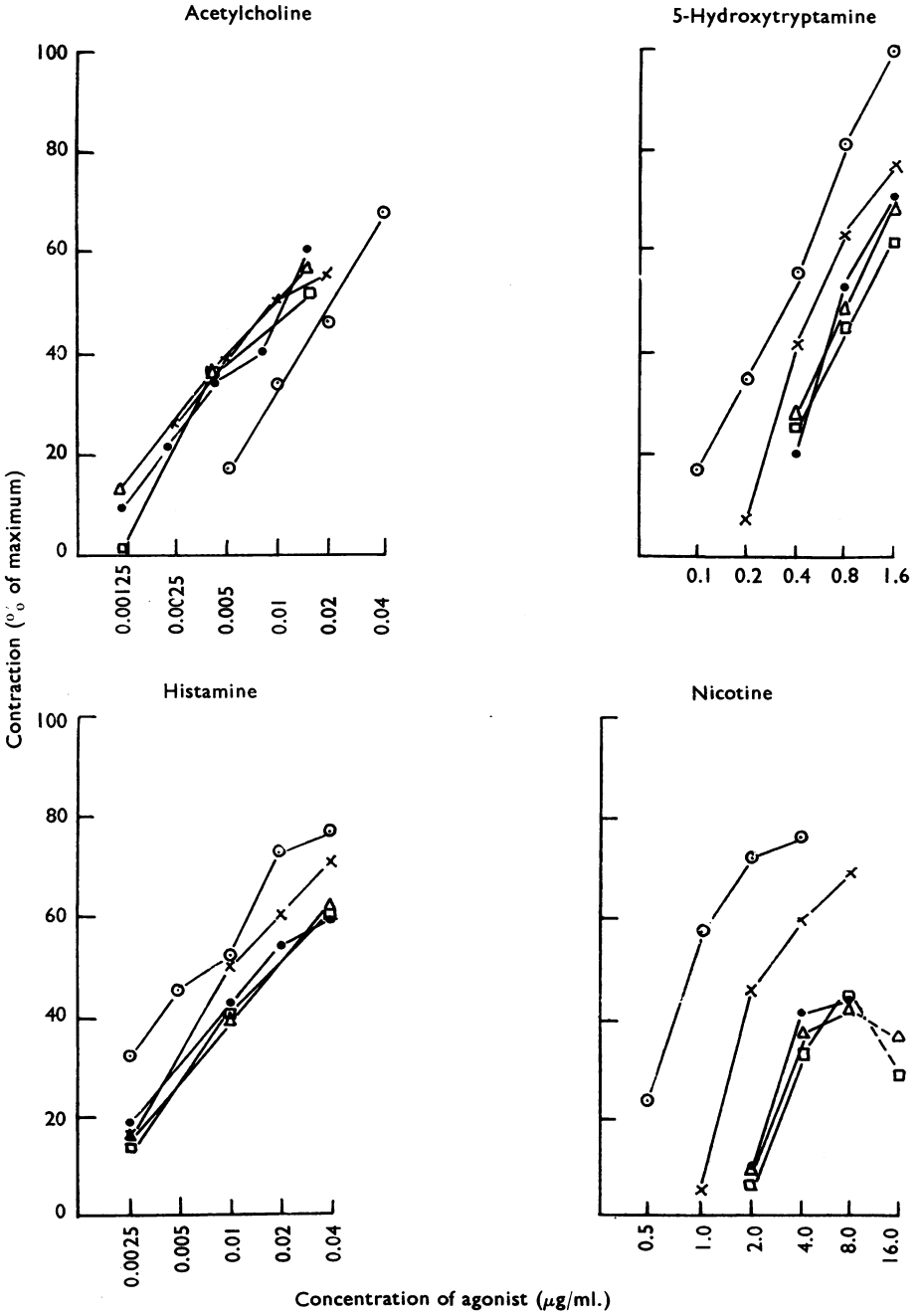

Fig. 5. The effects of increasing concentrations of morphine sulphate on dose/response curves to acetylcholine, 5-hydroxytryptamine, histamine and nicotine from a longitudinal muscle strip. Abscissa, concentration of agonist (μg/ml.) on log scale; ordinate, contraction expressed as percentage of maxima. ○——○ no morphine; ×——× 0.1 μg/ml.; ●——● 0.5 μg/ml.; △——△ 1.0 μg/ml.; □——□ 10.0 μg/ml. of morphine sulphate in organ-bath fluid.

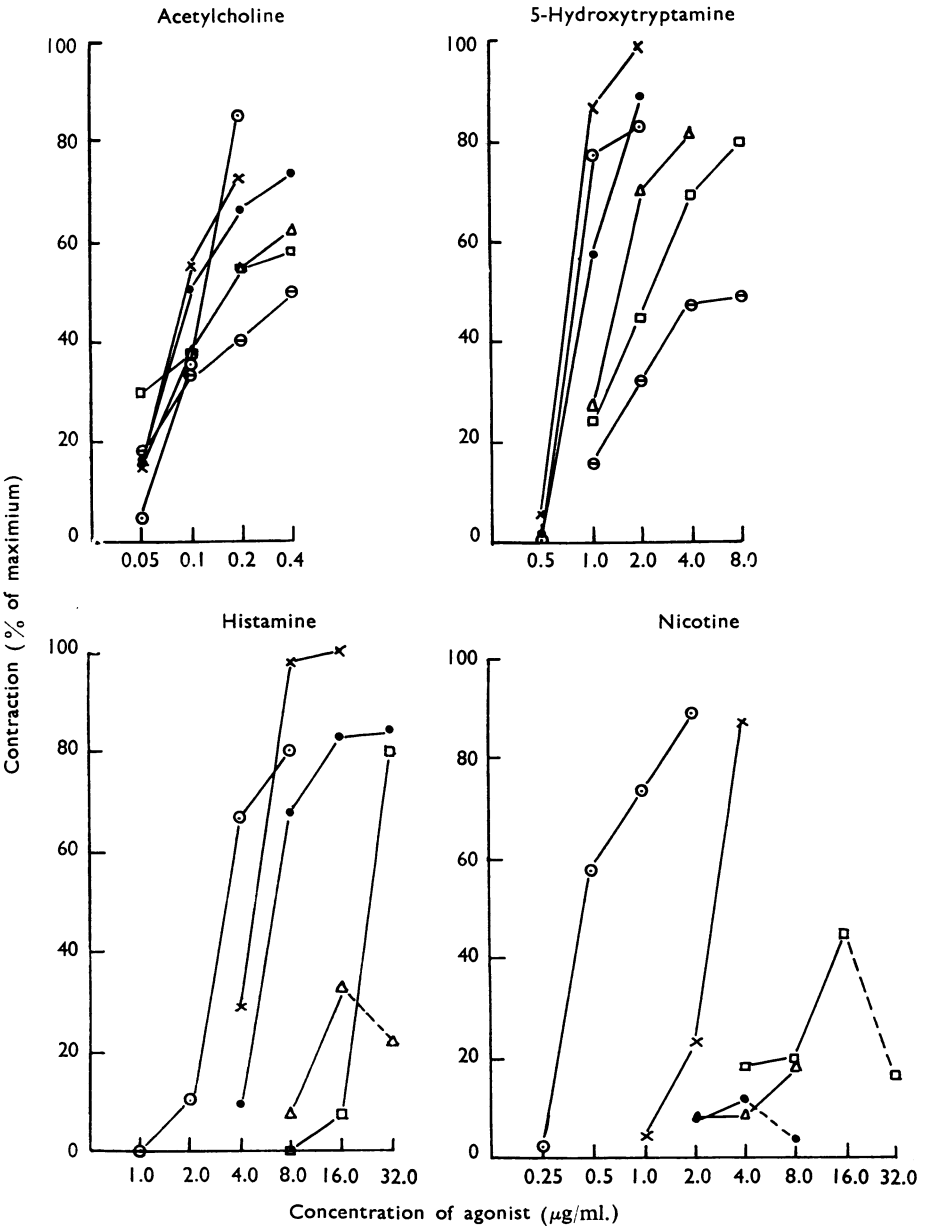

Fig. 6. The effects of increasing concentrations of morphine sulphate on dose/response curves to acetylcholine, 5-hydroxytryptamine, histamine and nicotine from a circular muscle strip. Abscissa, concentration of agonist (μg/ml.) on log scale; ordinate, contractions expressed as percentage of maxima. ○——○ no morphine; ×——× 0.1 μg/ml.; ●——● 0.5 μg/ml.; △——△ 1.0 μg/ml.; □——□ 5.0 μg/ml.; ⊖——⊖ 10.0 μg/ml. of morphine sulphate in organ-bath fluid.

contrast, mipafox produced a great increase in the sensitivity of the circular strip to acetylcholine, histamine and nicotine.

Bradykinin and substance P both strongly stimulated a longitudinal muscle strip (Fig. 3, lowest record).

The effect of hyoscine on the responses of the circular and longitudinal muscle strip to acetylcholine and histamine

Dose/response lines were constructed from measurements of the contractions of a circular muscle strip (Fig. 4, upper graphs) and of a longitudinal muscle strip (Fig. 4, lower graphs) to acetylcholine and histamine in the presence of several concentrations of hyoscine. The dose/response lines for acetylcholine were shifted in parallel to the right (competitive blockade) with both strips ; it can be seen from the dose/response lines that the effects of histamine on the longitudinal muscle strip were not modified by concentrations of hyoscine even as great as 10 μg/ml., while the dose/response lines from the circular strip showed complete antagonism.

The effect of morphine on the responses of the circular and longitudinal muscle strip

Dose/response lines were constructed for the effects of acetylcholine, 5-hydroxytryptamine, histamine and nicotine on a longitudinal muscle strip in the presence of increasing concentrations of morphine sulphate (0.1 to 10.0 μg/ml.). The lines for nicotine were shifted to the right (antagonism) (Fig. 5). Similar concentrations of morphine sulphate were needed on a circular muscle strip to produce a shift to the right of the nicotine and histamine dose/response lines (Fig. 6). Morphine antagonized the response to histamine on the circular muscle strip but not on the longitudinal muscle strip.

DISCUSSION

The evidence presented in this paper shows the longitudinal muscle strip preparation to differ from the circular muscle strip preparation in its qualitative response to drugs. Thus, the longitudinal preparation is sensitive to acetylcholine, methacholine, carbachol, 5-hydroxytryptamine, histamine and nicotine while the circular preparation is insensitive to 5-hydroxytryptamine, histamine and nicotine, and responds to the choline esters only in high concentrations. Incubation of the preparations with the anticholinesterase mipafox sensitizes both preparations to the action of acetylcholine ; potentiation of the longitudinal muscle is 16-fold ; that of the circular muscle 4,000-fold, yet the longitudinal muscle remains intrinsically the more sensitive to acetylcholine. Before mipafox the doses of acetylcholine needed to produce a 40% maximal contraction of the longitudinal muscle preparation and of the circular muscle preparation were 0.024 and 100 μg/ml. ; after mipafox they were 0.0015 and 0.025 μg/ml.

A third difference exists in the response to substance P and bradykinin ; these stimulate the longitudinal muscle strip but not the circular muscle strip. And treatment with mipafox does not alter this difference.

A fourth difference is provided by the responses to histamine. Stimulation of the longitudinal muscle strip by histamine is unaltered after mipafox, hyoscine or

morphine so that the action is directly on the smooth muscle cells. In contrast the action of histamine on the circular muscle preparation is potentiated by mipafox, and antagonized by hyoscine or morphine and is therefore mediated by the nervous plexuses.

Turning now to a consideration of the cellular sites in the guinea-pig ileum at which these differences may arise we may consider, as alternatives, the smooth muscle cells and the intramural nerve plexuses of Auerbach. An additional consideration is the role of cholinesterase. The ability of the longitudinal muscle strip preparation to respond readily to choline esters, to histamine by a direct action, to bradykinin and to substance P, all muscle stimulating drugs, whilst the circular muscle preparation does not, show the smooth muscle cells of the two layers to be intrinsically different.

The same range of concentrations of morphine antagonized the responses of both the longitudinal and circular muscle strips to plexus stimulating drugs. Similarly, nicotine stimulated both preparations in the same concentration ranges. Since both morphine and nicotine have actions wholly on the intramural plexuses, these observations suggest that the nerve fibres supplying both the circular and the longitudinal muscles possess similar pharmacological reactions.

Let us now consider the role of cholinesterase in the relative insensitivity of the circular muscle of the guinea-pig ileum to stimulating drugs. The fact that mipafox revealed the action of indirectly acting drugs suggests that more cholinesterase may be associated with the acetylcholine receptors in the circular muscle layer than in the longitudinal muscle layer (Harry, 1963). Further evidence for this is the potentiation of the acetylcholine responses on the circular muscle strip by 4,000-times; whereas that on the longitudinal muscle strip was only 16-times; again, carbachol, whose action on acetylcholine receptors is not modified by cholinesterases, stimulates the circular muscle strip more readily than does methacholine or acetylcholine; and finally, methacholine, whose action is more stable in the presence of cholinesterases than is that of acetylcholine, stimulates the circular muscle strip at lower concentrations than does acetylcholine.

Evidence from other workers, and in other animals, also supports a difference in the cholinesterases of the circular and longitudinal muscle of the intestine. Koelle, Koelle & Friedenwald (1950) showed on the cat ileum that specific cholinesterase was found in the circular muscle and that concentrations increased from outer to inner regions. Nonspecific cholinesterase was present in the longitudinal layer. Admiraal, Myers & Houten (1955) demonstrated that the circular muscle of the cat small intestine was less sensitive than the longitudinal muscle to intravenous injections of various anticholinesterases.

It is not our purpose to discuss here either the significance of the higher threshold of stimulation by acetylcholine of the circular muscle of the guinea-pig ileum or of the protection afforded by the cholinesterase against exogenous acetylcholine, except to say that both a physiological and survival role may be involved.

From a pharmacological standpoint the circular muscle strip preparation may prove a useful tool in a test of drug activity on the intramural nerve plexuses of

the ileum. Apart from the esters of choline, and perhaps those substances which release acetylcholine, only compounds with a plexus stimulating (indirect) action on the ileum appear to stimulate this preparation after treatment with mipafox. An old problem of the site of action of histamine on the guinea-pig ileum (Ambache, 1946, 1949 ; Emmelin & Feldberg, 1947 ; Feldberg, 1951 ; Ambache & Lessin, 1955) appears now to be solved. Histamine can stimulate the intramural plexuses of this ileum as shown by its action on the circular muscle strip. The longitudinal muscle preparation is more sensitive to the direct muscle stimulating action of histamine than to its nerve plexus stimulating action ; this makes it unlikely that any plexus stimulating action will contribute to the response.

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