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THE INHIBITORY ACTION OF MORPHINE ON THE CONTRACTION OF THE LONGITUDINAL MUSCLE COAT OF THE ISOLATED GUINEA-PIG ILEUM

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Morphine (0.05 to 0.1 μg./ml.) markedly inhibited the contractions of the isolated guinea-pig ileum caused by nicotine, barium, and 5-hydroxytryptamine while the actions of acetylcholine, carbachol, and histamine were affected only a little. Atropine (0.025 to 0.05 μg./ml.) had a similar effect, in addition to its known effects on acetylcholine and carbachol contractions. Morphine had no additional effect on the inhibitory action of atropine, while in the presence of morphine, atropine had a significant additional inhibitory action only on the contraction caused by nicotine.

The action of barium was complex. It caused contractions of a rhythmic type alternating with relaxations, a pattern which is similar to that produced by the emptying phase of the peristaltic reflex; these oscillations were inhibited by hexamethonium. On the other hand, that part of the sustained barium contraction which was inhibited by morphine may be explained by an action on the nerve cells innervating the muscle fibres (motor neurones). Similarly, the morphine-sensitive component of the 5-hydroxytryptamine (5-HT) contraction was probably also due to an action on the motor neurones. The morphine-insensitive contractions of barium, 5-HT, and nicotine were believed to be caused by a direct action on the muscle fibres.

The morphine inhibitors, nalorphine and levallorphan, had different effects with the different agonists. Their morphine-like action was particularly pronounced on the effect of 5-HT, which they antagonized, while their morphine-protecting action was most strongly present on the effect of nicotine.

In the isolated guinea-pig ileum, small concentrations (0.05 to 0.1 μg./ml.) of morphine inhibit both the preparatory and the emptying phases of the peristaltic reflex (Trendelenburg, 1917; Schaumann, 1955; Kosterlitz and Robinson, 1955, 1957). From the evidence available so far, it would appear that the inhibition takes place on the motor side of the reflex arc since drugs which cause contraction of the longitudinal muscle coat by stimulating the nervous structures of the efferent path are also antagonized by morphine. This was established for nicotine by Schaumann (1955), for 5-hydroxytryptamine by Kosterlitz and Robinson (1955) and Gaddum and Picarelli (1957) and for barium ions by Kosterlitz, Robinson, and Taylor (1957). In this paper the mechanism of morphine inhibition is subjected to further analysis.

METHODS

The contraction of the longitudinal muscle layer was recorded either isotonically by a conventional light lever or isometrically by a condenser myograph or a mechano-electrical transducer (Innes, Kosterlitz, and Robinson, 1957).

The bath fluid was Tyrode solution with a low $MgCl_2$ content (0.01 g./l.), aerated with O_2. There were intervals of 3 min. between the individual experimental procedures such as eliciting the reflex or the addition of drugs to the bath fluid (40 ml.), which was renewed at least every 6 min. to prevent a significant rise of the initial *p*H of 7.8.

The distal end of the guinea-pig ileum (7 to 8 cm.) was used after discarding a length of 10 cm. nearest to the ileo-caecal valve. The ileum was set up with an initial tension of 1 g. 1 to 2 hr. before the beginning of the experiment. Occasionally a preparation stored at 4° for 24 hr. was used.

The doses of the drugs are given in weight of base for acetylcholine chloride, carbachol (carbaminoylcholine chloride), histamine phosphate, 5-hydroxytryptamine creatinine sulphate (5-HT); in weight of salt for hexamethonium iodide, nicotine bitartrate, barium chloride, morphine chloride, atropine sulphate and (−)-hyoscyamine sulphate.

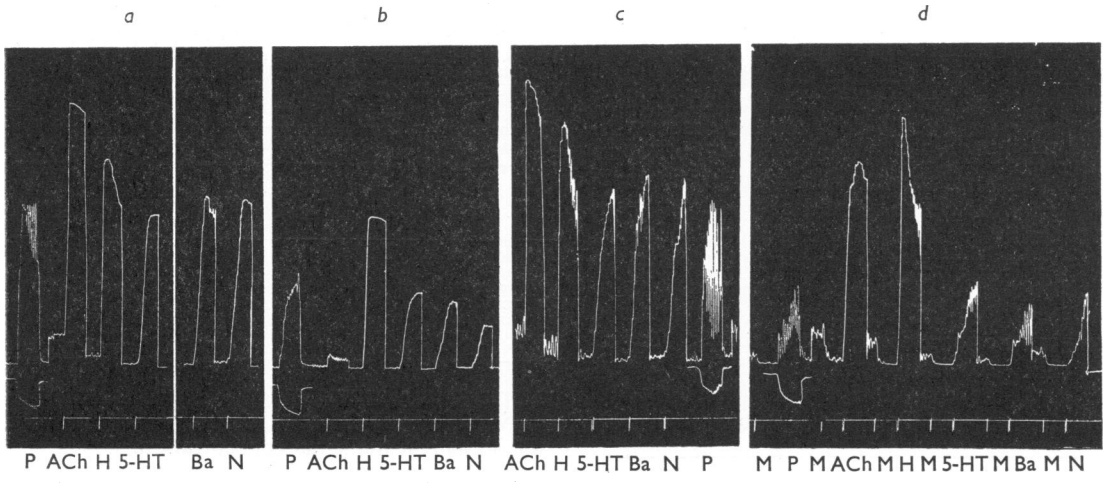

FIG. 1.—The effects of (−)-hyoscyamine and of morphine on isotonic contractions of the longitudinal muscle coat of the isolated guinea-pig ileum. The ileum had been stored at 4° for 24 hr. *a*, controls. *b*, in the presence of (−)-hyoscyamine in a concentration of 0.0005 μg./ml. for 53 min. and of 0.001 μg./ml. for 17 min. before *b*. *c*, 3½ hr. after washing out (−)-hyoscyamine. *d*, 18 min. after *c*, each test 30 sec. after addition of M, morphine (0.05 μg./ml.). P, intra-intestinal pressure raised by 2 cm. H_2O. ACh, acetylcholine (0.0013 μg./ml.). H, histamine (0.0038 μg./ml.). 5-HT, 5-hydroxytryptamine (0.015 μg./ml.). Ba, barium (38 μg./ml.). N, nicotine (1.3 μg./ml.).

RESULTS

Single-dose Experiments Showing the Inhibitory Action of Morphine and (−)-Hyoscyamine

When morphine in a concentration of 0.05 to 0.1 μg./ml. had been present in the bath for 30 sec., the contractions caused by acetylcholine or histamine were depressed only a little or not at all. On the other hand, the contractions due to distension, 5-HT, barium or nicotine were inhibited (Figs. 1*d* and 2). (−)-Hyoscyamine (0.001 μg./ml.) present in the bath fluid throughout the testing period caused a similar depression of the contractions after distension, 5-HT, nicotine and barium but not histamine; the acetylcholine contraction was, of course, inhibited (Fig. 1*b*). While the recovery after hyoscyamine was slow, the inhibitory action of morphine was more transient, recovery usually taking place in less than 15 min.

The contraction caused by barium was somewhat reduced by hexamethonium (25 μg./ml.), a fact already described by Toh (1951), but morphine was a much more powerful inhibitor (Fig. 2). It would appear that the main effect of hexamethonium on the barium contraction was the suppression of large alternating contractions and relaxations of the longitudinal muscle. Visual observation showed these to be associated with contraction of the circular muscle coat of the type found in the peristaltic reflex after intestinal distension.

The Effects of Morphine and Atropine on the Dose/Response Curves of Acetylcholine, Carbachol, Histamine, 5-HT, Barium, and Nicotine

Dose/response curves were obtained for the tensions developed in isometric contractions of the longitudinal muscle layer. In these experiments,

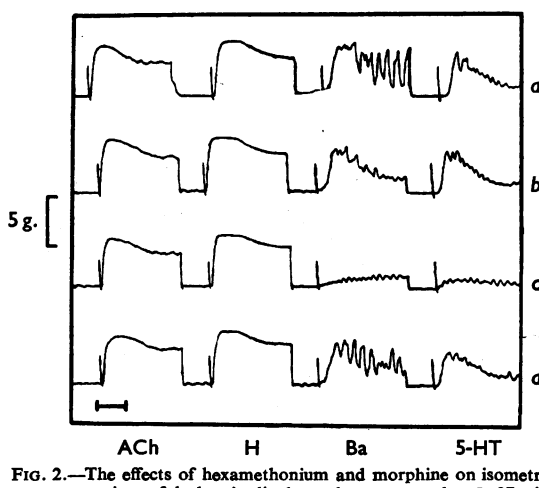

FIG. 2.—The effects of hexamethonium and morphine on isometric contractions of the longitudinal muscle. *a*, controls. *b*, 27 min. after addition of hexamethonium (25 μg./ml.). *c*, 81 min. after addition of hexamethonium and 27 min. after addition of morphine (0.025 μg./ml.). *d*, 27 min. after washing out. ACh, acetylcholine (0.01 μg./ml.). H, histamine (0.025 μg./ml.). Ba, barium (50 μg./ml.). 5-HT, 5-hydroxytryptamine (0.025 μg./ml.). Time, 10 sec.

the antagonists morphine and atropine were left in the bath fluid throughout the testing periods.

Usually as small a dose as 0.05 μg./ml. of morphine produced the maximum inhibition of nicotine, barium, and 5-HT contractions, doses up to 20 μg./ml. being no more effective (Fig. 3). In most experiments the actions of acetylcholine, carbachol, and histamine showed only little permanent depression; when a more pronounced inhibition was observed, this was smaller than the inhibition of 5-HT, barium, and nicotine (Fig. 4 and Table I). Only occasionally, there was a severe but transient depression of acetylcholine or carbachol. For instance, in the experiment shown in Fig. 3, the carbachol contractions were depressed during the first 27 min. after addition of morphine in a concentration of 0.05 μg./ml. and recovered during the next 27 min. with morphine still present in the same concentration.

A tolerance to morphine, which has been demonstrated by Schaumann (1955) and Paton (1957), did not develop under the conditions of these experiments although each concentration of morphine was maintained for 54 min. After the first application, the dose of morphine was raised

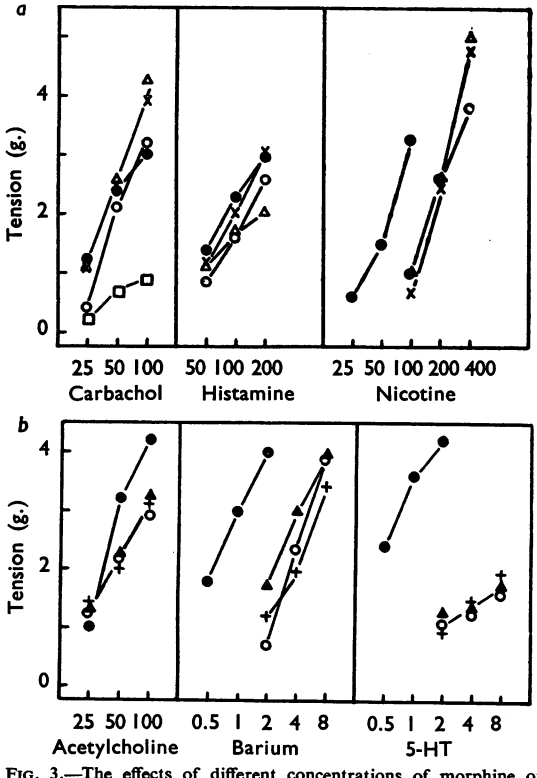

FIG. 3.—The effects of different concentrations of morphine on isometric contractions of the longitudinal muscle in a 40 ml. bath. Two pieces of ileum from two animals were used, *a* without and *b* with hexamethonium (25 μg./ml.). *a*, ● controls. After morphine: □ 3 to 27 min. after 0.05 μg./ml. (carbachol only); O 3 to 54 min. (in the case of carbachol 30 to 54 min.) after 0.05 μg./ml. X 3 to 54 min. after 0.15 μg./ml. △ 3 to 54 min. after 0.45 μg./ml. *b*, ●, controls. After morphine: O, 3 to 54 min. after 0.05 μg./ml. + 3 to 54 min. after 1 μg./ml. ▲, 3 to 54 min. after 20 μg./ml. Each point represents usually but not always the mean of two observations. In the cases of carbachol, histamine and acetylcholine the abscissae are in ng. With barium the abscissa is in mg. while with 5-HT and nicotine in μg.

TABLE I

RATIOS OF DOSES OF ACETYLCHOLINE, CARBACHOL, HISTAMINE, BARIUM, NICOTINE, AND 5-HYDROXYTRYPTAMINE CAUSING EQUAL CONTRACTIONS OF GUINEA-PIG ILEUM IN THE PRESENCE AND ABSENCE OF MORPHINE

The concentration of morphine used was 0·05 to 0·1 μg./ml. In 3 other experiments with 5-HT the dose ratios were >16, >150, >150.

Agonist	No. of Obs.	Dose Ratio	
		Mean	Range
Acetylcholine	16	1·1	0·7–1·4
Carbachol	11	1·4	1·0–3·1
Histamine	9	2·2	1·3–4·4
Barium	15	5·0	2·3–9·0
Nicotine	10	7·0	4·0–15
5-HT	12	23	9–65

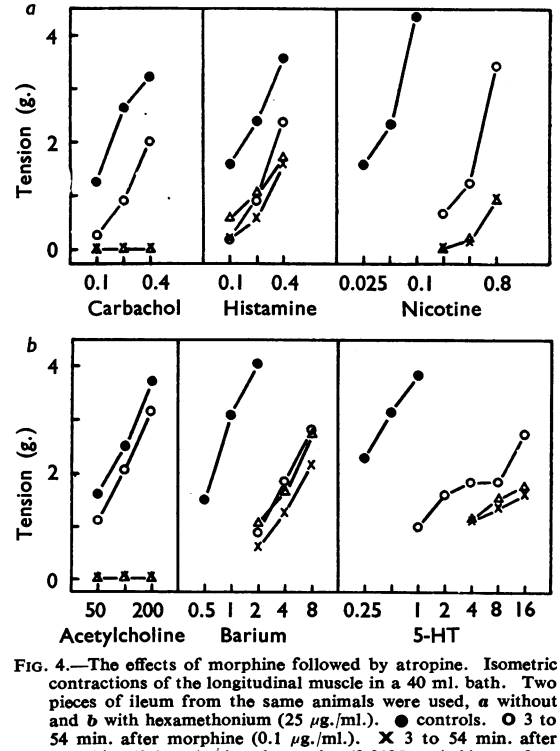

FIG. 4.—The effects of morphine followed by atropine. Isometric contractions of the longitudinal muscle in a 40 ml. bath. Two pieces of ileum from the same animals were used, *a* without and *b* with hexamethonium (25 μg./ml.). ● controls. O 3 to 54 min. after morphine (0.1 μg./ml.). X 3 to 54 min. after morphine (0.1 μg./ml.) and atropine (0.0125 μg./ml.). △ 3 to 54 min. after morphine (0.1 μg./ml.) and atropine (0.025 μg./ml.). Each point is the mean of two observations. Units of abscissae for acetylcholine ng., for carbachol, histamine and 5-HT μg. and for nicotine and barium mg.

twice so that the gut was in contact with morphine for a total of 162 min. (Fig. 3). However, occasionally observations were made which were possibly best explained by partial tolerance. Thus, in one experiment, after repeated applications, morphine lost its inhibitory effect on the nicotine contractions, while the contractions due to intestinal distension or to barium were still morphine-sensitive.

After morphine had been present in the bath for 54 min., addition of atropine (0.013 to 0.025 μg./ml.) reduced the residual contractions of nicotine and 5-HT somewhat but had scarcely any effect on barium (Fig. 4). The additional effect of atropine on the nicotine contraction was always more marked than on that of 5-HT. Conversely, when atropine (0.05 μg./ml.) was applied first, morphine (0.1 μg./ml.) had no additional inhibitory action (Fig. 5).

As was shown above (Fig. 2), hexamethonium depressed the action of barium in the absence of morphine; however, the residual morphine-insensitive barium contraction was unaffected by hexamethonium (Fig. 6). On the other hand, the nicotine contraction was further depressed by hexamethonium after it had been inhibited by a maximal dose of morphine.

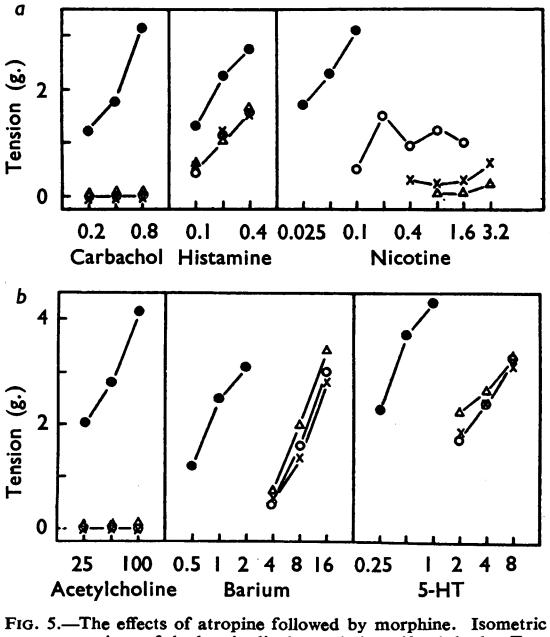

FIG. 5.—The effects of atropine followed by morphine. Isometric contractions of the longitudinal muscle in a 40 ml. bath. Two pieces of ileum from two animals were used (*a* and *b*). ● controls. O 3 to 54 min. after atropine (0.0125 μg./ml.). X 3 to 54 min. after atropine (0.05 μg./ml.). △ 3 to 54 min. after atropine (0.05 μg./ml.) and morphine (0.1 μg./ml.). Each point is the mean of two observations, except the points for nicotine after the first dose of atropine. Units in abscissae for carbachol, histamine, and 5-HT are μg., for acetylcholine ng. and nicotine and barium mg.

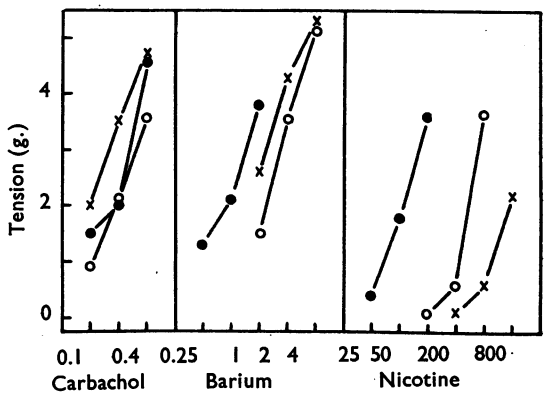

FIG. 6.—The effects of hexamethonium in the presence of morphine. Isometric contractions of the longitudinal muscle in a 40 ml. bath. ● controls. O 3 to 54 min. after morphine (0.1 μg./ml.). X 3 to 54 min. after morphine (0.1 μg./ml.) and hexamethonium (25 μg./ml.). Each point is the mean of two observations. Units of abscissa, for carbachol and nicotine μg. and for barium mg.

Although the dose/response curves were incomplete in that the maximum effect was never reached, an approximate calculation of the ratios of doses of equal potency in the presence and absence of morphine was made, in order to obtain a comparison with the results reported by Gaddum and Picarelli (1957). Whenever possible the dose ratios were determined from the values at the centre of the dose/response curves; however, when the slope of the curve was less steep, due to the addition of the antagonist, it was sometimes found necessary to compare the smallest dose before with the largest dose after the addition of the antagonist. For this reason, the estimates given can be used as a rough guide only. It was found that, in order to obtain the same effects in the presence of morphine (0.05 to 0.1 μg./ml.) as in its absence, the original concentration of acetylcholine, carbachol, and histamine had to be increased by a factor of between 1 and 2, while this factor varied between 5 and 23 for barium, nicotine, and 5-HT (Table I). A similar calculation for atropine (0.025 to 0.05 μg./ml.) as antagonist gave the following factors: histamine 2.8, barium 6, nicotine 160, and 5-HT 19.

The Effects of the Morphine Inhibitors, Nalorphine and Levallorphan

In the intact animal, the morphine inhibitors have a dual effect; while they protect against certain actions of morphine they may, in other

respects, exhibit morphine-like activity themselves (Woods, 1956).

The relationship between the agonists causing the contraction of the longitudinal muscle coat, the antagonists morphine and the morphine inhibitors, nalorphine and levallorphan, is necessarily rather complex. As a full investigation was not intended at this stage, the single-dose technique was employed, namely, the action of a given dose of morphine on the action of a given amount of agonist was compared in the presence and absence of the morphine inhibitors.

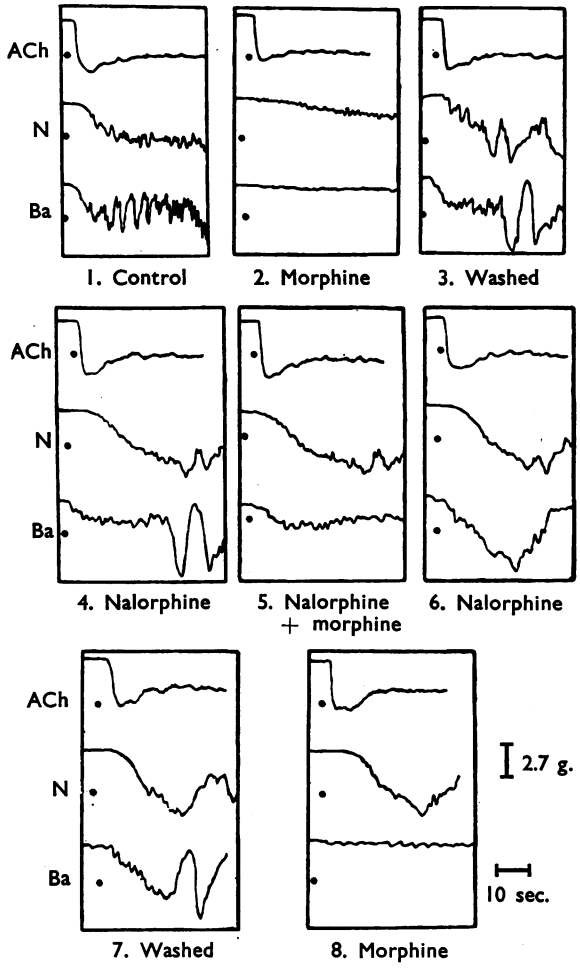

FIG. 7.—Inhibition of the morphine effect by nalorphine. Isometric contractions of the longitudinal muscle. Increase in tension downward. ACh, acetylcholine (0.0038 μg./ml.). N, nicotine (1.9 μg./ml.). Ba, barium (38 μg./ml.). Morphine (0.1 μg./ml.) was added 30 sec. before ACh, N or Ba. Nalorphine (0.075 μg./ml.) was left in the bath. 4, 12 to 18 min., 5, 27 to 36 min., 6, 48 to 54 min. after addition of nalorphine. 7, 27 to 36 min., 8, 39 to 48 min. after washing out nalorphine. The dots indicate the time when the drugs were added to the bath.

The assessment of the effects of the morphine inhibitors was made somewhat difficult by the considerable variation found between preparations. The general picture which emerged was that both nalorphine and levallorphan had a

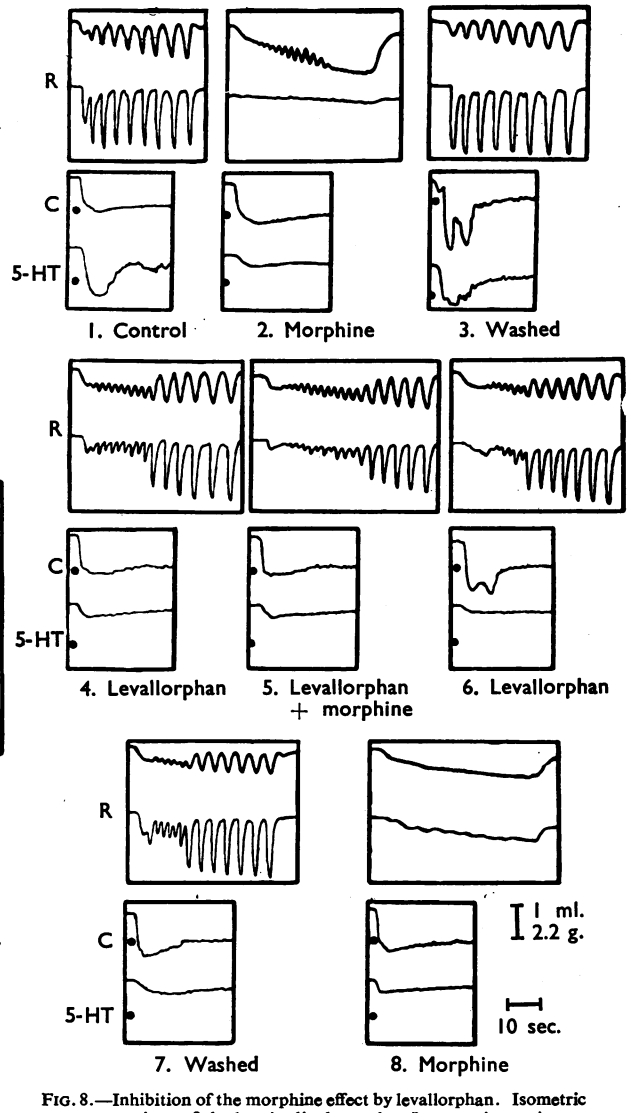

FIG. 8.—Inhibition of the morphine effect by levallorphan. Isometric contractions of the longitudinal muscle. Increase in tension or intestinal filling downward. R, peristaltic reflex after raising intra-intestinal pressure to 2.3 cm. H_2O. The upper tracing represents intra-intestinal volume changes and the lower tension changes in the longitudinal muscle. C, carbachol (0.0075 μg./ml.). 5-HT, 5-hydroxytryptamine (0.05 μg./ml.). Morphine (0.05 μg./ml.) was added 30 sec. before R, C, or 5-HT. Levallorphan (0.025 μg./ml.) was left in the bath. 4, 27 to 36 min., 5, 39 to 48 min., 6, 63 to 72 min. after addition of levallorphan. 7, 27 to 36 min., 8, 39 to 48 min. after washing out levallorphan. The dots indicate the time when the drugs were added to the bath.

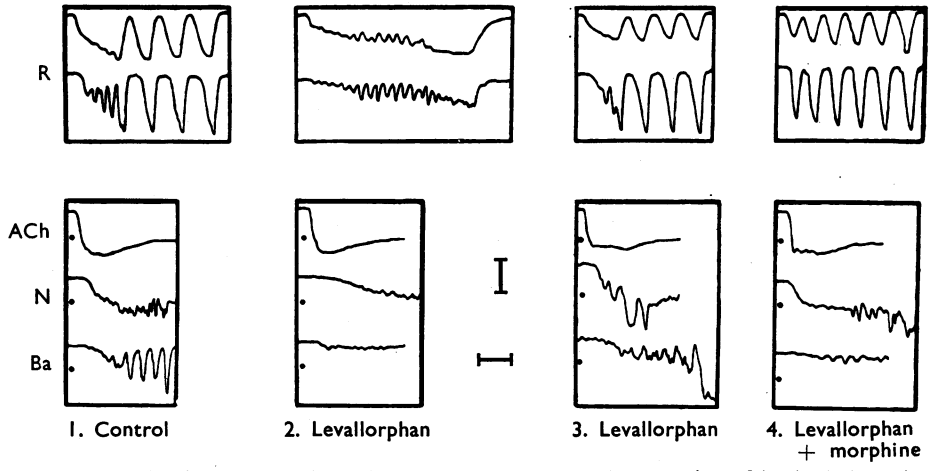

FIG. 9.—Transient morphine-like action of levallorphan. Isometric contractions of longitudinal muscle. Increase in tension or intestinal filling downward. R, peristaltic reflex after raising intra-intestinal pressure to 2 cm. H_2O. The upper tracing represents intra-intestinal volume changes and the lower tension changes in the longitudinal muscle. ACh, acetylcholine (0.006 μg./ml.). N, nicotine (2.5 μg./ml.). Ba, barium (50 μg./ml.). Morphine (0.05 μg./ml.) was added 30 sec. before R, ACh, N, or Ba. Levallorphan (0.05 μg./ml.) was left in the bath: 2, 9 to 18 min., 3, 27 to 39 min., 4, 42 to 51 min. after the addition of levallorphan. The dots indicate the time when the drugs were added to the bath. Vertical bar indicates 1 ml. or 3.6 g. Horizontal bar indicates 10 sec.

morphine-like and at the same time a morphine-inhibitory action and that the relative potencies of these actions varied with the agonists used. Thus, in the experiment shown in Fig. 7, nalorphine had no depressant effect on the contractions caused by nicotine and barium; it protected the nicotine contraction fully and the barium contraction partly against the effect of morphine. Half an hour after washing out the nalorphine, morphine scarcely affected the nicotine contraction but depressed the barium contraction.

In another experiment (Fig. 8) levallorphan partly depressed the peristaltic reflex and almost completely depressed the contraction caused by 5-HT. Morphine, in a concentration which in the absence of levallorphan abolished the peristaltic reflex, had only a small additional inhibitory effect on the reflex. The small residual contraction due to 5-HT was not further depressed by morphine. Half an hour after washing out the levallorphan, the peristaltic reflex was still partly inhibited and the 5-HT contraction showed only little recovery.

In some experiments, the depressant action of levallorphan was seen only during the first 10 to 20 min. after its addition to the bath; when the depressive phase had passed, the reflex and the nicotine contractions had become morphine-insensitive while the barium contraction was still inhibited by morphine (Fig. 9).

TABLE II

THE EFFECTS OF THE MORPHINE INHIBITORS, NALORPHINE AND LEVALLORPHAN, ON THE PERISTALTIC REFLEX AND THE CONTRACTIONS OF THE LONGITUDINAL MUSCLE CAUSED BY NICOTINE, BARIUM, AND 5-HYDROXYTRYPTAMINE

The numerals indicate numbers of experiments. Protection against morphine was tested when there was no or partial inhibition of the agonist by nalorphine or levallorphan; a 50% protection was considered positive. The number of experiments in which the morphine inhibitors produced a transient depression of the agonist are given in brackets. Doses: Morphine 0.025 μg./ml. Nalorphine 0.0125 to 0.0725 μg./ml. Levallorphan 0.0125 to 0.1 μg./ml.

Agonist	Nalorphine				Levallorphan			
	Antagonizes Agonist (Morphine-like Action)			Protects Agonist Against Morphine	Antagonizes Agonist (Morphine-like Action)			Protects Agonist Against Morphine
	Almost Completely	Partly	Not		Almost Completely	Partly	Not	
Intra-intestinal pressure (peristaltic reflex) ..	2	2	2 (1)	4	0	5	1 (1)	5
Nicotine	1	0	3	3	0	0	3 (1)	3
Barium	0	1	2	1	0	1	2 (1)	1
5-HT	2	0	0	—	5	0	0	—

The results of all experiments are summarized in Table II. Both nalorphine and levallorphan inhibited the 5-HT contractions in every preparation. The peristaltic reflex was at least partly depressed in 9 out of 12 experiments, showing a delayed onset of the peristaltic waves; on the other hand, the nicotine contractions were affected only occasionally. The modified reflex and the nicotine contractions were effectively protected against morphine. Although the barium contractions were depressed by the morphine inhibitors in only one third of the experiments, the protection against morphine was poor by either nalorphine or levallorphan.

Although morphine had very little effect on what was left of the 5-HT contractions after addition of either nalorphine or levallorphan (Fig. 8), this cannot at present be classed as a true protective effect. It is likely that this residual contraction is due to the action of 5-HT on the morphine-insensitive receptors.

Discussion

As the structure of the myenteric plexus and the relationship of most of its nerve cells to the smooth muscle fibres is by no means clear, the term "motor neurone" will be used to denote the nerve cells innervating the muscle fibres. It is felt that the term "post-ganglionic neurone" should be avoided since it is based on a preconceived idea of the anatomical arrangements in the myenteric plexus (Ambache, 1955).

In an analysis of the contraction of the longitudinal muscle coat during the preparatory phase of the peristaltic reflex it was found that this phase, which is not affected by ganglion blocking agents, is depressed by morphine, atropine, and lowering the bath temperature (Kosterlitz and Robinson, 1957). Therefore it was assumed that the reflex arc of the preparatory phase is probably non-synaptic.

Although an action of morphine on the afferent side of the reflex arc cannot be excluded, the evidence available so far suggests that at least most of its effects are on the efferent limb. There are obvious similarities between the contractions of the longitudinal muscle coat caused by intestinal distension on the one hand and those due to nicotine, barium, and 5-HT on the other. They are all depressed by lowering the bath temperature (Innes *et al.*, 1957) and by addition of morphine or atropine to the bath fluid. Further, botulinum toxin type D inhibits the action of nicotine, barium, and 5-HT (Ambache and Lessin, 1955).

The contractions of the longitudinal muscle layer caused by nicotine and barium are more complex than the contractions of the preparatory phase or that caused by 5-HT. When barium acts on fresh guinea-pig ileum, there is not only a sustained contraction of the longitudinal muscle but there are also contractions of a rhythmic type alternating with relaxations. This results in a pattern similar to that seen in the emptying phase of the peristaltic reflex and is often associated with a contraction wave of the circular muscle travelling in an aboral direction. Hexamethonium abolishes the oscillations of the longitudinal muscle and the contractions of the circular muscle but leaves the sustained contraction unchanged. It is assumed that the oscillations are caused by barium acting on cells of the intramural plexus involving hexamethonium-sensitive pathways to the "motor neurones," while the sustained morphine-sensitive contractions are due to a direct action on the "motor neurones." A similar analysis of the action of nicotine is impossible since hexamethonium seems to inhibit all nicotine receptors. 5-HT, which is not affected by hexamethonium, does not cause oscillatory contractions; the morphine-sensitive component of its action is believed to be on the "motor neurone."

Only part of the contractions caused by nicotine, barium, and 5-HT is inhibited by morphine and atropine. If it is assumed that the ratios of the doses which give identical contractions before and after the addition of a maximum dose of morphine are an indication of the susceptibility of the drug action to inhibition by morphine, then it follows that more than 80 to 85% of the original effects of nicotine and barium and over 95% of the original effect of 5-HT are inhibited by morphine. These figures are only very approximate as the shape of the dose/response curve is often altered by morphine. It should be noted that Gaddum and Picarelli (1957) found lower dose ratios for both 5-HT and nicotine; it is likely that the reason for this is to be found in different experimental conditions.

No definite evidence is available as to the nature of the morphine-resistant residual contractions. Gaddum and Picarelli (1957), investigating the inhibitory actions of morphine and phenoxybenzamine (dibenzyline) on contractions caused by 5-HT, concluded that the morphine-sensitive M receptors were probably in the nervous tissue and the morphine-resistant D receptors in muscle tissue. It therefore seems reasonable to assume that the morphine-resistant actions of nicotine and

barium are also on the muscle fibres themselves. This view is supported for barium, but not for nicotine, by the experiments of Ambache and Lessin (1955), who used botulinum toxin D to distinguish between action on neuronal and muscular receptors.

Once the actions of nicotine, barium, and 5-HT have been depressed by atropine, morphine has no further inhibitory effect. Conversely, atropine has only a small additive inhibitory effect on the morphine-treated ileum, a fact already observed for 5-HT by Gaddum and Picarelli (1957); only in the case of nicotine is this latter effect of some magnitude. When the special position of nicotine is disregarded, a likely explanation of the actions of morphine and atropine would be the assumption that both drugs act on different parts of the same neuro-effector unit. Since it has been shown that the amount of acetylcholine released after intestinal distension (Schaumann, 1956) or after electrical stimulation (Paton, 1956, 1957) is diminished by morphine, atropine seems to exert its effect more distally than morphine. This view has already been put forward by Gaddum and Picarelli (1957).

The fact that morphine inhibits the release of acetylcholine does not necessarily exclude the possibility of morphine interfering with the activity of the "motor neurone" at a site different from the point of acetylcholine release. This finds some support in the fact that the different agonists are affected in a different way by the morphine inhibitors. Thus, their morphine-like action decreases in the following order: 5-HT>intestinal distension>barium>nicotine and the degree of their morphine-inhibitory effect increases in approximately the same order: 5-HT<barium<intestinal distension<nicotine.

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