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THE PARALYSING ACTION OF MORPHINE ON THE GUINEA-PIG ILEUM

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Morphine has a paralysing action on the peristaltic reflex in isolated guinea-pig ileum, as first demonstrated by Trendelenburg (1917). Recent work (Schaumann, Giovannini and Jochum, 1952; Schaumann, 1954) has shown that all strong analgesics have this property and that their potency on this preparation runs parallel to their analgesic power. The *laevo* isomers are much more active than the *dextro* isomers, both as analgesics and on the guinea-pig gut. Levorphan, which is the strongest analgesic, is also the most effective on the peristaltic reflex; its *dextro* isomer is inactive in both tests. These findings suggest that the analgesics exert their specific paralysing action by a mechanism analogous to that which is responsible for their analgesic effects. If so, the guinea-pig ileum would be a suitable preparation on which to study the mode of action of morphine and its substitutes.

The present investigations were undertaken to determine the site of action of morphine on the guinea-pig ileum. Its paralysing activity was compared qualitatively and quantitatively with that of hexamethonium and atropine on the intestine stimulated by intraluminal pressure, nicotine, phenoxycholine, and acetylcholine. A few experiments were also done on the jejunum of the rabbit.

METHODS

Guinea-pigs or rabbits were killed by a blow on the neck. In the experiments on the guinea-pig the lower ileum was used, discarding the 10 cm. next to the caecum. A strip of jejunum was taken in the experiments on rabbits. The preparations were suspended in Mg-free Tyrode solution to which 0.3N-HCl was added to adjust the *p*H to 7.1–7.3. The bath was aerated with 95% O_2 and 5% CO_2 and the temperature kept at 36–37° C.

To elicit the peristaltic reflex the preparations were set up in a 45 ml. bath according to the method described by Trendelenburg (1917). Changes in volume, and isotonic contractions of the longitudinal muscle, were recorded on a smoked drum. Peristaltic waves were elicited by a rise in intraluminal pressure of 1.5 or 2 cm. Tyrode solution. In some experiments the gut was stored in Tyrode solution at 5.5° C. for 24 hr., then suspended at 37° C. and allowed to recover for at least 1 hr. before the experiment was started. For distension of the lumen a pressure of 3 or 4 cm. of Tyrode solution was used in these tests. In normal and in cooled preparations the rise in intraluminal pressure was maintained for 1 min. and followed by a rest period of 2 min.

Nicotine and phenoxycholine were added to the 45 ml. bath in doses of 30 or 45 μg., washed out after 45 sec. and given every 3 min.

Hexamethonium, atropine, and morphine were added 1 min. before a rise in intraluminal pressure or an application of nicotine or phenoxycholine; they were kept in the bath during several tests until their effect was maximal. The doses were increased from ineffective values in geometric steps of 1.5–2.0. The lowest dose was determined which inhibited peristaltic waves during a 1 min. rise in intraluminal pressure or reduced the contractions of the longitudinal muscle to less than 30%.

The drugs were used as the following salts: hexamethonium iodide, morphine sulphate, atropine sulphate, nicotine hydrogen tartrate, and β-dimethylamino-ethoxybenzene-iodomethylate (phenoxycholine iodate). All doses stated in the text refer to μg. of the salts added to the 45 ml. bath.

RESULTS

Guinea-pig Ileum

The peristaltic reflex is easily obtained in the guinea-pig intestine on distension of the lumen. It consists of two phases, termed by Trendelenburg preparatory and emptying. The preparatory phase consists of a contraction of the longitudinal muscle; the emptying phase, which follows, is a wave of contraction of the circular muscle, spreading aborally over the whole preparation. According to Feldberg and Lin (1949), different mechanisms are responsible for the two phases.

Emptying Phase.—It is known that the emptying phase of the peristaltic reflex is abolished by

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ganglionic blocking drugs such as hexamethonium (Paton and Zaimis, 1949), by analgesics such as morphine, and by atropine (Trendelenburg, 1917). Comparing the weight-for-weight potencies of these drugs in abolishing the emptying phase, atropine was the most and hexamethonium the least active. Atropine abolished the emptying phase in doses of 0.1–2.0 μg., morphine in doses of 0.5–5.0 μg., and hexamethonium in doses of 30–250 μg. The mean values from the results, together with the standard error of the mean and the number of experiments, are given in Table I, which shows that atropine was about three times as active as morphine and about 120 times as active as hexamethonium. The effects of morphine and of hexamethonium are illustrated in Fig. 1.

Nicotine and Phenoxycholine.—The nicotine contraction was also abolished by atropine, morphine or hexamethonium. Again atropine was the most and hexamethonium the least active drug, but the relative potencies were not the same as for the abolition of the emptying phase; both atropine and morphine were active in smaller, and hexamethonium in larger, doses than on the emptying phase. Further, whereas atropine was about three times as active as morphine in abolishing the emptying phase, it was about five times as active in abolishing the nicotine contraction. The relative potencies are given in Table I.

The ganglion-stimulating action of phenoxycholine has been described by Lévy, Michel-Ber, and Cafiot (1953). Weight for weight phenoxycholine produced in the guinea-pig ileum a stronger contraction than nicotine. This contraction was also abolished by atropine, morphine and hexamethonium, in the same order of potency as with nicotine (Table I).

TABLE I

DOSES (μG.) OF ATROPINE SULPHATE, MORPHINE SULPHATE, AND HEXAMETHONIUM IODIDE WHICH BLOCK THE EMPTYING AND PREPARATORY PHASES OF THE PERISTALTIC REFLEX AND NICOTINE AND PHENOXYCHOLINE CONTRACTIONS OF THE GUINEA-PIG ILEUM PREPARATION SUSPENDED IN A 45 ML. BATH

(No. of experiments in parentheses)

	Atropine	Morphine	Hexamethonium
Emptying phase 1·5 or 2 cm.	0·89±0 26 (8)	2·72±0·61 (16)	104±21 (11)
Nicotine 30 or 45 μg.	0·32±0·05 (9)	1·50±0 41 (10)	200±39 (7)
Phenoxycholine 30 or 45 μg.	0·32±0·13 (10)	2·70±0·61 (13)	170±60 (7)
Preparatory phase 3 or 4 cm.	0·5 ±0·14 (5)	3·14±0 38 (7)	No block

In some experiments the effect of atropine, morphine or hexamethonium was compared with equally strong contractions produced by nicotine or phenoxycholine. This is illustrated in the experiment of Fig. 2 in which 45 μg. phenoxycholine and 75 μg. nicotine were used. A given dose of morphine (3 μg.), of atropine (0.12 μg.), or of hexamethonium (not shown in the Fig.) reduced both contractions to the same extent. There was thus no quantitative difference in the paralysing action of these drugs on equal contractions produced by nicotine and phenoxycholine.

The effect of morphine in abolishing a nicotine contraction was overcome by increasing the

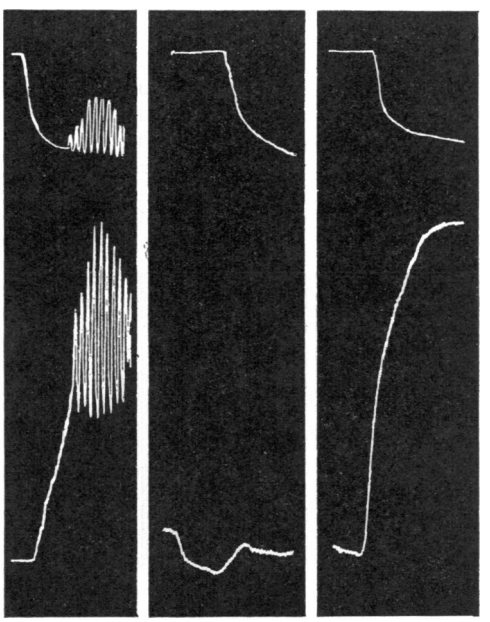

FIG. 1.—Guinea-pig ileum preparation suspended in 45 ml. Tyrode solution. In this and the following Figs. the upper tracing records intestinal volume, the lower tracing contractions of the longitudinal muscle. Intraluminal pressure raised to 2 cm. Tyrode soln. at (*a*), (*b*), and (*c*). At (*b*) in the presence of 3 μg. morphine and at (*c*) in the presence of 100 μg. hexamethonium.

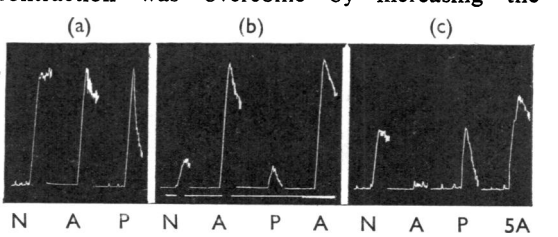

FIG. 2.—Guinea-pig ileum preparation suspended in 45 ml. Tyrode solution. Effect of morphine and atropine on equal contractions produced by 75 μg. nicotine (N), 0.18 μg. acetylcholine (A), and 45 μg. phenoxycholine (P). At (*b*), in the presence of morphine, at first 3 μg. (thin line) and later 15 μg. (thick line); at (*c*) in the presence of 0.12 μg. atropine; at (5A) dose of ACh increased to 0.9 μg.

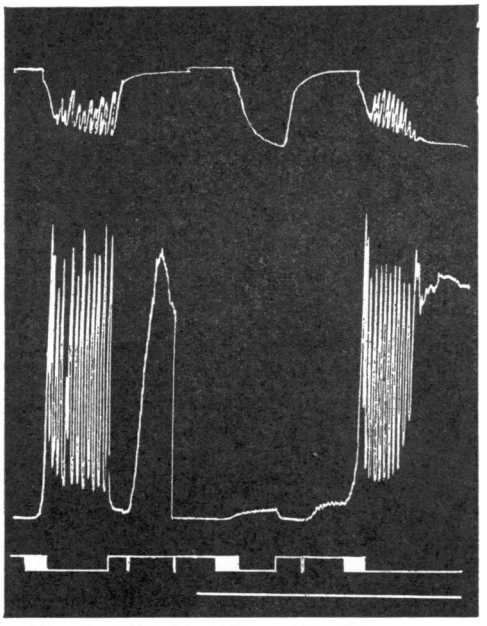

FIG. 3.—Guinea-pig ileum preparation suspended in 45 ml. Tyrode solution. Effect of morphine on peristalsis and nicotine contractions. The white marks at (*a*), (*d*), and (*f*) indicate that the intraluminal pressure was raised slowly to 2 cm. and maintained during the downward deflection of the signal. At (*b*) and (*e*) effects of 30 μg. nicotine; at (*c*) change of bath fluid. The bottom line indicates the presence of 3 μg. morphine in the bath.

nicotine concentration in the bath or by raising the intraluminal pressure of the intestine. This is illustrated in Fig. 3. It shows that 3 μg. morphine abolished the peristaltic reflex and the contraction produced by 30 μg. nicotine, but that when the pressure was raised in the presence of nicotine full peristaltic waves were again elicited. Similarly, peristaltic contractions blocked by morphine reappeared when nicotine was added whilst the pressure in the lumen was raised.

The Preparatory Phase.—Both atropine and morphine abolished the preparatory phase which, however, remained unaffected by hexamethonium. This difference between the action of morphine and hexamethonium on the peristaltic reflex is illustrated in Fig. 1, which shows that morphine abolished both the preparatory and the emptying phases, whereas hexamethonium abolished the emptying phase only. Previous storage of the preparation in the cold for 24 hr. had a similar effect to that of hexamethonium: it abolished the emptying phase but left the preparatory phase intact. On these preparations there was the same difference in the actions of hexamethonium and morphine. Hexamethonium in doses of up to 750 μg. was without any effect on the preparatory phase. On the other hand, morphine acted on these preparations in doses of 2–5 μg. Fig. 4 illustrates this effect of morphine and shows additional features. The tone of the muscle decreased on addition of small doses of morphine to the bath and any spontaneous movements ceased. When the intraluminal pressure was raised, the longitudinal muscle no longer contracted and the decrease in tone was then made further evident by a greater filling of the lumen, as seen from the volume record in Fig. 4. When the morphine was washed out, the tone not only reappeared but increased, particularly on raising the intraluminal pressure; the contraction of the longitudinal muscle was enhanced and the filling decreased. Further, when the intraluminal pressure was again lowered, the longitudinal muscle did not completely relax at once as in the untreated preparation; there was an initial, quick, partial relaxation followed by a prolonged period of gradual, slow relaxation so that the tone returned to normal only after about 5 min.

When the morphine was kept in the bath for a long time, its effect in abolishing the preparatory phase did not usually persist. This is illustrated in the experiment of Fig. 5, which shows the return of the preparatory phase in the continued presence of morphine in the bath. As seen from the volume record, the return of the preparatory phase was associated with a return of the normal distensibility of the intestine. When the preparatory phase had returned in the presence of small doses of morphine, addition of higher doses could again reduce it. This effect was transient and finally

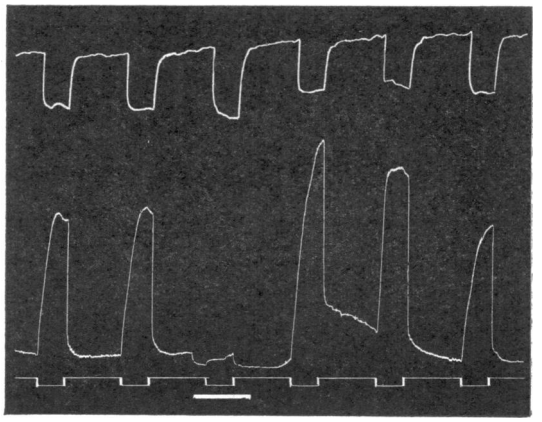

FIG. 4.—Guinea-pig ileum preparation, previously cooled for 24 hr., suspended in 45 ml. Tyrode solution. At signals, intraluminal pressure raised to 3 cm. for 1 min. The white line indicates the presence of 3 μg. morphine in the bath.

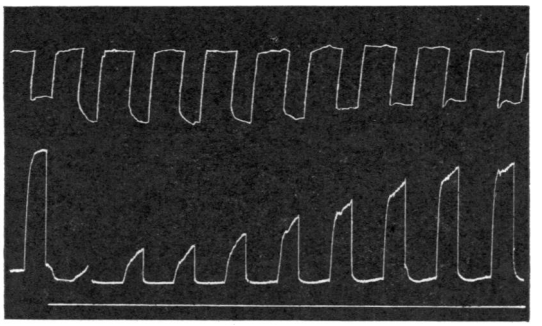

FIG. 5.—Guinea-pig ileum preparation, previously cooled for 24 hr., suspended in 45 ml. Tyrode solution. Effect of continued presence of 2 μg. morphine (white line) on the preparatory phase. Intraluminal pressure raised to 3 cm. for 1 min. every 3 min.

the preparatory phase became insensitive to morphine. Washing out the morphine in this condition produced no enhancement of the preparatory phase and no delay in the relaxation of the muscle.

The return of the preparatory phase in the continued presence of morphine in the bath did not occur in all experiments. In some there was only partial recovery, in others none at all.

Atropine, like morphine, abolished the preparatory phase in the cooled preparation. Weight for weight atropine was about six times as active as morphine; its action was more delayed, reached maximum within about 5 min., and then wore off in spite of its continued presence in the bath, and, as with morphine, the full preparatory phase was again obtained on raising the intraluminal pressure, which eventually could not be reduced by a tenfold increase in the dose of atropine. When atropine was washed out at a time when it was exerting its maximal effect, the sensitivity did not return at once to normal as after morphine; atropine never produced an enhancement of the preparatory phase or an increase in tone.

Acetylcholine.—The contractions produced by 0.1–0.3 μg. ACh were more sensitive to atropine than corresponding contractions produced by 30–75 μg. nicotine or by 30–45 μg. phenoxycholine. On the other hand, morphine did not abolish the ACh contractions (Fig. 2). In some experiments they were slightly reduced, but this reduction could not be increased by increasing the dose of morphine. It may be attributed to the slight ganglionic action of ACh described by Ambache (1946) and Feldberg (1951).

Rabbit Jejunum

Contractions elicited by *nicotine* are known to be relatively resistant to atropine (Lévy *et al.*, 1953). As shown in Fig. 6, 40 μg. atropine was required to prevent a response to 30 μg. nicotine, which was greatly reduced by 1 mg. hexamethonium. Morphine had no effect even when tested in doses of up to 1 mg.

The preparatory phase was studied in fresh preparations only. It was slightly reduced by 40 μg. atropine and was slightly increased by 400 μg. morphine (Fig. 6).

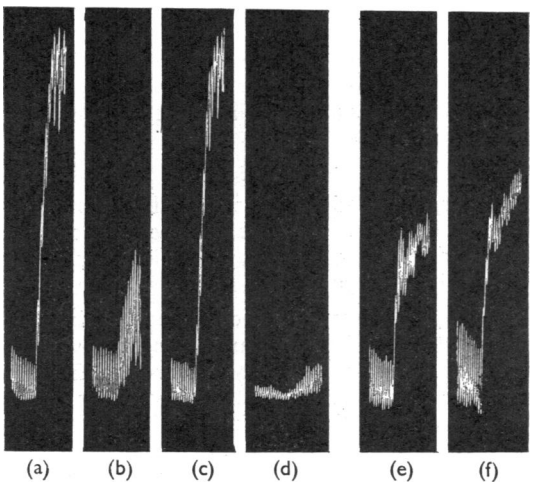

FIG. 6.—Rabbit jejunum preparation suspended in 45 ml. Tyrode solution. (*a*)–(*d*) Contractions produced by 30 μg. nicotine in the presence of 1 mg. hexamethonium at (*b*), of 1 mg. morphine at (*c*), and of 40 μg. atropine at (*d*). At (*e*) and (*f*) intraluminal pressure raised to 3 cm. in the absence (*e*), and presence (*f*), of 400 μg. morphine.

DISCUSSION

The present experiments show that the emptying phase of the peristaltic reflex and the contractions elicited by ganglion-stimulating drugs are abolished by about the same doses of atropine, morphine, or hexamethonium. Hexamethonium is a ganglion-blocking agent, and the results with hexamethonium thus agree with the assumption that stimulation of ganglion cells is involved in the emptying phase of the peristaltic reflex as well as in the nicotine contractions. The fact that atropine and morphine also abolish the emptying phase and the nicotine contractions, however, cannot be taken as evidence that they act as ganglion-blocking agents. Transmission in the superior cervical ganglion is readily blocked by hexamethonium, but atropine blocks only in high doses, and morphine, even in high doses, blocks only occasionally (Hebb and Konzett, 1950). Nevertheless, morphine might have a blocking action on the ganglion cells of the intestinal wall and not on the superior cervical ganglion, but there is no

necessity to postulate such an action to explain the effect of morphine on the emptying phase and on the nicotine contraction. Morphine may act by interrupting the pathway between ganglion cells and smooth muscle at a more peripheral point. This mode of action of morphine could explain why the preparatory phase—which is not blocked by hexamethonium and therefore not mediated by ganglion cells—is readily blocked by small doses of morphine. Further, as already shown by Henderson (1928), the preparatory phase is not affected by cooling, which abolishes the emptying phase of the peristaltic reflex. As Ambache (1946) found, the first effect of cooling an intestinal preparation is damage to the ganglion cells. The same argument as put forward for the site of action of morphine may apply for that of atropine, although it has some ganglion-blocking action in high concentrations, at least on the superior cervical ganglion.

We do not know the mechanism by which the preparatory phase is produced. Feldberg and Lin (1949) found that it was not abolished by hexamethonium or by cocaine, and therefore concluded that it is a myogenic response to a stretch of the longitudinal muscle fibres. This conclusion has not been generally accepted. There is, in fact, good evidence that stretching the longitudinal muscle does not in itself provoke a contraction, but that the preparatory phase is a reflex response to the stimulus of radial distension. Schaumann, Jochum, and Schmidt (1953) showed that when the longitudinal muscle was slowly stretched there was no increase in tone, and Kosterlitz, Pirie, and Robinson (1955), using isometric recording, proved that the increase in longitudinal tension produced by stretching the longitudinal muscle was insignificant compared with that elicited by distension of the lumen. A quick stretch also did not cause a contraction of the longitudinal muscle. Further, Schaumann, Jochum, and Schaumann (1953) found that the paralysing effect of morphine and its substitutes on the preparatory phase runs parallel with their analgesic and not with their spasmolytic activities ; indeed, not all of them have a spasmolytic action on the intestinal preparation (Haas, Hohagen, and Kollmannsperger, 1953).

It is therefore concluded that atropine and morphine act on the nervous elements involved in the preparatory phase. Nicotine and phenoxycholine probably stimulate some other nervous elements in addition to ganglion cells, and the resultant contraction may be mediated by the same nerve endings as the preparatory phase. These endings are probably the site of action of atropine and morphine. If we accept this concept, the finding by Feldberg and Lin (1949) that cocaine abolishes the emptying phase but not the preparatory phase of the peristaltic reflex suggests that cocaine in the doses used by these authors acted on the ganglion cells.

The effect of atropine and morphine in abolishing the preparatory phase of the peristaltic reflex need not necessarily be the result of the same mode of action of these drugs. Atropine could paralyse the preparatory phase by preventing the action of released acetylcholine ; if so, morphine, which is not an antagonist to acetylcholine, could act by preventing its release. Or both atropine and morphine could act on the same point in the efferent part of the reflex without the mediation of acetylcholine. This possibility may be less likely because of the difference of action of morphine and atropine on rabbit jejunum. On this preparation, only atropine has a paralysing effect on the preparatory phase and on nicotine contractions. Only guinea-pig intestine seems to be susceptible to the specific paralysing effect of morphine and its substitutes.

In addition to its action on the preparatory phase, morphine decreases the tone and abolishes spontaneous movements of the intestinal preparation. This may mean that these motor phenomena are also maintained by the same nervous elements as the preparatory phase. It is not possible at present to offer a satisfactory explanation for the two other effects of morphine in the present experiments on the intestinal preparation—tachyphylaxis, which also applies to atropine and which has also been observed *in vivo* for morphine and its substitutes (Schaumann, 1954), and the rise in tone which occurs after washing out the morphine.

Summary

1. Morphine and atropine abolish the preparatory and emptying phases of the peristaltic reflex and contractions produced by ganglion-stimulating drugs in isolated guinea-pig ileum.

2. The preparatory phase of the peristaltic reflex is still obtained when the ganglion cells have been inactivated. Therefore the paralysing effect of morphine cannot be attributed to paralysis of the ganglion cells in the intestinal wall.

3. It is suggested that atropine paralyses the preparatory phase by preventing the action of released acetylcholine, morphine by preventing its release ; or that atropine and morphine act on the same point in the reflex involved in the preparatory phase without the mediation of acetylcholine.

4. On the rabbit's jejunum morphine, in contrast to atropine, does not reduce the nicotine contraction or the preparatory phase of the peristaltic reflex.

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