THE INHIBITION BY MORPHINE OF THE ACTION OF SMOOTH MUSCLE STIMULANTS ON THE GUINEA-PIG INTESTINE

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Morphine and related analgesics depress the responses of the isolated guinea-pig ileum to nervous stimulation and to drugs which act by stimulating the nervous structures of the intestinal wall. The present experiments show that these analgesics also depress the responses to smooth muscle stimulants which act directly on the smooth muscle fibres.

Morphine has a depressant action on the isolated intestine which is generally explained by an action on the nervous structures of the intestinal wall (Schaumann, Giovannini, and Jochum, 1952; Schaumann, 1955, 1956a; Kosterlitz and Robinson, 1955). Morphine also inhibits the action of drugs which cause contraction of the intestine by stimulation of these nervous structures (Schaumann, 1955; Kosterlitz and Robinson, 1955, 1958; Gaddum and Picarelli, 1957; Kosterlitz, Robinson, and Taylor, 1957). Part of the inhibition which morphine exerts through its action on the nervous structures may result from inhibition of the release of acetylcholine from post-ganglionic cholinergic fibres (Schaumann, 1956b, 1957; Paton, 1956, 1957). Kosterlitz and Robinson (1958) found that morphine strongly inhibits the contractions of the isolated guinea-pig ileum to nicotine, barium and 5-hydroxytryptamine, but has only a slight effect on the response to acetylcholine, carbachol and histamine. They concluded that the strong inhibition of the responses to nicotine, barium and 5-hydroxytryptamine was due to an action on the nervous elements innervating the muscle fibres, whereas the responses to acetylcholine and histamine were mainly on the muscle fibres and therefore resistant to the depressant action of morphine.

The present experiments show that morphine apparently has a non-specific depressant action on the smooth muscle fibres as well, since it depresses the action of drugs which stimulate these fibres directly.

METHODS

The guinea-pig ileum preparation was suspended in 15 ml of Tyrode solution at 34°C. Acetylcholine chloride, histamine acid phosphate (calculated as base) and carbaminoyl choline chloride (carbachol) were added at intervals of either 2 or 3 min. and left in contact with the tissue for 15 sec. Nicotine acid tartrate, barium chloride, potassium chloride, 5-hydroxytryptamine creatinine phosphate, bradykinin (Elliott, Horton and Lewis, 1960) and substance P (11 U./mg.) were added at intervals of either 4 or 5 min. and left in contact for 30 sec.

When the action of morphine, of related drugs and of hexamethonium was examined, their concentration was maintained by additions to the bath after each washing. The following drugs were used for this purpose: morphine sulphate, diamorphine hydrochloride (heroin), methadone hydrochloride, dihydromorphinone hydrochloride, codeine sulphate, hexamethonium bromide (vegolysen).

RESULTS

Morphine reduced the contractions of the guinea-pig ileum to smooth muscle contracting substances. The experiment of Fig. 1 shows the depression of acetylcholine responses by morphine $10^{-7}$ at a, $10^{-6}$ at b, and $10^{-5}$ at c. The threshold concentration of morphine for this effect is between $5 \times 10^{-9}$ and $10^{-8}$, but the effect depends on the concentration of morphine only within narrow limits. When a concentration of $10^{-7}$ is reached no further depression occurs even when the concentration of morphine is raised to $10^{-5}$.
Fig. 1.—Responses of the guinea-pig ileum to acetylcholine 50 ng. alone and in the presence of morphine $10^{-7}$ g./ml. (a), $10^{-6}$ g./ml. (b), $10^{-8}$ g./ml. (c). Contractions every 3 min.; contact time 30 sec.

Usually the morphine has to be in contact with the tissue for 5 to 10 min. before it exerts its maximum effect, but some reduction in the responses is observed after morphine has been in contact for less than 1 min.

The duration of action of morphine after it is washed out will depend partly on the time it has been in contact and partly on the concentration used. With short contact times (of less than 2 min.) the time taken for the contractions to return to normal is proportional to the time of contact. Where the contact time is more than a few min. the recovery period depends on the concentration of morphine; at $10^{-8}$ recovery is almost immediate whereas at $10^{-5}$ the contractions do not return to normal for about 90 min.

Depression of the Action of Various Smooth Muscle Contracting Substances

The extent of the depressant action of morphine varies according to the drug used to contract the ileum, but in every experiment some depression was observed. The responses to acetylcholine and histamine were reduced about 20 to 40%. In the experiment of Fig. 2 morphine $2 \times 10^{-7}$ reduced the response to acetylcholine 0.03 µg. to that previously given by 0.02 µg., and in the experiment of Fig. 3 it produced the same reduction with the responses to histamine. The experiment of Fig. 2 also illustrates that the depressant action of morphine still occurred when the ganglia were blocked by hexamethonium.

Fig. 2.—Responses of the guinea-pig ileum to acetylcholine 30 ng. and 20 ng. given alternately every 2 min. Contact time 15 sec. The bars at the top indicate the presence of morphine $2 \times 10^{-7}$ g./ml. (M) or hexamethonium bromide $10^{-7}$ g./ml. (C6).
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Dose ratios for the other substances tested are shown in Table I, together with the standard errors. With the drugs in column 1, the dose ratios varied only between 1.42 and 1.82 and the variability of the morphine depression was relatively small, as shown by the standard errors. However, with nicotine, barium chloride and 5-hydroxytryptamine, the drugs in column 2, the dose ratios were not only greater but also more variable as shown by the large standard errors.

![Fig. 3](image)

**Fig. 3.**—Responses of the guinea-pig ileum to histamine 30 ng. and 20 ng. given alternately every 2 min. Contact time 15 sec. The bar at the top indicates the presence of morphine $2 \times 10^{-7}$ g./ml.

The experiment of Fig. 4 shows that the responses to both 5-hydroxytryptamine and nicotine were reduced to a much greater extent than those to histamine and acetylcholine, and the experiment of Fig. 5 shows that the responses to barium chloride were reduced much more than those to potassium chloride.

Morphine depressed the action of the various drugs to the same extent throughout their whole dose-response curves. In Fig. 6 the responses to acetylcholine are plotted on a graph to give the dose-response curve of acetylcholine alone and in the presence of morphine. The distance between the two curves gives the ratio of the doses of a substance causing equal contractions in the absence and in the presence of the inhibitor. This figure, which is a convenient measure of inhibition, has been called the dose ratio (Gaddum and Picarelli, 1957). In the experiment of Fig. 6 this figure was 1.7. The

![Fig. 4](image)

**Fig. 4.**—Response of the guinea-pig ileum to acetylcholine (A), 5-hydroxytryptamine (HT), histamine (H), and nicotine (N) alone and in the presence of morphine $2 \times 10^{-7}$ g./ml. (M). Doses are given in µg. added to the organ bath.

![Fig. 5](image)

**Fig. 5.**—Responses of the guinea-pig ileum to potassium chloride (K) and barium chloride (Ba) alone and in the presence of morphine $2 \times 10^{-7}$ g./ml. (M). Doses are given in mg. added to the organ bath.
Drugs Related to Morphine

The inhibition of the action of smooth muscle stimulants on the guinea-pig ileum is also shown by analgesic drugs related to morphine, such as methadone, heroin, codeine, and dihydro-morphinone. Usually methadone and heroin caused depressions at a slightly lower threshold concentration than morphine, whereas with codeine the threshold was higher than that for morphine. The experiment of Fig. 7 shows the depression of responses to carbachol and substance P by morphine $10^{-6}$ and by codeine $5 \times 10^{-6}$.

Adaptation to Morphine

In some experiments the guinea-pig intestine became tolerant to the action of morphine; after several doses, morphine no longer depressed the action of smooth muscle stimulants. This development of tolerance was more frequently obtained with large doses of morphine, but even under this condition it did not occur in all preparations.

When an intestine had become tolerant to morphine and larger doses were then applied, they did not decrease the responses to smooth muscle stimulants but increased them. Usually in these instances another peculiar effect was seen; when the morphine was withdrawn from the organ bath the responses were depressed, but could be restored on renewed addition of morphine to the bath, so that the intestine seemed to be dependent on the presence of morphine for its normal responsiveness. Such an experiment is illustrated in Fig. 8. Responses to barium chloride were at first inhibited by morphine $10^{-6}$. Later when morphine was continuously in contact with the tissue the responses gradually recovered, and when the dose of morphine was raised to $5 \times 10^{-6}$ and then to $10^{-5}$ the responses increased. On withdrawal of morphine the responses were depressed but were restored again when morphine was added during one contraction only. When the morphine was withdrawn again the responses were again depressed. In several intestinal strips tolerance with dependence was obtained and in some neither dependence nor tolerance could be produced.

Spontaneous Activity

When spontaneous activity of the intestine was present it was depressed by the concentrations of morphine which reduced the action of smooth muscle stimulants. This effect is seen in Figs. 4, 5 and 7. However, when the intestine became tolerant, morphine sometimes increased spontaneous movement, and on withdrawal of
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TABLE I
DOSE RATIOS
The ratio of the doses of various smooth muscle stimulating drugs giving equal response before and after morphine $2 \times 10^{-7}$ g./ml.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean</th>
<th>No. of Expts.</th>
<th>Standard Error of Mean</th>
<th>Mean</th>
<th>No. of Expts.</th>
<th>Standard Error of Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>1.61</td>
<td>10</td>
<td>0.086</td>
<td>Nicotine</td>
<td>3.00</td>
<td>6</td>
</tr>
<tr>
<td>Histamine</td>
<td>1.77</td>
<td>6</td>
<td>0.093</td>
<td>Barium chloride</td>
<td>4.17</td>
<td>6</td>
</tr>
<tr>
<td>Plasma kinin</td>
<td>1.75</td>
<td>4</td>
<td>0.171</td>
<td>5-Hydroxytryptamine</td>
<td>3.20</td>
<td>7</td>
</tr>
<tr>
<td>Substance P</td>
<td>1.42</td>
<td>5</td>
<td>0.120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbachol</td>
<td>1.60</td>
<td>4</td>
<td>0.131</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>1.82</td>
<td>4</td>
<td>0.075</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 7.—Responses of the guinea-pig ileum to carbachol 0.1 µg. (a) and substance P (b), alone (A), in the presence of morphine $10^{-7}$ (B), alone after 90 min. recovery (C) and in the presence of codeine $5 \times 10^{-4}$ (D). Time interval 3 min.; contact time 15 sec. for carbachol, 30 sec. for substance P.

Fig. 8.—Responses of the guinea-pig ileum to barium chloride 3 mg. given every 5 min. Contact time 45 sec. The period marked by bar (a) indicates the presence of morphine $10^{-4}$, (b) morphine $5 \times 10^{-4}$, (c) morphine $10^{-4}$.

It is evident from the present experiments that when we consider the action of morphine on the guinea-pig intestine we have to assume an action not only on the nervous structures of the intestinal wall, but also on the smooth muscle fibres themselves.

The finding that the contractions caused by substances which primarily stimulate smooth muscle directly are depressed to a smaller extent than those caused by substances which act on nervous structures is understandable because morphine during the phase of dependence, the contractions produced by barium chloride or acetylcholine were characterized by rhythmic activity as shown in Fig. 8.
morphine has a two-fold depressing effect on the responses to substances which act on the nervous structures. In the case of histamine, for instance, morphine has only one site of action—depression of the muscle fibre—whereas with nicotine it has two sites of action—reduction of acetylcholine released from postganglionic cholinergic fibres and depression of the action of the released acetylcholine on the muscle fibre. In the case of barium chloride morphine probably has a three-fold depressing action; it reduces the release of acetylcholine, and it inhibits the action of the released acetylcholine, and it depresses the direct smooth muscle stimulating effect of barium chloride. The wide range of dose ratios (as shown by standard error) for drugs which act on nervous structure or nervous and muscle structures is consistent with the view that morphine is acting at more than one site and therefore subject to greater variability.

It is established that acetylcholine, histamine, carbachol and potassium chloride contract the guinea-pig ileum mainly by direct stimulation of the muscle and that nicotine acts wholly in the ganglia whilst barium chloride acts both on the nervous structures as well as on smooth muscle (Feldberg, 1951; Ambache and Lessin, 1955). There is also evidence that 5-hydroxytryptamine causes contraction of the guinea-pig ileum by acting on a nervous structure, although this action is different from that of nicotine and barium chloride in being insensitive to the action of hexamethonium or large doses of nicotine (Rocha e Silva, Valle and Picarelli, 1953; Robertson, 1953). Gaddum and Picarelli (1957) have postulated the existence of two kinds of tryptamine receptors, one of which they call morphine, or M receptors which can be blocked with morphine. However, the results of the present experiments do not support the idea of such a specific action of morphine. Therefore the name morphine or M receptor would appear to be misleading, although the concept of tryptamine derivatives having two sites of action would not be invalidated.

The possibility must be considered that the cholinergic nerves in the intestine like cholinergic nerves elsewhere are continually releasing quanta of acetylcholine while "at rest." Such releases, themselves producing responses below threshold for contraction, could nevertheless sum with added acetylcholine or other smooth muscle contracting substances. Morphine, by abolishing the release of such subthreshold quanta, would increase by a slight amount the concentration of added smooth muscle stimulant required to produce a given contraction. An explanation of this kind would support the view that morphine has an entirely neuronal action. However, the dose-response curve of a smooth muscle stimulating substance such as acetylcholine is shifted to a parallel position on the right by morphine. But if morphine depressed only the quanta of acetylcholine released "at rest," then the shape of the dose-response curve would be altered to indicate a greater percentage depression with small doses than with large doses.

When discussing the action of morphine in reducing the release of acetylcholine from cholinergic nerve fibres, Schaumann (1957) concluded that morphine inhibits the excitatory processes which release acetylcholine from nerve endings. Recently Schaumann (1958) has suggested that analgesics like morphine might depress the excitability of the intestine by liberating noradrenaline within the intestinal wall. According to his results, noradrenaline inhibits the release as well as the action of adrenaline. The inhibition observed in the present experiments was similar in degree to the reduction in acetylcholine release found by Schaumann. There is also a remarkable similarity to the morphine depression described by Paton in his experiments with co-axial stimulation. The threshold concentration is the same, and above a concentration of about $2 \times 10^{-7}$ no further depression can be effected for the action on smooth muscle or for the release of acetylcholine. Onset and duration of both activities are similar, and both are effected not only by morphine but also by analgesic drugs related to morphine. In both cases it was sometimes possible to produce morphine tolerance and occasionally morphine dependence where, when morphine was withdrawn, the responses of the intestine were reduced and could be restored by fresh additions of morphine.

Thus morphine appears to act on the nervous structures of the intestine in much the same way as on smooth muscle and the underlying mechanism may well be an inhibition of a metabolic process common to both nervous and muscle structures.

References
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Robertson, P. A. (1953). J. Physiol. (Lond.), 121, 54P.