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Comparison of effect of morphine-like analgesics on transmurally stimulated guinea-pig ileum

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1. Morphine-like analgesic drugs caused depression of twitches of the isolated guinea-pig ileum in response to transmural electrical stimulation. The drugs tested were the narcotic analgesics codeine, diamorphine, fentanyl, morphine, morphine-N-oxide, normorphine, oxymorphone, pethidine, phenazocine and phenoperidine and the analgesic narcotic antagonists nalorphine and pentazocine.

2. With the first application of one of these drugs the extent of depression of twitches was proportional to concentration. Except in the case of pethidine, there was no further depression when additional drug was added to the organ bath. With the second application of a drug after washing out the first dose, the depressant effect was less; that is, tolerance developed. With pethidine, the depression of twitches was proportional to concentration and tolerance could not be observed.

3. When tolerance had been produced by cumulative addition of these drugs, a concentration was reached at which further addition resulted in increased activity of the ileum.

4. With codeine, morphine and normorphine, the twitches were increased in height and regular.

5. With diamorphine, fentanyl, oxymorphone, pentazocine, phenazocine and phenoperidine there were increased but irregular responses to transmural stimulation.

6. Having reached the concentration at which these effects were observed, washout of the drug resulted in reduction of activity; the twitches became smaller or the irregular responses ceased.

7. Readministration of a drug after activity of the ileum had been depressed by withdrawal of that drug resulted in restoration of activity, the ileum being dependent on the presence of the drug for its activity.

8. Codeine and nalorphine did not produce as great an increase in activity on readministration to a dependent ileum as did morphine: they seem to act as partial agonists in producing this effect.

9. In similar experiments with the isolated urinary bladder of the rat and guinea-pig, morphine was less active in depressing responses to stimulation than it was on the ileum, and tolerance to the drug and dependence on it did not occur.

10. These observations have been discussed in relation to analgesic activity, tolerance and dependence in man.

The decrease in twitches of the transmurally stimulated guinea-pig ileum produced by morphine was shown by Paton (1957a) to be due to a decreased output of acetylcholine. Schaumann (1957) also showed that morphine decreased the acetylcholine output from the resting ileum. Much further work has been done on the depressant action of morphine and related drugs on contractions of the transmurally stimulated guinea-pig ileum (de la Lande & Porter, 1967 ; Cox & Weinstock, 1966 ; Gyang & Kosterlitz, 1966). It has been suggested that the degree of inhibition of the ileum by morphine-like drugs is correlated with their analgesic potency (Paton, 1957a ; Cox & Weinstock, 1966).

Paton (1957a) showed that the isolated ileum developed tolerance to the depressant action of morphine. Then, after continued administration of morphine, a state was reached in which the twitches were reduced by withdrawal of morphine from the bath but were restored on readministration of the morphine. This phenomenon was called " morphine dependence " by Paton. In a later paper, Paton (1963) suggested that the inhibition of release of acetylcholine could explain dependence as well as depression of twitches.

This paper deals with the actions of a number of morphine-like analgesics in producing depression, tolerance and dependence in the isolated transmurally stimulated guinea-pig ileum. One of the objectives was to determine whether the preparation could provide a rapid and simple screening test for detection of addictive liability of these drugs. Experiments were also carried out with morphine on ilea from guinea-pigs chronically treated with morphine. Isolated innervated rat and guinea-pig bladders were also used to see whether tolerance and dependence to morphine could be induced in other smooth muscle preparations.

Methods

Transmurally stimulated guinea-pig ileum

Guinea-pigs of either sex weighing 250–400 g were killed by a blow on the head and 5 cm portions of the ileum were removed and attached to a transmural electrode. The ileum was placed in a 25 ml. organ bath containing McEwen's (1956) solution maintained at 34° C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The second electrode was immersed in the McEwen's solution. The tissue was connected to an auxotonic lever (Paton, 1957b) which gave a ten-fold magnification of the longitudinal movements of the ileum on a kymograph. The tissue was stimulated at 10 sec intervals with square wave pulses of 0·5 msec duration and of a strength greater than that necessary to produce maximal contractions.

Chronic treatment of guinea-pigs with morphine

Guinea-pigs were given daily intraperitoneal injections of morphine sulphate, beginning with a dose of 25 mg/kg, and with increases in the dose to 200 mg/kg over a period of 20 days. Guinea-pigs were killed during the next 20 days and ileal

preparations were taken; meanwhile the remainder were continued on a daily dosage of 200 mg/kg.

Isolated innervated rat and guinea-pig urinary bladders

The rat bladder was prepared as described by Huković, Rand & Vanov (1965). A similar procedure was followed for the guinea-pig bladder, except that in the guinea-pig the vesicular nerves are distinct and were dissected free of other tissue for 1·5 to 2 cm.

The isolated bladder was set up in a 30 ml. organ bath containing McEwen's solution at a temperature of 36° C and gassed with 95% oxygen and 5% carbon dioxide. The lever exerted a force of 1·5 *g* on the bladder and magnified the contractions 5–7 times. Submaximal contractions were obtained by stimulating the nerves with 2 msec pulses at a frequency of 10/sec for 10 sec at 2 min intervals.

Drugs

The following drugs were used: acetylcholine chloride, adrenaline hydrochloride, atropine sulphate, codeine phosphate, diamorphine hydrochloride (heroin), fentanyl citrate, morphine sulphate, morphine-N-oxide, nalorphine hydrobromide, normorphine, oxymorphone hydrobromide, pentazocine, pethidine hydrochloride, phenazocine hydrobromide, phenoperidine hydrochloride.

Drug solutions were prepared just before use, the salts being dissolved in distilled water and the bases in 0·1 M hydrochloric acid. Acid solutions were adjusted to pH 5 before use. In the volumes used for administering the drugs, neither distilled water nor water at pH 5 had any effect on the transmurally stimulated guinea-pig ileum. All drug concentrations are expressed as the base.

Results

Depression of responses to transmural stimulation by analgesic drugs

All the morphine-like drugs investigated, including the narcotic antagonists nalorphine and pentazocine, depressed the amplitude of the electrically induced twitches of the guinea-pig ileum. Representative records are shown in Fig. 1. Reproducible results were obtained with a given concentration of any one drug provided that the time for which it was allowed to act on the ileum did not exceed 3 min, and the preparation was allowed to recover for 15 min between successive tests. This confirms the observations of Gyang & Kosterlitz (1966). With each of the drugs, the depression of twitches was proportional to the logarithm of concentration. The concentrations producing a decrease in twitch height of 50% were calculated from log dose-response curves. At least five ileal preparations were used to determine the mean concentration producing 50% depression of twitch height for each drug. Morphine produced a 50% depression of twitch height in concentrations of 3 to 6 ng/ml. with a mean value of 3·5 ng/ml. This corresponds to $1{\cdot}25 \times 10^{-8}$M. The potencies of the other analgesic drugs in producing 50% depression of twitch height are given in Table 1.

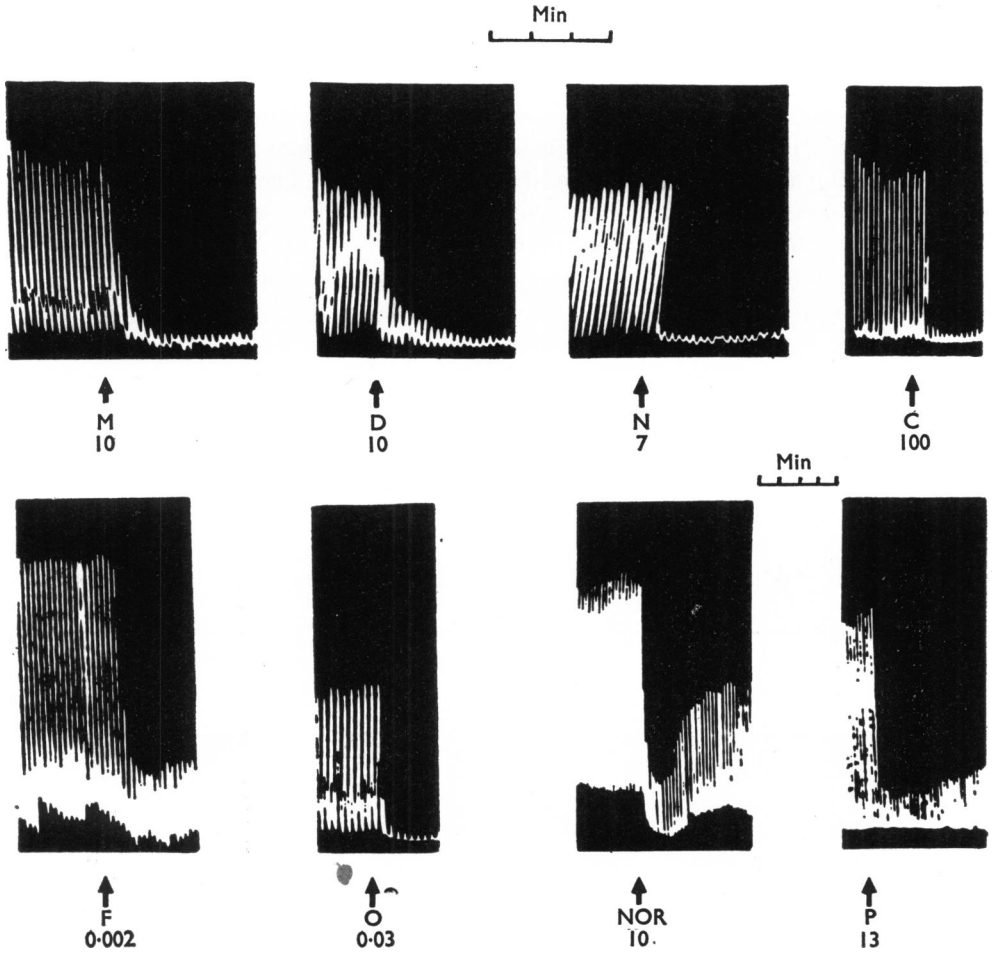

FIG. 1. Results of eight different experiments showing the depressant effects of morphine (M), diamorphine (D), nalorphine (N), codeine (C), fentanyl (F), oxymorphone (O), normorphine (NOR) and pentazocine (P) on twitches elicited by supramaximal transmural electrical stimulation of the isolated guinea-pig ileum. The time scale for the last two frames of the bottom row is different to the time scale for the rest of the figure, which appears at the top. The numbers underneath each arrow represent the final drug concentration expressed as ng/ml. of bath fluid.

TABLE 1. *Concentrations of narcotic analgesics required to cause 50% depression of contractions of the transmurally stimulated guinea-pig ileum*

Drug	Concentration in ng/ml. required to cause 50% depression of twitches	Molarity of drugs required to cause 50% depression of twitches
Fentanyl	0·0005	$1{\cdot}25\times10^{-12}$
Phenoperidine	0·002	$6{\cdot}3\times10^{-12}$
Oxymorphone	0·0075	$2{\cdot}5\times10^{-11}$
Nalorphine	2·0	$6{\cdot}3\times10^{-9}$
Diamorphine	2·5	$7{\cdot}35\times10^{-9}$
Normorphine	2·5	$8{\cdot}93\times10^{-9}$
Phenazocine	3·0	$1{\cdot}03\times10^{-8}$
Morphine	3·5	$1{\cdot}25\times10^{-8}$
Pentazocine	6·0	$2{\cdot}0\times10^{-8}$
Codeine	25	$8{\cdot}35\times10^{-8}$
Morphine-N-oxide	75	$2{\cdot}5\times10^{-7}$

Tolerance to the depressant action of analgesic drugs

When less than 15 min was allowed between successive tests, repeated administration of the same concentration of any one of the analgesic drugs tested resulted in a progressively diminishing depression of twitch height, the ileum becoming tolerant to the depressant action. This is illustrated in Fig. 2 with codeine, and shown graphically for morphine, codeine and fentanyl in Fig. 3. The rate of development of tolerance was increased by increasing the contact time of the drugs and by reducing the interval between successive doses.

Tolerance developed at about the same rate with each drug when they were compared on a standard regime. In our experiments tolerance did not develop as rapidly as described by Paton (1957a). After tolerance to the depressant action had developed, the sensitivity of the ileum was restored by allowing it to remain in a drug-free solution for about 30 min (Fig. 2a).

When the concentration of an analgesic drug was increased without previously washing out the bath, there was little or no further depression of twitch height after the first of the series of doses (Fig. 2b). Tolerance produced in this way to the depressant action of each of the analgesics developed at about the same rate as with tolerance produced by successive separate doses. Cross tolerance was observed

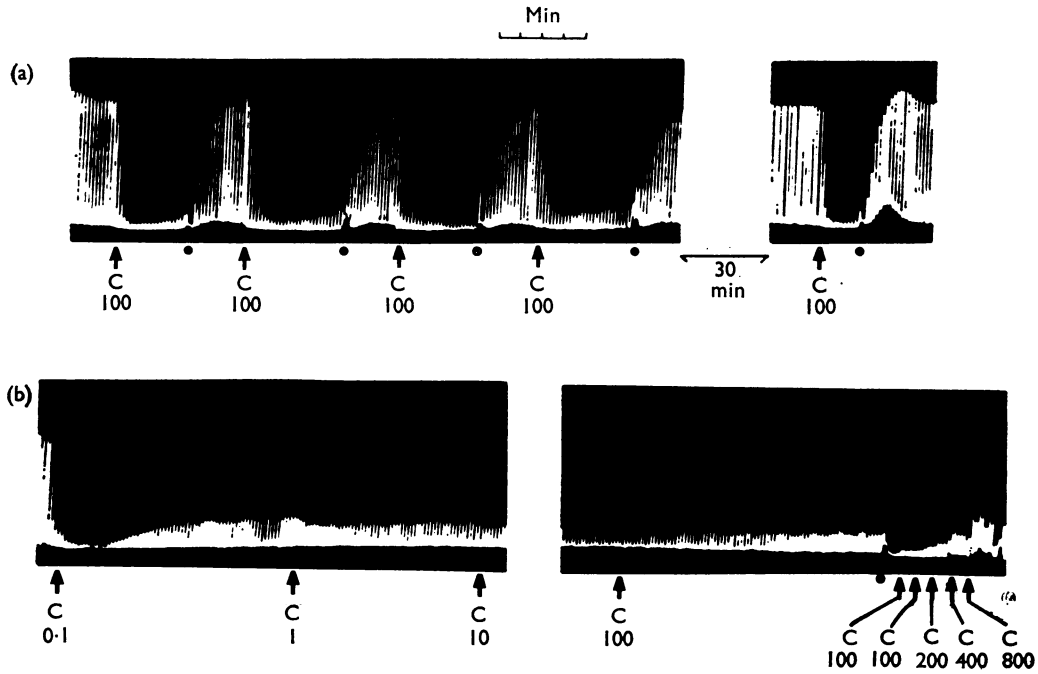

FIG. 2. (a), Development of tolerance to the depressant action of codeine (C) on twitches elicited by supramaximal electrical stimulation of the guinea-pig ileum. After a rest period of 30 min the sensitivity of the ileum had been restored to its original level. The numbers underneath each arrow represent added drug concentration expressed as ng/ml. of bath fluid. (b), Development of physical dependence to codeine. There is a period of 3 min between the frames. The lower record is a continuation of the upper record. The numbers underneath each arrow represents added drug concentration expressed as μg/ml. of bath fluid. At ● the bath fluid was changed.

between each of the analgesics tested and morphine. Tolerance to a depressant action on twitches did not occur with non-analgesic drugs, such as atropine and adrenaline. Tolerance to pethidine was not observed. This agrees with the observations of Paton (1957a), who attributed the failure of development of tolerance to pethidine to the atropine-like action of this drug.

Increased activity produced by increased concentrations of analgesic drugs in tolerant ileum preparations

After tolerance to the depressant action had developed by giving cumulatively increasing concentrations of an analgesic drug, a further increase in concentration produced an increase in activity of the ileum. This effect was observed with all the drugs tested except morphine-N-oxide. The nature of the increased activity depended on the drug. With morphine (Fig. 4), normorphine, codeine and nalorphine, the twitches were increased in height and uniform; whereas with diamorphine, fentanyl, phenoperidine, oxymorphone, phenazocine (Fig. 5) and pentazocine,

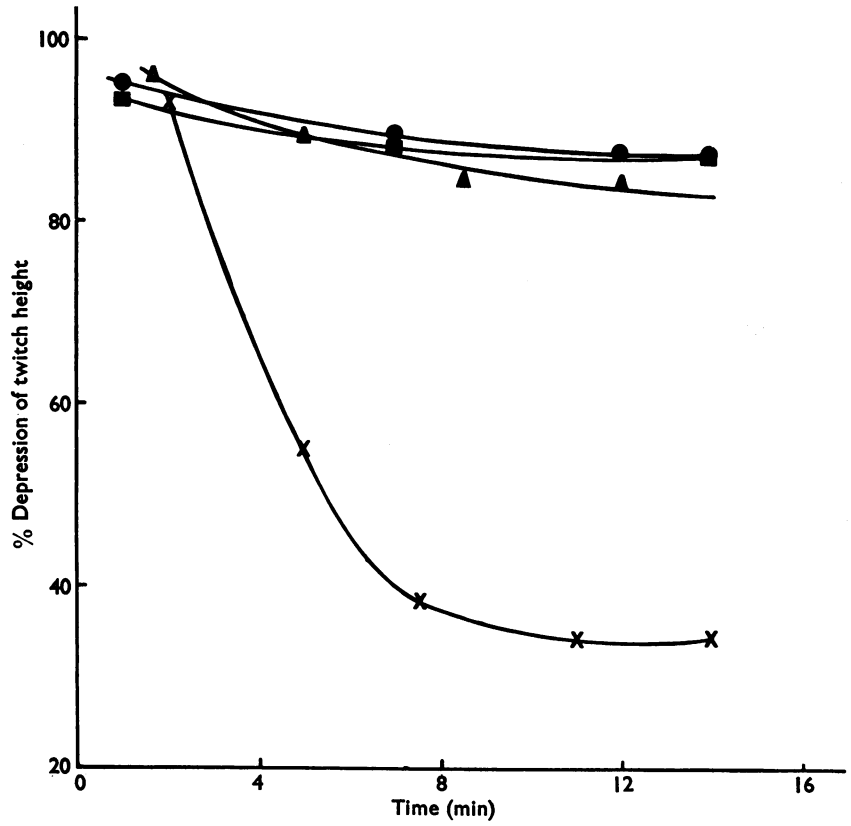

FIG. 3. Graph of four different experiments showing the rate of development of tolerance to successive doses of morphine 0·01 μg/ml. (●), codeine 0·1 μg/ml. (■) and fentanyl 1 pg/ml. (▲) in ilea of untreated guinea-pigs. The curves for these three drugs differ markedly from the curve obtained with morphine 0·01 μg/ml. on ileum of guinea-pigs treated chronically with morphine (×). The vertical axis shows percentage depression of twitch height, the horizontal axis shows time in min.

the contractions of the ileum in response to transmural stimulation were increased but irregular in height. The movements of the ileum appeared to be arrhythmic, but when the transmural stimulation was turned off the preparation was quiescent.

Depression of ileal activity on withdrawal of analgesic drugs

Once a drug had produced a regular or an irregular increase in twitch height, there was a marked reduction in the activity of the ileum when the drug was washed out.

Regular twitches which were reduced by withdrawal of a drug did not recover during a period of up to an hour. Irregular activity was only temporarily decreased by withdrawal of a drug, however, and returned within about 10 min, as shown in Fig. 5 with phenazocine.

Table 2 shows the threshold concentrations for the analgesics tested at which withdrawal of the drug resulted in reduction of twitches.

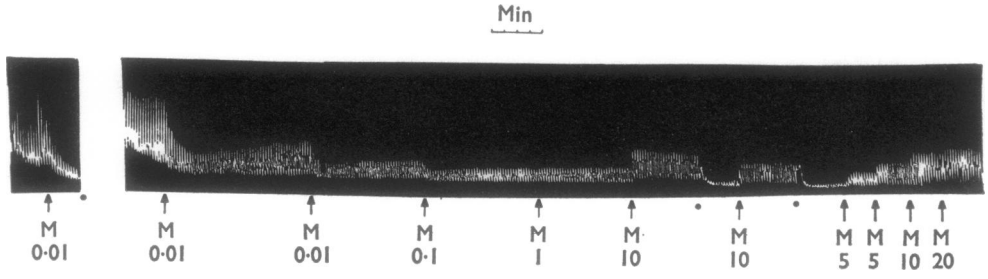

FIG. 4. Development of tolerance and physical dependence to morphine (M). At ● the bath fluid was changed. Time interval between frames is 4 min. The numbers underneath each arrow represent added drug concentration expressed as μg/ml. of bath fluid.

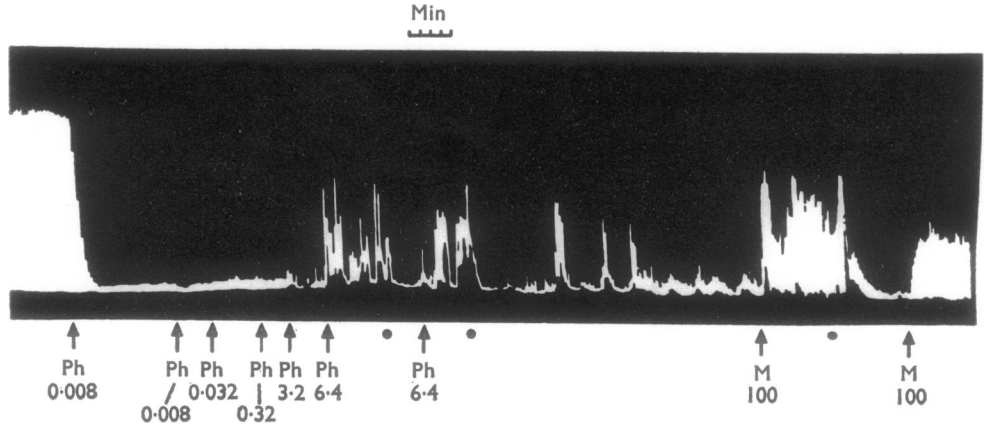

FIG. 5. Development of acute tolerance to phenazocine (Ph) and cross dependence with morphine (M). At ● the bath fluid was changed. The numbers underneath each arrow represent added drug concentration expressed as μg/ml. of bath fluid.

Restoration of responses decreased by withdrawal

Regular twitches which had been depressed by withdrawal of morphine, normorphine, codeine and nalorphine could be restored, almost to control levels, by adding the drug which had been withdrawn in a concentration equal to or greater than the threshold concentration at which it produced a withdrawal effect. The effect with morphine is illustrated in Fig. 4. At this point, the ileum might be described as "dependent" on morphine for twitches in response to transmural stimulation: Paton (1957a) used the term "morphine dependence" for the effect. The increase in height of twitches restored by readministration of drug was dose dependent and log dose-response curves are shown in Fig. 6. Twitches that had been depressed by

TABLE 2. *Concentrations of drugs at which withdrawal resulted in reduction of responses to transmural stimulation*

Drug used	Concentration in μg/ml. capable of inducing withdrawal response	Molar potency relative to morphine in inducing withdrawal response
Fentanyl	0·0045	4,400
Oxymorphone	0·35	63·6
Phenoperidine	3·0	6·54
Phenazocine	3·2	5·93
Pentazocine	8·0	2·46
Normorphine	8·0	2·38
Diamorphine	18	1·44
Morphine	20	1·00
Nalorphine	36	0·61
Codeine	150	0·14

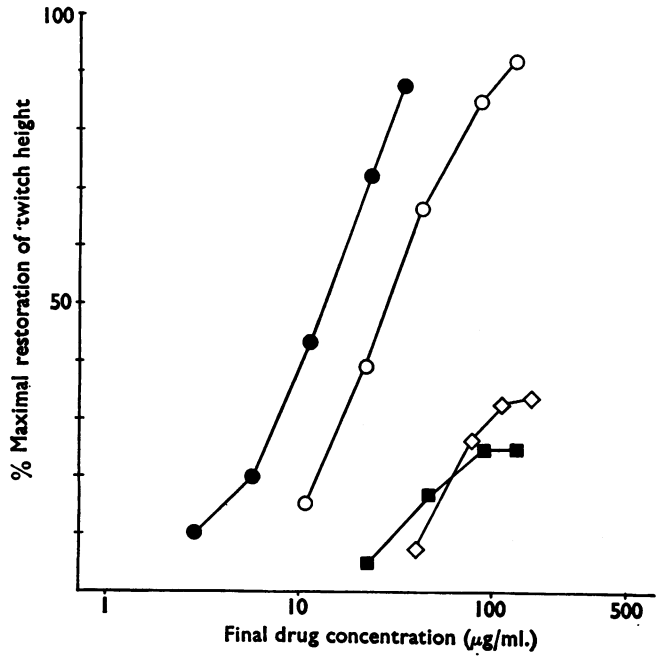

FIG. 6. Dose-response curves for the readministration responses of dependent ileum to normorphine (●), morphine (○), codeine (✧) and nalorphine (■). The vertical axis depicts percentage maximal restoration of twitch height, figures on the horizontal axis represent final drug concentrations in μg/ml. of bath fluid.

withdrawal of any one of these drugs were restored by morphine, the maximum height of the restored twitches being independent of the drug withdrawn.

Codeine and nalorphine were less potent than morphine in restoring depressed twitches and their maximal effects were considerably less. The form of the log dose-response curves (Fig. 6) suggests that they are partial agonists. A comparison of morphine and codeine in restoring twitches in a morphine dependent ileum is shown in Fig. 7. The maximal effect was produced by about 50 μg/ml. of morphine, but the maximal effect with codeine (about 200 μg/ml.) was very much less. Further evidence that codeine may be a partial agonist is provided in the last panel of Fig. 7, which shows that it reduces twitches restored by morphine ; that is, codeine is having an antagonistic action on the effect of morphine. Similarly, nalorphine reduced twitches that had been restored by morphine.

Drugs which produced irregular activity of the ileum in high concentrations also produced irregular activity when they were added to the ileum that had been depressed by their withdrawal. The effect with phenazocine is illustrated in Fig. 5. Similar results were obtained with diamorphine, fentanyl, oxymorphone, pentazocine and phenoperidine. Morphine restored twitches that had been depressed by withdrawal of each of these drugs. The restored twitches were less regular than those produced in morphine dependent ileum, but were more regular than those restored by drugs producing irregular activity. An example of morphine restoration in an ileum depressed by withdrawal of phenazocine is shown in Fig. 5.

Morphine-N-oxide did not cause an increase in twitch height after addition of high concentrations to a tolerant ileum, nor did withdrawal cause depression of twitches ; rather, the twitches returned to their control height. It did not restore twitches that had been depressed by withdrawal of another drug. Twitches that were maintained by the presence of morphine in a dependent preparation were depressed by morphine-N-oxide (Fig. 8).

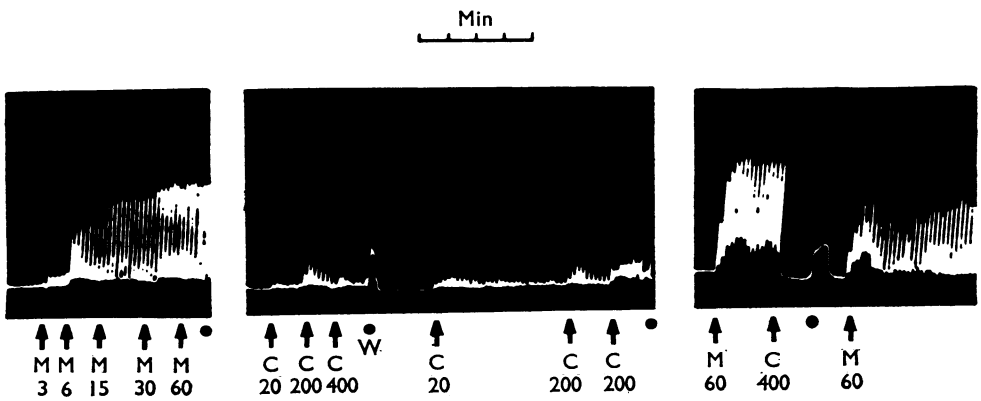

FIG. 7. Partial agonist nature of codeine. The ileum was made morphine dependent by repeated morphine administration until a morphine withdrawal response was induced. The first frame shows the dose dependent readministration response to morphine (M). The middle frame depicts the cumulative readministration response to codeine (C). The final frame depicts the antagonistic action of a high dose of codeine on the morphine administration response. Similar pictures to these could be obtained using ileum made dependent to codeine by repeated codeine administration. At ● the bath fluid was changed, and at W there was a wash artefact on changing the bath fluid. The numbers underneath each arrow represent added drug concentration expressed as μg/ml. of bath fluid.

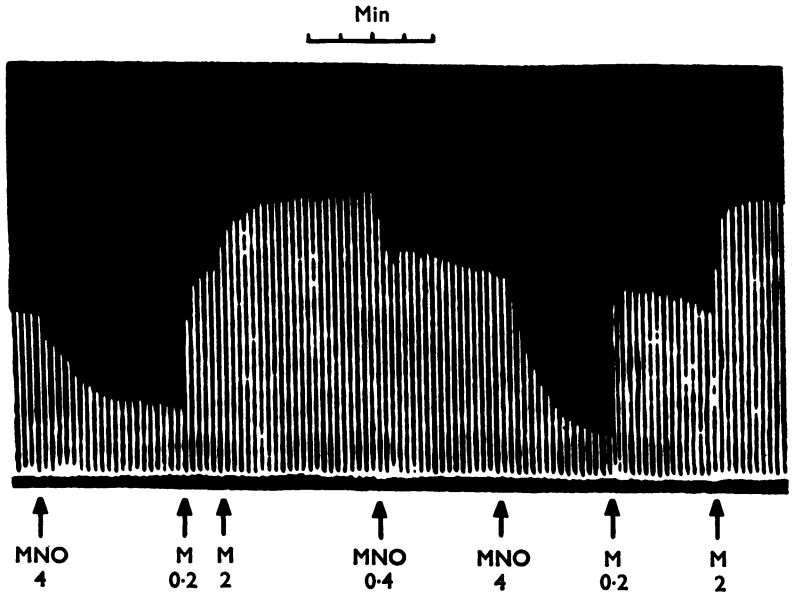

FIG. 8. Effects of morphine (M) and morphine-N-oxide (MNO) after the development of dependence to morphine. The numbers underneath each arrow represent added drug concentration expressed as μg/ml. of bath fluid.

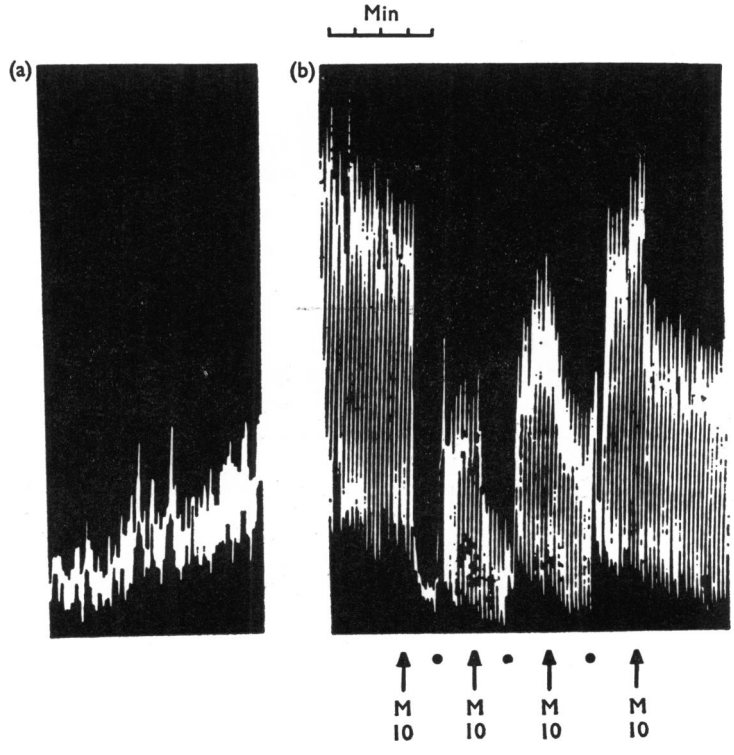

FIG. 9. Ileum taken from guinea-pigs chronically pretreated with morphine as described in **Methods** section. (a), Marked activity in the absence of electrical stimulation characteristic of this preparation. (b), Very rapid tolerance to morphine (M) as seen in these preparations. By the fourth administration, the inhibitory response to morphine 10 ng/ml. had declined from an initial almost 100% to only 30%. At ● the bath fluid was changed. The numbers underneath each arrow represent added concentration expressed as ng/ml. of bath fluid.

Ilea from guinea-pigs chronically treated with morphine

Preparations of ileum from these guinea-pigs had marked spontaneous activity (Fig. 9a). They responded to transmural stimulation with twitches of the usual height, but the twitches were somewhat more irregular than usual. Morphine depressed the twitches in the same doses as it did in ileum from untreated animals, and it also reduced or abolished the spontaneous activity. Tolerance to the depressant action of morphine developed much more rapidly than usual (Fig. 9b). Figure 3 provides a comparison of development of tolerance of ileum from untreated and morphine treated guinea-pigs. Dependence developed at the same rate and occurred with similar doses as in ileum from untreated animals.

Vesicular nerve stimulation of rat and guinea-pig bladders

Morphine reduced the size of the contractions of the guinea-pig and rat bladders evoked by electrical stimulation, but it was less active than on the ileum, a dose of about 100 μg/ml. of morphine being required in either preparation to produce a 50% reduction in twitch height. The onset of inhibition was slow and after 60 min the average decrease in height was about 25%. When doses of morphine of 200 μg/ml. were left in contact with the bladder for 30 min before washing out, tolerance gradually developed over a 5–6 hr period. Dependence on morphine could not be demonstrated in these preparations.

Discussion

Each one of the twelve morphine-like analgesics tested depressed the twitch of the transmurally stimulated guinea-pig ileum. The depressant effect of pethidine is due, at least partly, to its atropine-like activity (Paton, 1957a).

With the exception of pethidine, there is a reasonable correlation between analgesic potency of the drugs tested and their depressant activity on the guinea-pig ileum as shown in Table 3. This correspondence has been pointed out previously by Paton (1957a) and by Cox & Weinstock (1966). The present findings extend to the more recently developed and extremely powerful narcotic analgesics fentanyl and phenoperidine. Tolerance to the depressant action of morphine, codeine, phenadoxone

TABLE 3. *Comparison of analgesia in mice and man, and potency in depressing contractions of the isolated ileum*

Drug	50% depression of contractions of ileum. Molar potencies relative to morphine	Analgesia in mice (hot plate method). Relative molar potencies to morphine*	Analgesia in man. Molar dose relative to morphine*
Fentanyl	10,000	250	100
Phenoperidine	2,000	41·5	10
Oxymorphone	500	13	7·1
Nalorphine	2·0	0·03	1·1
Diamorphine	1·7	2·8	2·4
Normorphine	1·4	0·05	0·25
Phenazocine	1·22	19	4·0
Morphine	1·0	1·0	1·0
Pentazocine	0·63	0·30	0·42
Codeine	0·15	0·16	0·09
Morphine-N-oxide	0·05	0·10†	—

*From figures quoted in review by Mellett & Woods (1963). †Fennessy (1968).

and nalorphine was described by Paton (1957a), and a similar effect has now been obtained with other morphine-like drugs.

Tolerance to the depressant action of each of these drugs appeared to develop at about the same rate, even though they differ widely in their depressant potency. The development of tolerance to the depressant action seems to be confined to morphine-like analgesic drugs, for it did not develop to atropine or adrenaline, two non-analgesic drugs which also cause depression of twitches by two separate mechanisms.

Ileum taken from guinea-pigs chronically treated with morphine had spontaneous activity, as had been noted by Takagi, Takayanagi, Irikura, Nishino, Ichinoseki & Shishido (1965). The increased spontaneous activity may be analogous to the enhanced intestinal movements occurring in human addicts on withdrawal of morphine. These withdrawal symptoms in man are relieved by a low dose of morphine (Reynolds & Randall, 1959) and spontaneous activity and twitches of ileum from chronically treated guinea-pigs were depressed by the same dose of morphine that depressed twitches of ileum from untreated guinea-pigs.

The rate of development of tolerance to the depressant action of morphine in ileum from morphine treated guinea-pigs was much greater than in those from untreated guinea-pigs. There was, however, no difference in the concentration of morphine required to produce dependence in the ileum. These findings suggest that the effects of morphine on the ileum *in situ* differ from those observed in isolated ileum. It had been anticipated that ileum from guinea-pigs treated with large doses of morphine would have behaved like ileum exposed to high concentrations in the organ bath, and been dependent on morphine for twitch response. The explanation for this difference will require further experiment, but the observations do not support the view that morphine dependence as observed in the ileum is analogous to dependence as seen in man.

All the morphine-like analgesic drugs except pethidine and morphine-N-oxide produced a state of dependence in the ileum in that twitches were depressed by withdrawal of high concentrations and were restored by adding the drug again. Pethidine cannot be tested in this way because of its pronounced atropine-like activity. Morphine-N-oxide had no atropine-like activity, and tolerance developed to its depressant action on twitches. It differs in a number of other respects: thus, in an equally analgesic dose to morphine it has no narcotic effect, does not produce vomiting or depress respiratory movements in dogs (Fennessy, unpublished observations). None of these differences explains why it does not cause dependence in the ileum.

Both cross tolerance and cross dependence were demonstrated between morphine and most of the other analgesics, but morphine-N-oxide was an exception in that there was no cross dependence although there was cross tolerance. Collier (1965) has pointed out that tolerance and dependence are not necessarily related.

Twitches in the presence of high concentrations of morphine, normorphine, codeine, nalorphine and morphine-N-oxide were regular in height, however they were irregular with the remaining drugs tested. The difference is not related to their depressant potency on the ileum nor to their analgesic potency (Table 3). Replacement of a drug producing irregular activity in a dependent ileum with morphine tended to regularize the twitches. The findings indicate a difference in action of the two groups of drugs, but do not provide a clue to its nature.

Codeine and nalorphine behaved as partial agonists in restoring responses of the ileum when they were readministered after the development of acute tolerance. This was observed both in ileum made dependent on morphine and in ileum made dependent on codeine or nalorphine. The finding may be related to the observation that codeine has a low addiction liability and a lower maximal addiction intensity than morphine in man (Eddy, Halbach & Braenden, 1956), and nalorphine precipitates a withdrawal syndrome in morphine addicts. However, there was no correlation between the minimum concentration of analgesic drug required to cause a reduction in twitch height on drug withdrawal and the figures for addiction liability of these drugs quoted by Mellett & Woods (1963). Pentazocine is reported not to cause dependence in man (Fraser & Rosenberg, 1964), but it produces dependence in the ileum. These observations indicate that the transmurally stimulated ileum cannot be used as a reliable screening method for the evaluation of addiction liability of opiates in man.

The absence of results with the guinea-pig or rat bladder comparable with those obtained with the guinea-pig ileum indicates that the nerves to the bladder are insensitive to morphine. There are considerable differences in the effects of morphine on different organs from the same species, and on the same organ from different species. Thus Vanov (1965) demonstrated that morphine (100 μg/ml.) partly reduced the contractions of the isolated rat bladder evoked by electrical stimulation of the vesical nerves. Morphine has been shown to decrease the negative chronotropic response to stimulation of the vagus nerve in rats and rabbits, but this effect was present only to a small extent in cats, and was not present in guinea-pigs (Kosterlitz & Taylor, 1959). Reduction of the nictitating membrane response of the cat to either pre- or postganglionic stimulation by morphine has been reported by Trendelenberg (1957) and by Cairnie, Kosterlitz & Taylor (1961). These authors observed, however, that the effects of morphine on responses of the spleen to splenic nerve stimulation were inconsistent, and that increases in heart rate caused by stimulation of the cardiac sympathetic nerves were unaffected by morphine.

These findings create a difficulty in attempting to elucidate the mechanisms of action of morphine. If the effects on the ileum are due simply to depression of transmitter release, it would be expected that it would act similarly on other tissues. At the present time, it is not clear what determines whether the effects of stimulation of a nerve will be depressed by morphine.

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REFERENCES

CAIRNIE, A. B., KOSTERLITZ, H. W. & TAYLOR, D. W. (1961). Effect of morphine on some sympathetically innervated effectors. *Br. J. Pharmac. Chemother.*, **17**, 539–551.

COLLIER, H. O. J. (1965). A theory of drug dependence. In *Hashish: Its Chemistry and Pharmacology*, ed. Wolstenholme, G. E. B., Biol, F. I. & Knight, J., pp. 83–87. London: Churchill.

COX, B. M. & WEINSTOCK, M. (1966). The effect of analgesic drugs on the release of acetylcholine from electrically stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **27**, 81–92.

DE LA LANDE, I. S. & PORTER, R. B. (1967). Factors influencing the action of morphine on acetylcholine release in the guinea-pig intestine. *Br. J. Pharmac. Chemother.*, **29**, 158–167.

EDDY, N. B., HALBACH, H. & BRAENDEN, O. J. (1956). Synthetic substances with morphine-like effect. *Bull. Wld Hlth Org.*, **14**, 353–402.

FENNESSY, M. R. (1968). The analgesic action of morphine-N-oxide. *Br. J. Pharmac.*, **34**, 337–344.

FRASER, H. F. & ROSENBERG, D. E. (1964). Studies on the human addiction liability of 2'-hydroxy-5,9-dimethyl-2(3,3-dimethylallyl)-6,7-benzomorphan (Win 20,228): a weak narcotic antagonist. *J. Pharmac. exp. Ther.*, **143**, 149–156.

GYANG, E. A. & KOSTERLITZ, H. W. (1966). Agonist and antagonist actions of morphine-like drugs on the guinea-pig isolated ileum. *Br. J. Pharmac. Chemother.*, **27**, 514–527.

HUKOVIĆ, S., RAND, M. J. & VANOV, S. (1965). Observations on an isolated innervated preparation of rat urinary bladder. *Br. J. Pharmac. Chemother.*, **24**, 178–188.

KOSTERLITZ, H. W. & TAYLOR, D. W. (1959). The effect of morphine on vagal inhibition of the heart. *Br. J. Pharmac. Chemother.*, **14**, 209–212.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678–689.

MELLETT, L. B. & WOODS, L. A. (1963). Analgesia and addiction. *Fortschr. Arzneimittelforsch.*, **5**, 155–267.

PATON, W. D. M. (1957a). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **12**, 119–127.

PATON, W. D. M. (1957b). A pendulum auxotonic lever. *J. Physiol., Lond.*, **137**, 35–36*P*.

PATON, W. D. M. (1963). Cholinergic transmission and acetylcholine output. *Can. J. Biochem. Physiol.*, **41**, 2637–2653.

REYNOLDS, A. K. & RANDALL, L. O. (1959). In *Morphine and Allied Drugs*, 1st ed., pp. 124–144. Toronto: University of Toronto Press.

SCHAUMANN, W. (1957). Inhibition by morphine of the release of acetylcholine from the intestine of guinea-pig. *Br. J. Pharmac. Chemother.*, **12**, 115–118.

TAKAGI, K., TAKAYANAGI, I., IRIKURA, T., NISHINO, K., ICHINOSEKI, N. & SHISHIDO, K. (1965). Responses of the isolated ileum of the morphine tolerant guinea-pig. *Archs int. Pharmacodyn. Thér.*, **158**, 39–44.

TRENDELENBERG, U. (1957). The action of morphine on the superior cervical ganglion and on the nictitating membrane. *Br. J. Pharmac. Chemother.*, **12**, 79–85.

VANOV, S. (1965). Pharmacological actions of nicotine on the peripheral autonomic nervous system. Ph.D. Thesis, Univ. London.

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