Br. J. Pharmac. Chemother. (1966), **27**, 81–92.

THE EFFECT OF ANALGESIC DRUGS ON THE RELEASE OF ACETYLCHOLINE FROM ELECTRICALLY STIMULATED GUINEA-PIG ILEUM

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(*Received January* 10, 1966)

Paton (1957) has shown that the contractions of transmurally stimulated guinea-pig ileum were depressed by morphine and related analgesic drugs. He found some difficulty in comparing the potencies of drugs because there was a tendency for the effects of a particular dose of drug to decline on repeated administration. This problem was overcome in the present experiments by leaving the drugs in contact with the gut only long enough for the maximal effect of a particular dose to be produced. In addition, the administration of drugs was so arranged as to allow the responses of the gut to return to their original height before the next dose was given.

A study has been made of the relative potencies of a number of structurally related analgesic drugs. The activities of optical isomers of analgesic drugs in depressing the gut contractions have been compared, and the effects of analgesic antagonists were investigated.

METHODS

Transmurally stimulated guinea-pig ileum

Male guinea-pigs weighing 300–1,000 g were killed by a blow on the head and bled out. The ileum was removed and placed in Krebs solution. A length of about 7 cm of gut in which no food residues were present was cut from a point approximately midway down the ileum.

The tissue was set up in a 5 or 15 ml. organ bath containing Krebs solution at 37° C, and oxygenated with 95% O_2 and 5% CO_2. The arrangement was similar to that described by Paton (1955), except that the lower tubular support was left open, thus allowing intraluminal contents to be extruded slowly during the course of the experiment. Gut movements were recorded on a kymograph by means of a gimbal lever, and the weighting on the gut was adjusted so that a level baseline was obtained.

The tissue was stimulated by single shocks with a pulse-width of 0.5 msec or less, delivered every 20 sec from a Palmer square wave stimulator. The voltage was adjusted initially to give a maximal response. Drugs related to morphine or levorphanol were left in contact with tissue for 2 min. Drugs of the M series required a contact time of 3 min for their maximal effects to be produced.

To obtain constant depressions of contraction by a given concentration of analgesic drug over a period of several hr, doses inhibiting the contractions by more than 85% were avoided, and the contractions were allowed to return to their initial height between each dose. An interval between

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doses of 15 min was usually sufficient for this, but in some preparations it was necessary to extend this time to 20 min.

Measurement of acetylcholine release

The effects of three different concentrations of the analgesic drugs under test were first determined in duplicate on the responses of the ileum to electrical stimulation. A contact period of 3 min was allowed, so that for every drug concentration used the effect on contraction height was measured at equilibrium. Stimulation was then discontinued, and physostigmine sulphate at a concentration 5 μg/ml. was added to the Krebs solution. Thirty to forty min later, the gut was again stimulated for periods of 3 min every 10 min.

The concentration of acetylcholine in the bath fluid was determined after each period of stimulation. Drugs were added to the bath for 3 min before a period of stimulation, and again during stimulation. The amount of acetylcholine released in the presence of each drug concentration was expressed as a percentage of the mean control release determined before and after the application of the drug.

The samples of bath fluid for acetylcholine assay were diluted in the ratio 1:1.5 with distilled water, and the physostigmine sulphate content increased to 50 μg/ml., to make them suitable for direct application to the leech muscle. They were stored at 0° C together with known amounts of acetylcholine diluted similarly. After storage for 48 hr the mean recovery of acetylcholine was 99.25%.

Acetylcholine assay

The acetylcholine was assayed on the dorsal muscle of the leech (*hirudo medicinalis*). A strip of the dorsal muscle of the leech was set up in an organ bath of 1 ml. capacity in Krebs solution diluted 1:1.5 with distilled water. The fluid was at room temperature, and contained physostigmine sulphate at a concentration of 50 μg/ml. The tissue was aerated with 95% O_2 and 5% CO_2. The acetylcholine content of each sample was determined by bracketing, and expressed in terms of acetylcholine chloride.

The presence of analgesic drug in the concentrations used in these experiments did not affect the responses of the leech muscle to acetylcholine. Several samples of bath fluid were assayed by standard methods for 5-hydroxytryptamine and histamine. These were never found present in sufficient quantities to modify the responses of the leech muscle.

Drugs and solutions

Krebs solution having the following composition was used: NaCl 0.69%, KCl 0.035%, $CaCl_2$ 0.028%, $MgCl_2$ 0.011%, $NaHCO_3$ 0.21%, NaH_2PO_4 0.014%, glucose 0.2%.

The following drugs have been used: morphine sulphate, codeine phosphate, diacetyl morphine HCl, normorphine HCl, nalorphine HBr, levorphanol tartrate and HBr, dextrorphan tartrate, levomethorphan HBr, dextromethorphan HBr, (−)-3-hydroxy-morphinan base, levallorphan tartrate, dextrallorphan HBr, M.99 HCl [3-hydroxy-6,14-*endo*-etheno-5,7,8,8-tetrahydro-7(2-hydroxypent-2-yl) oripavine HCl, B.P.937214] and related drugs M.183 HCl (B.P.937214) and M.53 HCl (B.P.925723) (for the relationship of these drugs to M.99 see Table 1 and Fig. 3), physostigmine sulphate, atropine sulphate, L-adrenaline base, acetylcholine chloride, and D-tubocurarine chloride. The doses of analgesic drugs were calculated in terms of their molar concentration.

RESULTS

The effect of morphine on stimulated ileum

Morphine reduced the responses of the guinea-pig ileum to transmural stimulation. In the most sensitive preparations a concentration of morphine as low as 12.5 pmole per ml. (4.7 ng/ml.) produced an appreciable depression of the contraction height. The depression usually reached a maximum within 60 sec.

There was, however, considerable variation in the effectiveness of morphine in different preparations. Out of a total of 55 experiments, morphine or a similar drug was able to depress the contractions by 70% or more of their initial height in 36 preparations. In 8 preparations morphine was completely without effect even when doses up to 1 μmole (379 μg) were added to the organ bath. In the remaining 11 preparations morphine reduced the contraction height by only 20 or 30%, and an increase in dose produced little increase in response. In such a preparation, the usual sensitivity to morphine could be achieved if the gut was stimulated submaximally. The results of alterations in voltage and duration of stimulus on the sensitivity to morphine are shown in Fig. 1.

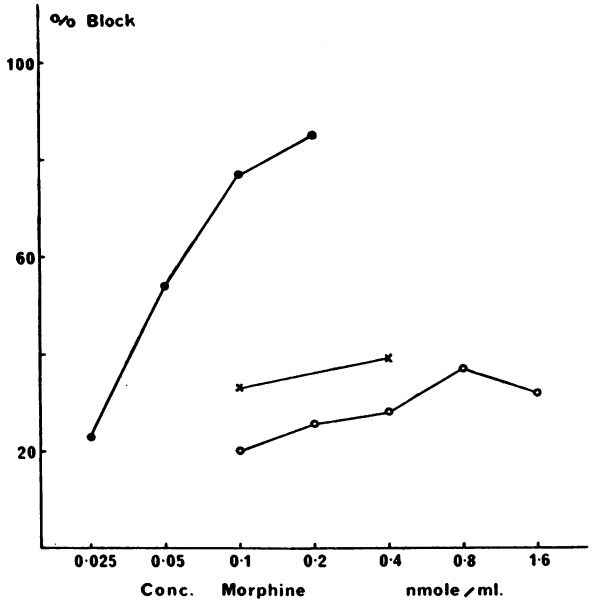

Fig. 1. *Increased sensitivity to morphine produced by alteration of electrical stimulus.*—○, Stimulus 500 μsec, 20 v., giving maximal contractions; ●, Stimulus 60 μsec, 20 v., giving submaximal contractions; ×, Stimulus 60 μsec, 60 v., giving maximal contractions. Ordinate: % reduction of contraction height. Abscissa: concentration of morphine in nmole/ml.

Identification of the active substance released as acetylcholine

Application of the fluid bathing the ileum to the leech muscle resulted in a contraction only when eserine was present. The responses to a standard solution of acetylcholine and to a pooled sample of bath fluid were both reduced to one-third of their original size in the presence of D-tubocurarine (1 μg/ml.), and abolished completely when the concentration of D-tubocurarine was raised. No activity remained in the pooled sample after boiling at *p*H 11.0 for one min.

The effect of morphine on the release of acetylcholine

The effect of morphine on the contractions of the stimulated ileum and on the release of acetylcholine is shown in Fig. 2. The histograms represent the mean results from

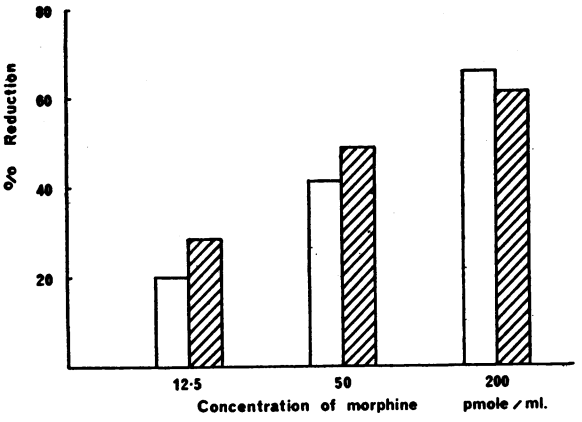

Fig. 2. *Effects of morphine on contraction height and acetylcholine release.*—Open histograms represent % reduction of contraction height, hatched histograms represent % reduction of acetylcholine released. Ordinate: % reduction. Abscissa: concentration of morphine pmole/ml.

three experiments. A very close correlation was found between the percentage reductions of contraction height and acetylcholine release.

Structure-activity relationships

The basic structures of the series of compounds used to study the relationship between chemical structure and ability to depress the responses of the ileum to electrical stimulation are shown in Fig. 3. Three groups of compounds were used. The first group was based on morphine (I), and the other two groups were derivatives of morphinan (II) and oripavine (III) respectively (Lister, 1964).

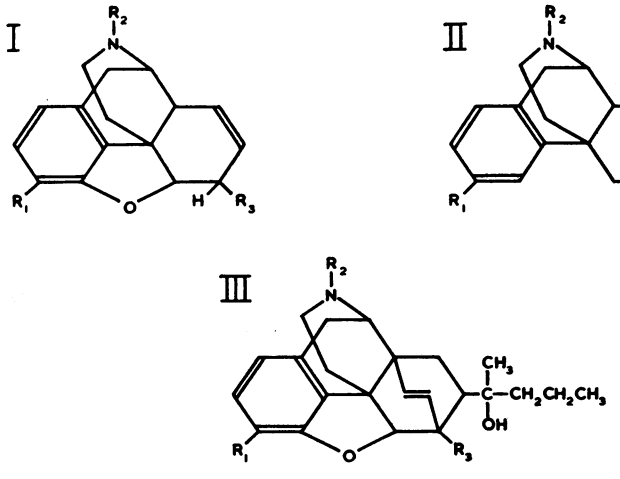

Fig. 3. *Basic formulae of drugs used.*—I, morphine series (morphine: $R_1=OH$, $R_2=CH_3$, $R_3=OH$); II, morphinan series (levorphanol: $R_1=OH$, $R_2=CH_3$); III, M series (M.99: $R_1=OH$, $R_2=CH_3$, $R_3=OCH_3$).

It had been found previously that none of the analgesic drugs, including the M compounds, affected the contractions of the gut produced by applied acetylcholine in the concentrations used.

Dose-response curves were constructed for the drug under study and the reference member (i.e., morphine, levorphanol or M.99) of its series. From these the concentrations of drug required to depress the contractions to 50% of their initial level were calculated. The potency of a drug was then expressed relative to that of its parent compound. Two or more determinations of the relative potencies of each compound were made.

Replacement of the 3-hydroxyl group by a methoxy group was found to reduce the ability of the drugs to depress the contractions of the stimulated ileum in all three series of compounds studied (Table 1). On the other hand, introduction of an acetyl group into the -3- position increased the inhibitory action. Thus, both heroin and M.183 were more

TABLE 1

EFFECT OF MODIFICATION OF THE SUBSTITUENT IN THE 3 POSITION (R_1)

	I		II		III	
R_1	Compound	Mean R.P.	Compound	Mean R.P.	Compound	Mean R.P.
$-OH$	morphine	1	levorphanol	1	M.99	1
$-OCH_3$	codeine	0.011	levomethorphan	0·107	M.53	0.063
$-OCOCH_3$	diacetylmorphine*	1·70	—	—	M.183	1·28

* In this compound, R3 is also $-OCOCH_3$
Mean R.P.=mean relative molar potency

active than morphine and M.99 respectively. In the case of M.183 the determination of its relative potency was complicated by the fact that it hydrolyses rapidly to M.99 in aqueous solution and hence its activity may have been underestimated.

When the nitrogen atom of the piperidine ring was changed from tertiary to secondary as in normorphine, or (-)-3-hydroxy-morphinan, the potency of the compounds was not markedly changed, although it was surprising that normorphine should be a little more potent than morphine, while (-)-3-hydroxy-morphinan was less active than levorphanol (Table 2). When the methyl substituent on the nitrogen atom was replaced by an allyl

TABLE 2

EFFECT OF MODIFICATION OF THE SUBSTITUENT ON THE NITROGEN ATOM (R_2)

	I		II	
R_2	Compound	Mean R.P.	Compound	Mean R.P.
$-CH_3$	morphine	1	levorphanol	1
$-H$	normorphine	1·36	(—)-3-hydroxy morphinan	0·65
$CH_2-CH=CH_2$	nalorphine	1·53	levallorphan	1·40

Mean R.P.=mean relative molar potency

group a small increase in potency was observed in both the morphine and morphinan series. However, modification of the substituents of the nitrogen atom has, in general, made only small changes in the potencies of the drugs.

TABLE 3

COMPARISON OF THE POTENCIES OF DRUGS IN DEPRESSING CONTRACTION OF ELECTRICALLY STIMULATED ILEUM AND THEIR ANALGESIC POTENCIES

	Mean relative molar potency	
Drug	Depression of contraction height*	Analgesia†
Morphine	1	1
M.183	992	1575
M.99	775	1280
Diacetylmorphine	1.70	2·50
Levorphanol	0·778	2·50
Levomethorphan	0·075	0·74
Codeine	0·011	0·114

* Mean of two or more determinations.
† Results calculated from mouse hot-plate experiments (Weinstock, 1961; Cox & Weinstock, 1964; and unpublished observations).

A comparison of the relative potencies of drugs in depressing gut contractions with that of their analgesic activities is made in Table 3. A close correlation was found between the two actions.

Stereochemical considerations

Analgesic activity resides in only one isomer of pairs of optical enantiomorphs of morphine-like drugs (Beckett & Casy, 1954). The effects of optical stereoisomers of analgesic drugs have been compared on the responses of guinea-pig ileum to electrical stimulation. The isomeric pairs levorphanol and dextrorphan, levomethorphan and dextromethorphan, and levallorphan and dextrallorphan have been used. In each case the laevo-isomer produced a depression of the contractions similar to the effect of morphine. The dextro-isomers on the other hand were inactive in doses up to one hundred times greater than effective doses of the laevo-isomers (Fig. 4).

The effect of levorphanol and dextrorphan on the quantity of acetylcholine liberated from the stimulated ileum was also investigated. The experiments were carried out in the way described previously for similar experiments with morphine. The results (Table 4) show that while levorphanol reduced both the contraction height and the quantity

TABLE 4

EFFECTS OF LEVORPHANOL AND DEXTRORPHAN ON CONTRACTION HEIGHT AND ACETYLCHOLINE RELEASED

		% reduction of	
Drug	Concentration pmole/ml.	Contraction height	Acetylcholine released
Levorphanol	50	47·5	45·5
Levorphanol	200	69·5	56·0
Dextrorphan	200	0	—8·5*
Dextrorphan	2000	0	11·0

Means of duplicate determinations
* The negative sign indicates that the amount of acetylcholine released was slightly increased by this treatment.

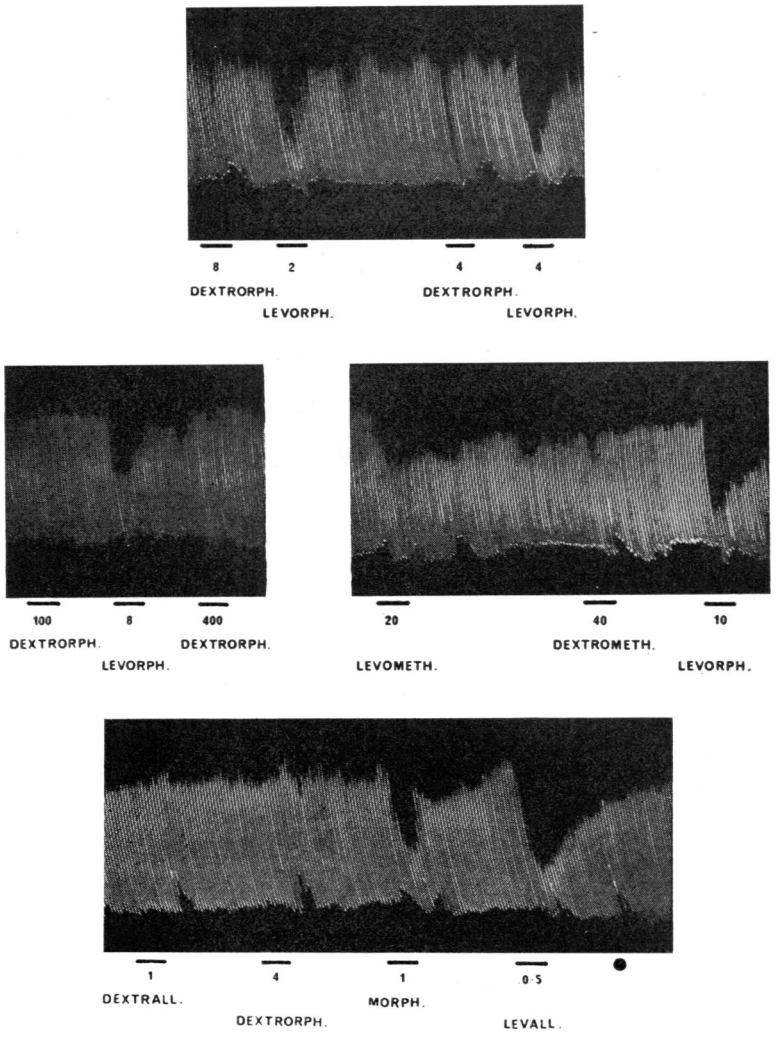

Fig. 4. *Comparison of the activities of optical isomers on the stimulated ileum preparations.*— Stimulus: 20 v., 0.5 msec, 0.05/sec. Bath volume 15 ml. Drugs were present during the periods indicated by the bars. Doses in nmoles. Dextrorph = dextrorphan ; Levorph. = levorphanol ; levometh. = levomethorphan ; Dextrometh. = dextromethorphan ; Dextrall. = dextrallorphan ; Leval. = levallorphan ; Morph. = morphine. The dot indicates a change of bath fluid.

of acetylcholine released, dextrorphan in the same or higher concentrations, did not alter the contraction height, and produced only inconsistent changes in the quantity of acetylcholine released.

Antagonism of the effects of analgesic drugs

The effects of morphine were not modified if the antagonist, nalorphine or levallorphan was added to the tissue at the same time as morphine. However, the inhibitory action

of morphine on the ileum was significantly reduced by the antagonist if the latter was left in contact with the tissue for 12 min before the morphine was given. Fig. 5 shows that a concentration of levallorphan of 1.67 pmole/ml. reduced the effects of different doses of morphine. It can be seen that levallorphan itself had slightly depressed the contraction height. This was not necessary for antagonistic activity since it occurred in only 5 of 10 successful experiments.

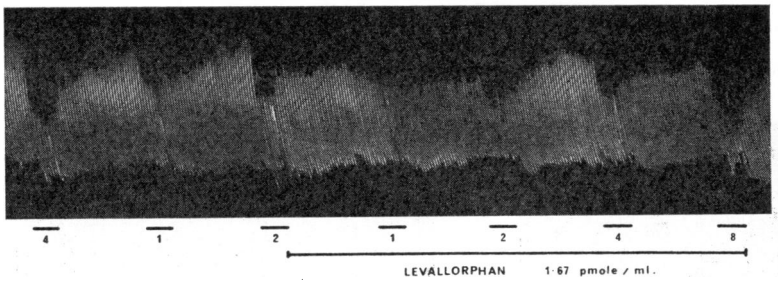

Fig. 5. *Antagonism of morphine by levallorphan.*—Stimulus: 10 v., 0.5 msec, 0.05/sec. Bath volume 15 ml. Morphine was added to the bath during the periods indicated by the short bars; the doses were measured in nmoles. Levallorphan was present in the bathing fluid during the period indicated by the long bar.

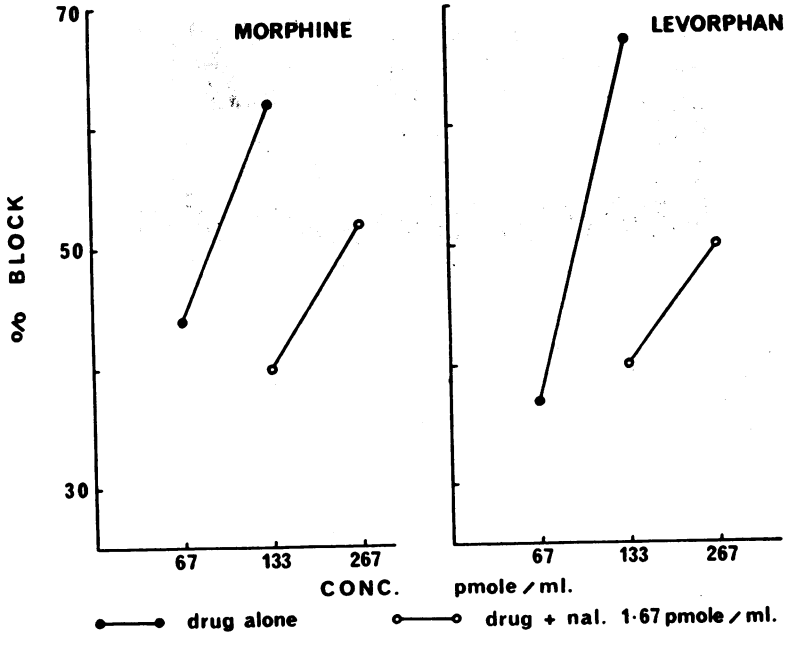

Fig. 6. *Antagonism of morphine and levorphanol by nalorphine.*—Stimulus: 20 v., 0.5 msec, 0.05/sec. Bath volume 15 ml. ●, drug alone; ○, drug plus nalorphine 1.67 pmole/ml. Ordinate: % reduction of contraction height. Abscissa: concentration of drug, pmole/ml.

The antagonism was reversible ; the effects of the active drugs had usually returned to their control values within 30 min of the removal of the antagonist. The dose response curve for morphine was shifted to the right in the presence of nalorphine, and the maximum effect obtainable with morphine could not be reduced by increasing the dose of antagonist.

Other analgesic drugs were also antagonized by nalorphine. Fig. 6 shows the results of an experiment in which the effects of nalorphine on the responses to levorphanol and morphine were compared. From the graphs the doses of each drug required to reduce the contraction height by 50%, in the absence and presence of nalorphine, were determined. The ratios of the dose in the presence of nalorphine to that in its absence were calculated. The value for morphine was 2.88, while the value for levorphanol 2.96. Thus nalorphine was equiactive in antagonizing morphine and levorphanol. The very potent compound M.99 was also antagonized by a similar concentration of nalorphine.

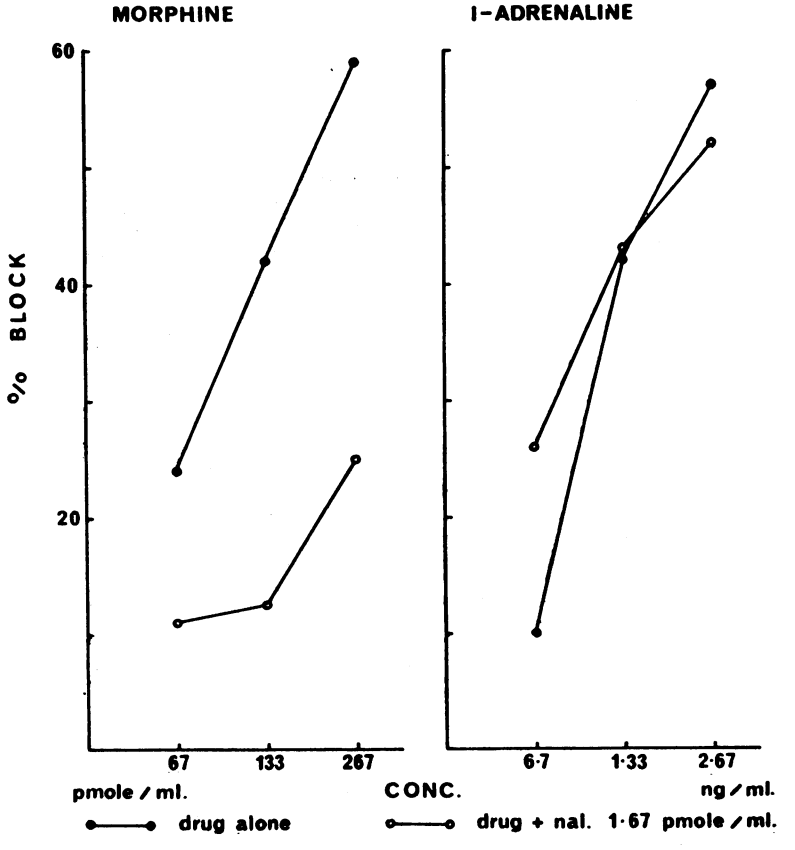

Fig. 7. *Effects of nalorphine on responses to morphine and adrenaline.*—Stimulus: 20 v., 0.5 msec, 0.05/sec. Bath volume 15 ml. ●, drug alone ; ○, drug plus nalorphine 1.67 pmole/ml. Ordinate: % reduction of contraction height. Abscissa: concentration of drug, pmole/ml. for morphine, ng/ml. for L-adrenaline.

Specificity of antagonism

Adrenaline also reduced the contractions of guinea-pig ileum produced by electrical stimulation (Schaumann, 1958). The ability of nalorphine to antagonize the inhibition produced by adrenaline was compared with its ability to antagonize the inhibitory action of morphine on the stimulated ileum. Fig. 7 shows the results of such an experiment.

The adrenaline inhibition was not reversed by nalorphine although on the same preparation it antagonized the action of morphine. Thus the antagonism appeared to be specific for analgesic drugs.

The antagonistic activities of both optical isomers of 3-hydroxy-N-allylmorphinan (levallorphan and dextrallorphan) have been compared. It has been shown previously that levallorphan antagonized the inhibitory effect of morphine on the ileum at about the same concentration as nalorphine. However, at this concentration the dextro-isomer, dextrallorphan, was completely without effect. A four-hundred-fold increase in the concentration of dextrallorphan was required to reduce the effects of morphine. Stereochemical factors would therefore also seem to be important in determining the antianalgesic activity of drugs on the electrically stimulated guinea-pig ileum preparation.

The morphine-like depressant effects of nalorphine on contractions of the ileum were not antagonized by the presence of a low concentration of morphine in the bathing fluid.

DISCUSSION

The release of acetylcholine from isolated segments of guinea-pig ileum produced by electrical stimulation was reduced by concentrations of morphine as low as 12.5 pmole/ml. (4.7 ng/ml.). Paton (1957) reported some difficulty in obtaining constant responses to morphine in this preparation. This may have been due to the fact that he used concentrations approximately ten times larger than in the present investigation. By avoiding such high concentrations, and by ensuring that the contractions had returned to their control height between doses, it was possible to obtain constant responses to the analgesic drugs over a period of several hr. A very close correlation was found between the inhibition of the contractions of the stimulated ileum by analgesic drugs and their effects on the acetylcholine released from the ileum during stimulation. The reduction in contraction height appeared to be the result of an inhibition of the release of acetylcholine.

In the few experiments in which morphine was completely inactive, the contractions could still be reduced by a low concentration of atropine or adrenaline. Furthermore, the contractions could also be abolished by anoxia or by lowering the temperature to 27° C. Thus it is unlikely that the muscle was being stimulated directly.

Beckett & Casy (1954) suggested that morphine-like drugs combined with specific receptors in the central nervous system to elicit an analgesic response. If the action of analgesic drugs on the stimulated ileum is also mediated through a combination with specific receptors, the structural requirements for inhibitory activity should resemble those for analgesia.

It has been shown that analgesic potency was reduced when the hydroxyl substitute on the -3- position (R_1) was replaced by a methoxy group ; on the other hand, if an acetyl

group was substituted in this position analgesic potency was increased (Braenden, Eddy & Halbach, 1955). Substitution of the phenolic hydroxyl group by a methoxy group also reduced the inhibitory action of the compound on the stimulated ileum. In addition, the acetyl derivatives showed increased inhibitory activity.

A difference between the analgesic receptors and those concerned with the depression of acetylcholine release in the ileum was observed when substituents on the nitrogen atom were changed. Whereas the analgesic activity of morphine was abolished in animals but not in man, by replacement of the *N*-methyl group with an *N*-allyl group (Green, Ruffell & Walton, 1954), there was little difference in the ability of morphine and nalorphine to reduce the release of acetylcholine from guinea-pig ileum. However, in both analgesic tests and in experiments using the stimulated ileum, the change of the *N*-methyl group to an *N*-allyl group resulted in the production of an antagonist. It may be significant that nalorphine is an analgesic approximately equipotent with morphine in man (Lasagna & Beecher, 1954).

The analgesic activity of compounds which can be resolved into optical enantiomorphs was found to reside exclusively in those isomers having the D configuration (Beckett & Casy, 1954). When isomeric pairs of drugs were tested it was found that only the isomers with analgesic activity reduced the responses of the gut to electrical stimulation. The dextro-isomers (L-configuration) were completely ineffective in the stimulated ileum. This observation suggested that the drugs were acting at a specific site which was similar to the three-dimensional analgesic receptor postulated by Beckett & Casy (1954).

Paton (1957) was unable to show that nalorphine antagonized the effects of morphine on the release of acetylcholine from the stimulated guinea-pig ileum, since in the doses used nalorphine itself depressed the contractions. In the present experiments much lower concentrations of nalorphine and levallorphan have been used, which in themselves produced very little inhibitory effect. However, both nalorphine and levallorphan specifically antagonized the actions of morphine-like drugs on the ileum. Dextrallorphan (L-configuration) was inactive as an antagonist. This finding also indicated the close similarity between the analgesic receptors and those in the ileum.

There is some evidence that morphine is also capable of reducing the release of acetylcholine from certain brain neurones. At a concentration of 1 μg/ml. morphine reduced the release of acetylcholine into fluid perfusing the cerebral subarachnoid space of anaesthetized cats (Beleslin, Polak & Sproull, 1965). Similarly, at a slightly higher concentration, morphine reduced the amount of acetylcholine released into fluid perfusing the left lateral ventricle of anaesthetized cats (Beleslin & Polak, 1965). If the brain contains cholinergic nerve endings which are as sensitive to the depressant actions of morphine-like drugs as the nerve endings in guinea-pig ileum, then the reports of several workers (Szerb & McCurdy, 1956; Mellett & Woods, 1959; Milthers, 1962) suggest that sufficient drug would be present in brain tissues after administration of analgesic doses, to depress the release of acetylcholine from these nerve terminals.

It would therefore be of interest to compare the effects of analgesic drugs and their antagonists on the release of acetylcholine from brain neurones, with their action on the stimulated guinea-pig ileum.

SUMMARY

1. Morphine and some related analgesic drugs were shown to inhibit the contractions of the transmurally stimulated guinea-pig ileum.

2. The degree of inhibition was proportional to the concentration of analgesic drug.

3. This effect of morphine was due to a reduction in the release of acetylcholine.

4. Structural requirements for inhibitory action on the ileum were found to resemble closely those for analgesic activity.

5. Stereospecificity was demonstrated for optical isomers having the D configuration.

6. Low concentrations of nalorphine and levallorphan, which themselves were without inhibitory effect on the ileum, antagonized the action of morphine and related drugs on the release of acetylcholine.

The authors wish to thank Dr M. W. Parkes of Roche Products Ltd. for supplying the morphinan derivatives, and Dr G. F. Somers of Reckitt and Sons Ltd. for supplying the M compounds. The help of Professor H. C. Stewart in the production of this paper is gratefully acknowledged. This work was undertaken by one of us (B.M.C.) in partial fulfilment of the requirements for the Ph.D. degree of the University of London. B.M.C. was a Nicholas Research Fellow.

REFERENCES

BECKETT, A. H. & CASY, A. F. (1954). Synthetic analgesics: stereochemical considerations. *J. Pharm. Pharmac.* **6**, 986–999.

BELESLIN, D. & POLAK, R. L. (1965). Depression by morphine and chloralose of acetylcholine release from the cat's brain. *J. Physiol. (Lond.)* **177**, 411–419.

BELESLIN, D., POLAK, R. L. & SPROULL, D. H. (1965). The release of acetylcholine into the cerebral subarachnoid space of anaesthetised cats. *J. Physiol. (Lond.)* **177**, 420–428.

BRAENDEN, O. J., EDDY, N. B & HALBACH, H. (1955). Synthetic substances with morphine-like effect. Rela tionship between chemical structure and analgesic action. *Bull. Wld. Hlth. Org.* **13**, 937–998.

COX, B. M. & WEINSTOCK, M. (1964). Quantitative studies of the antagonism by nalorphine of some of the actions of morphine-like analgesic drugs. *Br. J. Pharmac. Chemother.*, **22**, 289–300.

GREEN, A. F., RUFFELL, G. K. & WALTON E. (1954). Morphine derivatives with antianalgesic action. *J. Pharm. Pharmac.*, **6**, 360–397.

LASAGNA, L. & BEECHER, H. K. (1954). The analgesic effectiveness of nalorphine and nalorphine-morphine combinations in man. *J. Pharmac. exp. Ther.*, **112**, 356–363.

LISTER, R. E. (1964). Structure-activity requirements in some novel thebaine-derived analgesics. *J. Pharm Pharmac.*, **16**, 364–366.

MELLETT, L. B. & WOODS, L. A. (1959). The intracellular distribution of N-C^{14}—methyl levorphanol in brain, liver and kidney tissue of the rat. *J. Pharmac. exp. Ther.*, **125**, 97–104.

MILTHERS, K. (1962). The in vivo transformation of morphine and nalorphine into normorphine in the brain of rats. *Acta pharmac. Copenh.*, **19**, 235–240.

PATON, W. D. M. (1955). The response of the guinea pig ileum to electrical stimulation by coaxial electrodes. *J. Physiol. (Lond.)*, **127**, 40–41P.

PATON, W. D. M. (1957). 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.

SCHAUMANN, W. (1958). Zusammenhange zwischen der Wirkung der Analgetica und Sympathicomimetica auf dem Meerschweinchen-Dünndarm. *Arch. exp. Path. Pharmak.*, **233**, 112–124.

SZERB, J. C. & MCCURDY, D. H. (1956). Concentration of morphine in blood and brain after intravenous injection of morphine in nontolerant and neostigmine-treated rats. *J. Pharmac. exp. Ther.*, **118**, 446–450.

WEINSTOCK, M. (1961). Similarity between receptors responsible for the production of analgesia and lenticular opacity. *Br. J. Pharmac. Chemother.*, **17**, 433–441.