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The effect of morphine pretreatment on the sensitivity of mouse and guinea-pig ileum to acetylcholine and to morphine

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Guinea-pigs and mice were subjected to morphine pretreatment for at least 14 days culminating in subcutaneous injections of 1 g/kg daily in 2 divided doses. In mice, tolerance to the effects of morphine on gastrointestinal propulsion had developed by the end of the pretreatment course. The sensitivity to acetylcholine of the ilia from pretreated animals and the effects of morphine on transmural stimulation were compared with the ilia from normal animals. Ilia from pretreated guinea-pigs were marginally more sensitive to frequency of stimulation but significantly less sensitive to the depressant effect of morphine than ilia from normal animals. There was no significant difference in the sensitivity to acetylcholine. There was a highly significant decrease in sensitivity to morphine of ilia from pretreated mice, but this also was not associated with any alteration in sensitivity to acetylcholine. It is concluded that on the isolated ileum preparation the development of tolerance to morphine is not associated with an increased sensitivity to the transmitter whose release morphine inhibits.

Morphine and pharmacologically related drugs suppress the release of acetylcholine from some postganglionic parasympathetic nerve endings (Paton, 1957). This peripheral site of action has been used as a model of morphine's central activity. The relative potencies of morphine analogues (Harris, 1970), and the ratios of agonist to antagonist activities (Kosterlitz & Watt, 1968) found with this model agree with those found for analgesia in man.

The possibility that the transmurally stimulated guinea-pig ileum might also act as a model for narcotic tolerance and physical dependence has been explored by Fennessy, Heimans & Rand (1969). Tachyphylaxis to morphine could be induced *in vitro* and after a time the presence of morphine in the tissue bath was necessary for electrical stimulation to elicit a contraction. Fennessy and his colleagues termed this 'dependence'.

In 1966, Collier suggested that the primary event of central narcotic action is depression of transmitter release (using the peripheral mechanism as his precedent), that tolerance is due to induction of receptors to the transmitter, and that physical withdrawal symptoms are due to an exaggerated response to the transmitter when its release returns to normal.

Attempts to show differences between normal ileum and that removed from morphine-pretreated guinea-pigs have generally been unsuccessful (e.g. Fennessy & others, 1969), but it should be noted that in the guinea-pig tolerance develops very slowly to the action of morphine (Seevers & Deneau, 1963). We have therefore investigated the drug-sensitivity of ilia removed from guinea-pigs that had received

an intense morphine pretreatment course. A similar study with the mouse is also reported since this is a species in which we have found a considerable tolerance to morphine develops quickly.

METHODS

Male guinea-pigs (500–600 g) and male and female mice (50–60 g) were used.

The morphine pretreatment course in mice and guinea-pigs. The pretreatment course was identical in both species. Initially 3 subcutaneous injections of morphine hydrochloride were given each day commencing with a dose of 10 mg/kg, then increasing on the third injection of each day until a dose of 500 mg/kg had been reached (within 10 days). After this, 1 g/kg daily was given in 2 divided doses at 09.00 and 17.00 h until the day of challenge which occurred after 28 to 36 injections for guinea-pigs, and 40 to 54 injections for mice.

In vivo *assessment of tolerance in mice.* Groups of not less than 8 mice that had been fasted for 24 h were given 0·2 ml of a suspension of charcoal orally and 45 min later were killed. The maximum distance the charcoal travelled was expressed as a percentage of the length of the dissected tract (Keranen, Zaratzian & Coleman, 1961). Mice were given an intraperitoneal injection of morphine, 50 mg/kg, 15 min before the charcoal meal. The effects of this dose of morphine was also examined in mice that had been pretreated with morphine (27 injections).

The tolerance development to the depressant effect of morphine upon respiratory rate was also examined. Respiratory rate was measured 20 min after each of 3 daily intraperitoneal injections of morphine, 100 mg/kg. The experiment was continued for a total of 6 injections.

The effect of drugs and electrical stimulation on the isolated ileum. Sections of ileum were usually taken from animals 17 h after the last morphine injection, though in a few experiments this time interval varied between 7 and 18 h. Guinea-pig ileum was transmurally stimulated using supra-maximal field stimulation (15–20 Hz, 20 V and 0·3 ms width) applied from stainless steel electrodes, one of which was situated in the lumen, for periods of 5 s at 30 s intervals. Changes in tension were recorded potentiometrically using a mechano-electrical transducer (Ether UFI 2 oz).

A 6 cm length of mouse ileum was similarly set up for field stimulation. On both preparations the contact time for spasmogens was 30 s, and the effects of drugs on transmural stimulation were examined until equilibrium had been reached, or spontaneous recovery occurred (usually about 2 min).

Drugs. Acetylcholine chloride (Laboratoires Lematte et Boinot); histamine phosphate (BDH); morphine sulphate (B.P. Injection, BDH). All *in vivo* doses are expressed in terms of the above salts/kg body weight (unless otherwise stated). All *in vitro* final bath concentrations are in terms of the salt.

RESULTS

Observations on the pretreatment course

Mice. After 7 days pretreatment there was little indication of a sedative effect of morphine, and as the schedule progressed the mice showed increasing excitation after injection. The Straub tail response was much in evidence throughout.

Physical withdrawal symptoms were seen before the morning injection after 14 days pretreatment. The symptoms were diarrhoea, agitation, and after 3 weeks, tremor. These symptoms were quickly quelled by injection of morphine.

During the pretreatment period there was a fall in mean body weight from 54 to 31 g.

The mortality rate during the course was about 5%. Death followed prolonged convulsions. Gross post mortem examination of the injection site showed neither tissue damage nor infection.

When the abdominal cavity was opened before removal of the ileum there was little evidence of constipation.

Guinea-pigs. Despite the massive doses of morphine given, the drug had negligible sedative effects either initially or towards the end of the pretreatment course.

No evidence of physical withdrawal symptoms were observed at any stage.

During the pretreatment course there was a fall in mean body weight from 561 to 487 g.

Pretreated animals showed spectacular constipation.

In vivo *assessment of tolerance in mice.* On opening the abdominal cavity of a control mouse given morphine the normal spontaneous activity of the intestine was absent, though in a morphine-pretreated mouse there was some movement.

The effect of morphine on intestinal propulsion in control and pretreated groups of mice gave values (mean % $\pm$ s.e. of 8 readings) of 78 $\pm$ 1, 5 $\pm$ 2, 28 $\pm$ 2 for control, control given morphine and morphine-pretreated mice respectively. Thus morphine caused a marked inhibition of gastrointestinal propulsion in the control group. The same dose given to the pretreated group produced a significant depression of propulsion but this was significantly less than in the control group ($P < 0{\cdot}001$).

Mice treated with morphine, 100 mg/kg 3 times a day showed significant tolerance to its depressant action on respiratory rate by the third injection ($P < 0{\cdot}02$). 20 min after the sixth injection of morphine, respiratory rate was not significantly different from saline controls ($P > 0{\cdot}7$).

The effects of drugs and electrical stimulation on the isolated ileum from normal and from morphine-pretreated guinea-pigs. Whilst there appeared to be a little more spontaneous movement *in vitro* of ileum removed from pretreated animals, this was not marked, and had usually waned after the preparation had been in use for about 1 h.

The log frequency response relation of pretreated preparations lay to the left of that obtained with normal preparations, but this was not significant at any of the frequencies examined ($P > 0{\cdot}05$). The supramaximal frequency used in subsequent experiments was the same in both groups of preparations.

Fig. 1 shows log concentration effect lines for the inhibitory effect of morphine upon the responses to transmural stimulation of ilia from normal and morphine-pretreated animals. Because the concentration regimens investigated within each group differed, the log concentration effect relation was determined by the method of least squares. Ilia from pretreated animals were significantly less sensitive to the effects of morphine than ilia from normal animals ($P < 0{\cdot}005$). The log concentration effect line was shifted to the left without deviation from parallelism ($P > 0{\cdot}8$).

When the sensitivity of the two groups of preparations to acetylcholine was measured, there was no significant difference between the mean tensions at any of the

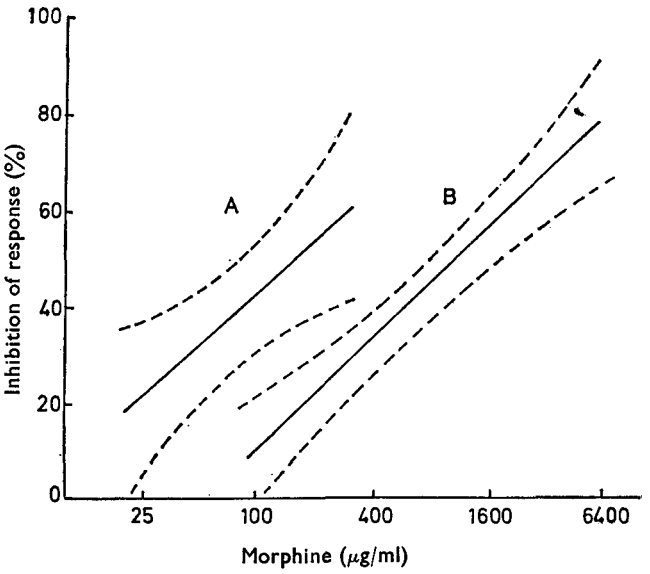

FIG. 1. The effect of morphine upon contractions elicited by electrical stimulation of guinea-pig ileum removed from normal animals (A), and morphine pretreated animals (B). The log concentration effect lines were fitted by the method of least squares. The broken lines represent the 95% confidence limits.

concentrations examined ($P > 0{\cdot}2$). Similarly there was no significant difference in the effects of histamine between the two groups ($P > 0{\cdot}2$).

Cumulative additions of morphine to normal preparations usually produced a progressive depression of response to electrical stimulation to 100% inhibition. In very few instances was tachyphylaxis seen, but on those occasions when further addition of morphine failed to cause further depression of response, the sensitivity of the preparation to spasmogens was unaltered.

The effects of drugs and electrical stimulation on the isolated ileum from normal and morphine-pretreated mice. There was marginally more spontaneous activity of ileum from pretreated mice than that seen in normal preparations, but this was unimpressive. In preparations from pretreated mice the log frequency response relation lay to the left of controls, but this was only significant at 1 of the 5 frequencies examined—5 Hz ($0{\cdot}05 > P > 0{\cdot}025$).

Log concentration effect relations for morphine upon the responses to transmural stimulation of ileum from normal and pretreated mice are shown in Fig. 2. Ileum removed from pretreated mice were significantly less sensitive to the depressant effect of morphine ($P < 0{\cdot}001$), though there was no significant difference in slope ($P > 0{\cdot}05$).

Log concentration effect curves for acetylcholine, 20–640 μg/ml, in the two groups revealed that whilst the pretreated ileum appeared more sensitive to acetylcholine than the control, this was not significant at any of the concentrations investigated ($P > 0{\cdot}2$).

DISCUSSION

The animals were pretreated with morphine in high doses at frequent intervals for a long time, and it is unlikely that they could have been rendered more tolerant.

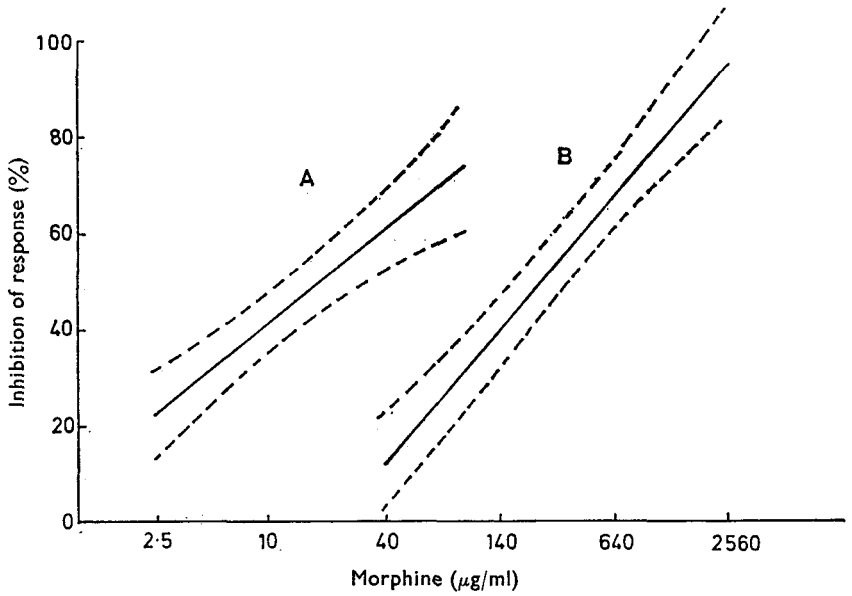

FIG. 2. The effect of morphine upon contractions elicited by electrical stimulation of mouse ileum removed from normal animals (A), and morphine-pretreated animals (B). The log concentration effect lines were fitted by the method of least squares. The broken lines are the 95% confidence limits.

Pretreatment terminated with at least 14 days of twice daily injections of 500 mg/kg—the LD50 in mice (Barnes & Eltherington, 1964). Two unsuccessful attempts were made to render the animals more tolerant. Firstly a third daily injection of 500 mg/kg morphine was introduced at mid-day but this caused convulsions. Since increasing the morphine dose was limited by the water solubility of morphine hydrochloride, a second approach was to switch the pretreatment drug to heroin hydrochloride which is more potent on a weight for weight basis, is more water-soluble, and to which the animals would have been cross tolerant. Whilst guinea-pigs survived the exchange from 500 mg/kg morphine to 200 mg/kg heroin, when this latter dose was doubled, convulsions occurred. Since little tolerance develops to the stimulant effects of morphine (Foldes, Swerdlow & Siker, 1964) further increases of the dose might not have been possible.

Our pretreatment course was more drastic than that used by others. For instance, Fennessy & others (1969) used a pretreatment schedule commencing at 25 mg/kg morphine sulphate, then rising over a period of 20 days to daily intraperitoneal injections of 200 mg/kg. These authors were unable to detect any alteration in the sensitivity of guinea-pig ileum to morphine. With our more drastic pretreatment a significant alteration in morphine sensitivity was seen. But tolerance development was not associated with any alteration in sensitivity to acetylcholine or histamine.

Tachyphylaxis to morphine is well documented (e.g. Paton, 1957) and one of us has often observed the phenomenon when investigating the effect of morphine upon responses of the transmurally stimulated guinea-pig ileum recorded isotonically. In the present experiments however, on the few instances on which tachyphylaxis was seen, this was not accompanied by any alteration in sensitivity to acetylcholine.

Guinea-pigs do not develop tolerance to morphine quickly (Seevers & Deneau,

1963). For this reason the experiments were repeated using mice—a species in which a very rapid development of tolerance occurs.

The fact that some degree of tolerance also developed to the effects of morphine on intestinal propulsion is an unusual observation, since tolerance to this action is not usually considered to occur (Foldes & others, 1964). The reason for this might be in terms both of the massive doses used in the pretreatment course, and that intestinal propulsion, rather than the more gross measure of constipation was assessed.

The mouse ileum is sensitive to acetylcholine and to field stimulation, but only to pulse trains, not to single impulses—hence the use of pulse trains in these experiments. Such contractions are inhibited by atropine (2 ng/ml), personal observation.

There was a highly significant difference in the sensitivity to morphine of ilia removed from normal and from morphine-pretreated mice. This depression of morphine sensitivity was not associated with any alteration in sensitivity to acetylcholine.

Amongst the many theories advanced to explain tolerance to narcotic analgesics is that of Collier (1966) who suggested that tolerance is due to an increased sensitivity of the post-synaptic membrane to the synaptic transmitter. Whilst extrapolation of the mechanism from postganglionic parasympathetic neuroeffector junctions to synapses in the central nervous system might be unwise, we can conclude that at the one site where morphine's mechanism of action is relatively well understood, the development of tolerance is not associated with an increase in sensitivity to the transmitter whose release morphine inhibits.

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