J. Pharm. Pharmacol. 1983, 35: 54–56
Communicated May 19, 1982

0022–3573/83/010054–03 $02.50/0

The failure of morphine to depress selectively non-adrenergic neural inhibition of the guinea-pig taenia caeci

R. C. SMALL*, V. W. YONG, *Department of Pharmacology, Materia Medica and Therapeutics, University of Manchester, M13 9PT, U.K.*

Tetrodotoxin and some other toxins of animal origin can interrupt the process of non-adrenergic inhibitory neuroeffector transmission in the guinea-pig taenia caeci. Apart from these toxins, few agents are known which can modify the transmission process without altering the tone of the preparation. The claim that morphine might be such an agent (Shimo & Ishii 1978) was therefore of great interest.

The present experiments were carried out in the hope that we would be able to confirm the observations of Shimo & Ishii (1978) and then perhaps to extend them by determining which of the opiate receptors (Kosterlitz 1980) was involved in this action of morphine. Our initial experiments were carried out in Krebs solution and we were surprised to find that morphine lacked an action against the non-adrenergic inhibitory responses of the taenia to field stimulation in this medium. We now report the failure of morphine to affect such responses even when the experimental conditions of the original authors are closely mimicked and when concurrent experiments with the transmurally stimulated ileum demonstrate the effectiveness of morphine against cholinergic transmission in that tissue.

Materials and methods

Tricoloured guinea-pigs (David Lewis colony) or albino animals (Dunkin Hartley strain) of either sex, weight 200–400 g, were killed by stunning and bleeding. Isolated tissues were mounted in Krebs solution (Small & Weston 1979) or Tyrode solution (Shimo & Ishii 1978) maintained at 37·5 °C and gassed with 5% CO_2 in O_2.

Taenia caeci was set up in either of the salt solutions containing 0·2 μM atropine and 5 μM guanethidine. Each segment of taenia was arranged for the stimulation of intramural nerves and the recording of tissue length changes as described by Shimo & Ishii (1978). Field stimulation was carried out using pulses of width 0·3 ms and maximal voltage. In some experiments these pulses were delivered at frequencies of 0·5 or 1 Hz in trains of 4 s duration every 3 min. In other experiments the taenia was stimulated continuously at a pulse frequency of 0·02 Hz.

Ileum was routinely taken from the same animals which provided taenia caeci. Ileal segments were set up in either of the salt solutions but no atropine or guanethidine was added. Transmural stimulation of ileum was carried out as described by Small & Weston (1979) except that tissue length changes were measured using isotonic transducers and an applied load of 1 g.

Acute concentration/effect curves for morphine were constructed on each piece of ileum using tenfold concentration increments. Each concentration of morphine was allowed 2 min contact before washout, and the tissue was subsequently challenged with morphine only when the twitch height had returned to the previous control value or showed no further improvement. This required a minimum of 10 min. Morphine's action on the ileum was measured as the maximal percentage reduction in twitch height occurring during each drug exposure.

The construction of acute concentration/effect curves for morphine was also attempted in the taenia using tenfold concentration increments. When pulse trains were used the drug contact time was 9 min, but when continuous stimulation was used the contact time was 3 min. In either case the effect of morphine was assessed by measuring the three control relaxations evoked immediately before morphine and the first three test relaxations evoked in the presence of morphine (Fig. 1). A mean value was calculated for both the control and test relaxations. The mean test relaxation was then expressed as a percentage of the mean control relaxation for each concentration of morphine used.

In experiments with tetrodotoxin, cumulative concentration/effect curves for noradrenaline and ATP were constructed (Small & Weston 1979) both before,

Table 1. Effects of morphine on relaxations evoked by field stimulation of the non-adrenergic inhibitory nerves of the taenia caeci. The data represent the means of responses obtained from tissues from 7 animals, where response signifies the relaxation observed in the presence of morphine expressed as a percentage of the control value before morphine. Within each horizontal row of data, analysis of variance revealed no significant differences ($P > 0·05$) between means. * indicates the use of pulse trains.

Pulse frequency Hz	Mean response (% of control) Concentration of morphine 1 nM	10 nM	100 nM	1 μM	10 μM
Krebs solution					
0·02	105·5	98·1	101·4	86·7	86·3
0·5*	104·2	107·1	96·3	98·7	100·2
1·0*	100·5	107·5	102·6	112·1	101·8
Tyrode solution					
0·02	98·7	101·4	100	94·7	95·3
0·5*	104·7	106·4	114·3	103·5	93·4
1·0*	107·9	103·5	129·3	100·5	90

* Correspondence.

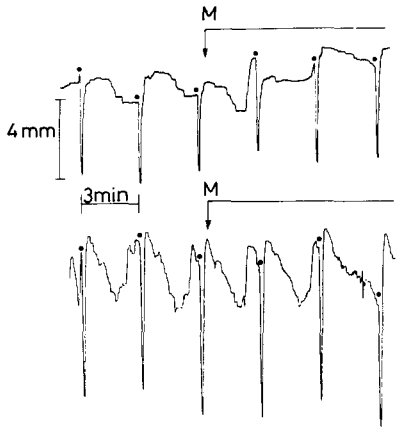

FIG. 1. Atropine- and guanethidine-treated guinea-pig taenia caeci: failure of morphine 1 μM (added at arrow) to suppress the relaxations evoked by field stimulation (●) using pulse trains of 4 s duration every 3 min. Pulse frequency 0·5 Hz. Calibration of time axis × 3 min. Calibration of tissue length change axis = 4 mm. Upper trace = Krebs-bathed tissue. Lower trace = Tyrode-bathed tissue.

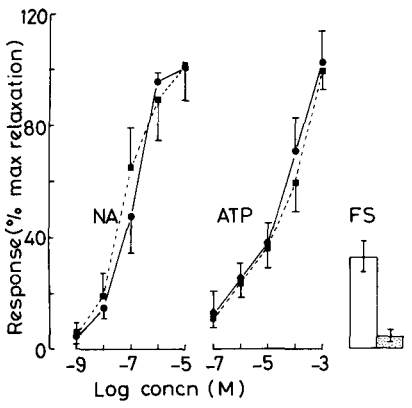

FIG. 2. Tyrode-bathed taenia caeci: effect of 0·3 μM tetrodotoxin on relaxant responses to noradrenaline (NA), ATP and field stimulation (FS) using pulse trains and a pulse frequency of 1 Hz. ●——● and unstippled column represent control responses. ■- - - -■ and stippled column represent responses in presence of tetrodotoxin. Each point represents the mean of responses from 7 tissues ± s.e.

and in the presence of the toxin. Equilibration of toxin with the tissue was observed by adding the toxin while the tissue was stimulated using pulse trains as described above and a pulse frequency of 1 Hz.

Drug concentrations are throughout expressed in terms of the molar concentration of base. Drugs used were atropine sulphate (Sigma), the disodium salt of ATP (Sigma), guanethidine sulphate (Ciba), morphine hydrochloride (McFarlan-Smith), (−)-noradrenaline bitartrate (Koch Light), tetrodotoxin (Sigma). Dilutions of noradrenaline were prepared from a stock solution in 0·1 M HCl on the day of use. Each noradrenaline dilution contained 1 mM ascorbic acid as an antioxidant. Dilutions of ATP were prepared by dissolving the solid in distilled water immediately before use.

Results

Experiments with transmurally stimulated ileum. Tissues in Tyrode solution often required a prolonged period of incubation before beginning to twitch in response to each applied pulse. Such twitches were almost always smaller than those observed in Krebs solution and were sometimes complicated by spontaneous changes in the resting length of the tissue.

Morphine (1 nM–10 μM) caused a concentration-dependent depression of ileal twitches, complete twitch abolition occurring with adequate drug concentration in all tissues. The mean $\log_{10}$ IC50 ± standard error for morphine on Tyrode-bathed tissues was −8·34 ± 0·08 (n = 13) whilst that for Krebs-bathed tissues was −7·36 ± 0·09 (n = 21). Morphine was significantly ($2P << 0{\cdot}05$, unpaired *t*-test) more potent on Tyrode-bathed tissues.

Concentration/effect studies with morphine in taenia caeci. Segments of taenia mounted either in Tyrode or Krebs solution exhibited myogenic tone and field stimulation elicited relaxations which were sometimes followed by a secondary contraction. In many preparations it was evident that the amplitude of the evoked relaxation was dependent on the tone of the preparation at the instant of stimulation.

Concentrations of morphine (1nM–10 μM) demonstrably effective against twitches of ileum from the same animal were apparently without effect on the relaxations of the taenia evoked by the trains of pulses described by Shimo & Ishii (1978). Morphine was also without effect on the relaxations evoked by subjecting the taenia to continuous stimulation at a pulse frequency of 0·02 Hz. Morphine's lack of acitivity against relaxations evoked by single pulses or pulse trains was seen both in tissues bathed by Krebs solution and in those bathed by Tyrode solution (Table 1).

Studies with morphine-naive taenia. The effects of 1 μM morphine were examined on morphine-naive taenia stimulated using the pulse trains described above and a pulse frequency of 1 Hz. The data (mean response % of control) show that morphine did not depress the relaxations (Krebs 113·6 ± 8·4, Tyrode 110·5 ± 7·1) evoked by field stimulation and that its effects were not significantly different from those of a vehicle control (Krebs 103·3 ± 3·3, Tyrode 104·2 ± 4·7). This held true whether the tissue was bathed by Krebs solution or by Tyrode solution.

Studies with tetrodotoxin. Fig. 2 shows that 0·3 μM tetrodotoxin markedly reduced responses of Tyrode-bathed taenia to field stimulation without modifying the relaxant actions of noradrenaline or ATP. A similar result was obtained for tissues bathed by Krebs solution.

Discussion

Our experiments have failed to confirm the report by Shimo & Ishii (1978) that morphine can depress the relaxations evoked by stimulation of non-adrenergic inhibitory nerves in the taenia caeci without modifying tissue tone.

The explanation for this discrepancy is not that our sample of morphine lacked characteristic pharmacological activity. In experiments with the transmurally stimulated ileum we were always able to demonstrate the well-documented inhibitory action of morphine against ileal twitches. Furthermore, our estimates of the IC50 for morphine are comparable to those reported by earlier workers (Paton 1957; Cox & Weinstock 1966; Lord et al 1977).

The difference between our results and those of Shimo & Ishii (1978) is unlikely to be a consequence of their reported inverse relationship between the effectiveness of morphine in the taenia and the pulse frequency. We used not only the stimulus parameters suggested by the original authors but also attempted, without success, to demonstrate an effect of morphine against responses to single pulses of lower frequency.

The development of acute tolerance can be a problem when attempting to observe the relationship between concentration and effect for morphine in the transmurally stimulated ileum (Paton 1957). Some evidence of acute tolerance was obtained in our ileal experiments in that morphine's effect often reached a peak and began a decline within the 2 min contact period. The illustration of Shimo & Ishii (1978) indicates that acute tolerance was not a prominent feature of their reported action of morphine on the taenia. In that illustration morphine is shown to cause a well-sustained (in excess of 12 min) depression of relaxations to field stimulation. Furthermore our studies using 1 μM morphine on morphine-naive tissues would seem to rule out the possibility that acute tolerance was responsible for our failure to detect an action of morphine on the taenia.

Our electrode design and parameters used for field stimulation of the taenia were essentially similar to those employed by Shimo & Ishii (1978). Despite this we wondered whether the relaxations of the taenia seen in our experiments might be due to a direct effect of the voltage field on the muscle cells and might be resistant to morphine for that reason. Two pieces of evidence argue against this. Firstly, stimulation at an appropriately high voltage evoked immediate contraction. This implies that the direct effect of field stimulation on the muscle cells is excitatory rather than inhibitory. Secondly, the relaxant response to field stimulation which we observed was profoundly reduced by a concentration of tetrodotoxin which did not modify the relaxant actions of noradrenaline or ATP. Evidence of this kind strongly suggests that we were observing a phenomenon which was dependent on Na^+ channel activation and was therefore a neurogenic rather than a direct myogenic response to the stimulus.

Our ileal experiments showed that ileum is more sensitive to morphine when bathed by Tyrode than when bathed by Krebs solution. No such difference in sensitivity was seen in the corresponding taenia experiments. Where Tyrode-bathed taenia was used our experimental conditions mimicked those of Shimo & Ishii (1978) in almost every respect. However, the original authors used tissue only from male animals while we used tissues from animals of either sex. Nevertheless examination of our records revealed no sex-based difference in the sensitivity of taeniae to morphine.

Despite our attempts to display any action of morphine on the taenia to its best advantage and closely mimicking the conditions of the original authors, we have failed to detect their reported action of morphine. Moreover we have been unable to pinpoint a methodological difference which might account for this. Since the results of independent studies in other laboratories (Coates, J., Spedding, M. & Weetman, D. F., personal communication, 1981) also indicate that morphine is unable selectively to depress non-adrenergic inhibitory neuroeffector transmission in the taenia, we are left wondering whether Shimo & Ishii (1978) may have worked with a strain of animal whose taeniae are uniquely sensitive to morphine. Strain-linked differences in the sensitivity of tissues to morphine are not unknown for Henderson & Hughes (1976) have reported such differences for the mouse vas deferens preparation.

Supported in part by a grant from the Wellcome Trust. We thank Mr R. Richards for expert technical assistance.

REFERENCES

Cox, B. M., Weinstock, M. (1966) Br. J. Pharmacol. Chemother. 27: 81–92

Henderson, G., Hughes, J. (1976) Ibid. 57: 551–557

Kosterlitz, H. W. (1980) in: Turner, P. (ed.) Proceedings of the First World Conference on Clinical Pharmacology and Therapeutics, Macmillan, London, pp 33–48

Lord, A. H., Waterfield, A. A., Hughes, J., Kosterlitz, H. W. (1977) Nature (London) 267: 495–499

Paton, W. D. M. (1957) Br. J. Pharmacol. Chemother. 12: 119–127

Shimo, Y., Ishii, T. (1978) J. Pharm. Pharmacol. 30: 596–597

Small, R. C., Weston, A. H. (1979) Br. J. Pharmacol. 67: 301–308