J. Pharm. Pharmacol. 1986, 38: 24–27
Received July 26, 1985

Can the effects of meptazinol on the guinea-pig isolated ileum be explained by inhibition of acetylcholinesterase?

CHRISTINE ENNIS*, FAIZAL HAROUN AND NORMAN LATTIMER

Department of Pharmacology, Wyeth Research (UK), Huntercombe Lane South, Taplow, Maidenhead, Berks, UK

It has previously been shown that there is a cholinergic component in the antinociceptive action of the opioid analgesic drug meptazinol. In the present study meptazinol was shown to be an inhibitor of acetylcholinesterase in-vitro with a potency one hundredth that of physostigmine. This activity was found to reside only in the (−)-enantiomer of meptazinol. The anticholinesterase activity of meptazinol may explain the increase in the size of the electrically-evoked contraction of the guinea-pig isolated ileum preparation since by using a long pulse width (5 ms) it was found that the (−)-enantiomer of meptazinol modified only the component of the response due to neuronally released acetylcholine and had no direct effect on the smooth muscle. This property of meptazinol may also be responsible for the cholinergic effects of the drug in-vivo.

The new analgesic agent, meptazinol, is classified as an opioid drug because it has been shown to displace [^{3}H]naloxone from specific μ binding sites in rat brain in low concentrations (Blurton et al 1982), has an antinociceptive action that is antagonized by naloxone (Stephens et al 1978) and is structurally related to established opioid analgesic drugs (e.g. pethidine and profadol). Meptazinol, however, has been found to possess several properties which appear to involve cholinergic mechanisms that are not shared by other analgesics. Thus the antinociceptive effect of meptazinol, unlike that of other opioid analgesic drugs, is antagonized markedly by low doses of hyoscine (scopolamine) (Bill et al 1983) and large doses of meptazinol induce overt signs of parasympathetic stimulation, i.e. salivation and tremor. Furthermore, the action of meptazinol on the guinea-pig isolated ileum preparation, a tissue of established importance in the study of opioid receptor pharmacology, is not typical of opioid drugs in that meptazinol has been found to increase the size of the twitch response of this tissue to electrical stimulation (Stephens et al 1978). Recently, this effect has been shown to be associated selectively with the (−)-enantiomer of meptazinol, whereas the (+)-enantiomer behaved like a typical opioid agonist (Duchesne et al 1984).

The precise nature of this cholinergic component of the pharmacological profile of meptazinol is unclear. In view of the similarity between the effects of meptazinol on the guinea-pig ileum with that reported for inhibitors of acetylcholinesterase (Cox & Stobart 1972) it was decided to compare the effects of meptazinol directly with those of physostigmine on this preparation. Also, although an early indirect assessment (Stephens et al 1978) failed to demonstrate evidence of anticholinesterase activity, the possibility that meptazinol might possess this property was re-investigated using a colorimetric method to measure cholinesterase activity directly in-vitro.

* Correspondence.

METHODS

Guinea-pig ileum

Male Tuck guinea-pigs (250–300 g) were killed by cervical dislocation and a length of ileum removed discarding the 10 cm proximal to the caecum. A strip of ileum, 2 cm in length, was suspended in a 6 ml organ bath containing Krebs bicarbonate solution of the following composition (mM); NaCl 118·0; KCl 4·7; $CaCl_2$ 2·5; $MgSO_4$ 1·2, KH_2PO_4 1·2, glucose 11·0, $NaHCO_3$ 25·0; maintained at 37 °C and aerated with 95% O_2 and 5% CO_2. The contractions were measured by means of isotonic transducers (Ealing) under a 1 g resting tension. The strips of ileum were washed with Krebs solution at 5 min intervals and were left to equilibrate for 30 min before the start of stimulation. The tissues were stimulated at 10 min intervals for 16 s at 0·5 Hz with a pulse width of 5 ms at 100 mA current and responses were recorded on a Servogar 220 pen recorder.

The agonist drugs under investigation were added directly to the organ bath 90 s before the start of stimulation. The bathing fluid was changed after each period of stimulation. Antagonists were added

to the Krebs reservoir and left to equilibrate with the tissue for 1 h. Concentration-effect curves to the agonists were constructed in the absence and presence of the antagonists and the results calculated as percentage change from the control contraction before addition of agonists.

Acetylcholinesterase assay

The method used to determine acetylcholinesterase activity was a modification of that originally described by Rappaport et al (1959). It is based on the colour change of an acid-base indicator, *m*-nitrophenol, to measure the amount of acetic acid produced by the enzymatic hydrolysis of acetylcholine (ACh). The colour change of *m*-nitrophenol is linearly proportional to the cholinesterase activity present in the sample. The final incubation volume was 2·8 ml and consisted of 1 u ml^{-1} acetylcholinesterase from bovine erythrocytes (E.C. 3.1.7), *m*-nitrophenol (2·0 nM) in 0·1 M sodium phosphate buffer, test drug solution in appropriate concentration and ACh (30 mM). The reaction was initiated by the addition of ACh and the samples were incubated at 37 °C for exactly 30 min. The samples were then filtered and the absorbance (A) of *m*-nitrophenol in each sample was read at a wavelength of 420 nm in a spectrophotometer using water as a reference blank. An enzyme blank was prepared from denatured enzyme obtained by incubating the enzyme at 60 °C for 10 min. A calibration curve was also prepared using denatured enzyme and known amounts of acetic acid ranging from 0·4 to 20 nM. All determinations were performed in duplicate.

The change in absorbance (ΔA) from the enzyme blank was calculated for each sample in the calibration curve and a plot of ΔA against cholinesterase activity (u ml^{-1}) prepared. The test samples were treated identically and from the ΔA value the cholinesterase activity in each sample was read directly from the calibration curve. A sample containing no inhibitor was included to establish 100% enzyme activity and the percentage inhibition of this activity calculated for each drug concentration.

All drugs were dissolved in distilled water and dilutions prepared using Krebs solution. All drug solutions were neutralized as far as possible before the acetylcholinesterase assay.

Statistical analysis of results was performed using Student's *t*-test.

Drugs used: Acetylcholine chloride (Sigma), acetylcholinesterase from bovine erythrocytes (Sigma), atropine sulphate (Sigma), buprenorphine HCl (Reckitt and Colman), meptazinol HCl and (±)-enantiomers (Wyeth), morphine sulphate (Macfarlan-Smith), naloxone HCl (Endo Labs), *m*-nitrophenol (Sigma), pentazocine base (Winthrop), pethidine HCl (Macfarlan-Smith), profadol HCl (Parke-Davis).

RESULTS

Guinea-pig ileum

Electrical stimulation of the guinea-pig isolated ileum preparation using a pulse width of 5 ms evoked contractions of the ileum which were stable for up to 5 h. The contractions were reduced in the presence of atropine by a maximum of 66% of control. Morphine and the (+)-enantiomer of meptazinol inhibited the contraction of the ileum by a maximum of 60 and 40%, respectively (Fig. 1). The IC50 value for morphine was 0·23 ± 0·05 μM (n = 10). This inhibition was blocked by naloxone (3 μM). In contrast, meptazinol, the (−)-enantiomer of meptazinol and physostigmine, produced a concentration-related increase in the size of the electrically-evoked contraction (the concentrations required to increase the size of the contraction by 50% (EC50) were 0·78 ± 0·08, 0·39 ± 0·05 and 0·075 ± 0·008 μM, respectively) (Fig. 1). Atropine (0·3 μM) produced a rightward shift (5-fold) in the concentration-effect curve to all three compounds (Fig. 1). Naloxone (3 μM) had no effect on the response produced by meptazinol, (−)-meptazinol or physostigmine. In concentrations approximately ten-fold greater than the EC50 values, all three agents produced a contraction of the ileum in the absence of electrical

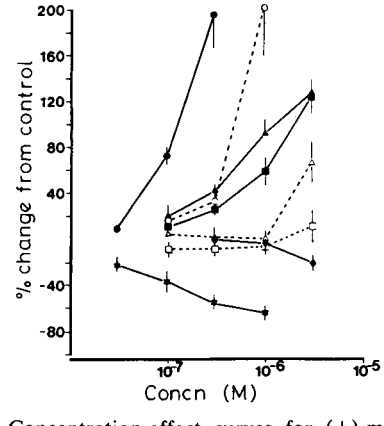

FIG. 1. Concentration-effect curves for (±)-meptazinol (■), (−)-meptazinol (▲), (+)-meptazinol (◆), physostigmine (●) and morphine (★) on the electrically stimulated guinea-pig isolated ileum preparation. Closed symbols represent the response to these agents in the absence and open symbols the response in the presence of atropine (3×10^{-7} M). Vertical bars show the standard error of the mean, n = 3–6 determinations.

stimulation. These compounds did not modify contractions produced by exogenous ACh (0·1 μM).

Acetylcholinesterase assay

Meptazinol (0·1 to 1000 μM) produced a concentration-related inhibition of enzyme activity. The IC50 (concentration producing 50% inhibition) was 6·4 ± 2·5 μM (n = 3). The (−)-enantiomer of meptazinol was also an effective inhibitor of the enzyme (IC50 3·3 ± 1·2 μM), but the (+)-enantiomer was much less potent producing only 30% inhibition at 1·0 mM. Physostigmine was effective over the concentration range 1·0 nM to 100 μM with an IC50 of 67 ± 11 nM (Fig. 2). Of a range of opioid agonists and antagonists tested, only pentazocine showed any significant activity, producing 37% inhibition at 1·0 mM. Morphine, buprenorphine, pethidine, profadol and naloxone were all virtually inactive at 1·0 nM (18, 12, 16, 7 and 6% inhibition, respectively).

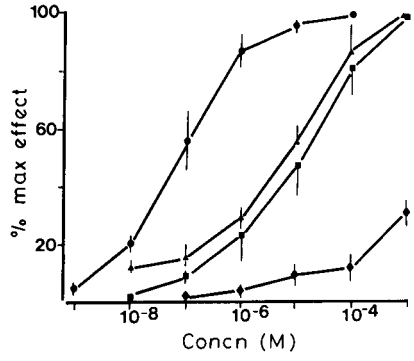

FIG. 2. Concentration effect curves for the inhibition of acetylcholinesterase activity produced by physostigmine (●), (±)-meptazinol (■), (−)-meptazinol (▲) and (+)-meptazinol (◆). Each point is the mean of at least 3 determinations performed in duplicate. Vertical bars represent the standard error of the mean.

DISCUSSION

When the isolated ileum preparation is electrically stimulated with a conventional short pulse width of 0·5 ms, the contraction produced is completely blocked by atropine, implying that the response is entirely due to the release of ACh (Wikberg 1977). In contrast, the contraction produced by a longer pulse width (5 ms) was only partially reduced by atropine since a component of the contraction is due to direct electrical stimulation of the smooth muscle in addition to that due to the release of ACh. Thus, by using a longer pulse width in the absence and presence of atropine it was possible to distinguish between pre- and post-synaptic effects of drugs.

Using this technique it was found that meptazinol and the (−)-enantiomer of meptazinol increased the size of the contraction of the guinea-pig ileum to electrical stimulation in the same way as physostigmine. The effect of all three compounds was blocked by atropine indicating that they modified only the component of the contraction due to ACh release and had no direct effect on the smooth muscle except at high concentrations. The opiate antagonist, naloxone had no effect on the response to any of these three compounds. In contrast, the (+)-enantiomer of meptazinol and morphine both produced a naloxone-sensitive inhibition of the electrically-evoked contraction which is an effect consistent with opioid agonist activity.

The response to exogenous ACh was unaffected by either physostigmine or (−)-meptazinol. This finding is difficult to explain but may be due to the fact that electrical stimulation of the ileum produces ACh release predominantly from the myenteric plexus (Paton & Zar 1965) an area which is rich in acetylcholinesterases (Ambache et al 1969). Exogenous ACh may act at a different site to the neuronally released ACh and may therefore be less susceptible to the action of acetylcholinesterase

In view of the similarity between the response to the (−)-enantiomer of meptazinol and to physostigmine, an investigation was made into the possibility that (−)-meptazinol may act as an inhibitor of acetylcholinesterase. Using a simple in-vitro method for the determination of acetylcholinesterase activity it was shown that meptazinol did inhibit this enzyme. The concentration-inhibition curve to meptazinol was parallel to that obtained to physostigmine and both compounds produced complete inhibition of the enzyme. Although K_I values were not obtained, the fact that the concentration-inhibition curves were parallel allowed a rough estimate of the potency ratio between meptazinol and physostigmine to be made.

Thus, meptazinol was shown to be approximately one hundred times less potent than physostigmine. This activity of meptazinol appeared to be confined to the (−)-enantiomer since the (+)-enantiomer was much less effective, producing only 30% inhibition at the highest concentration tested (10^{-3} M). Furthermore, the (−)-enantiomer was twice as potent as the racemate in inhibiting the enzyme.

Thus, it is possible that many of the atypical effects of meptazinol and the (−)-enantiomer on the guinea-pig isolated ileum and also in the whole animal (Bill et al 1983) may be explained in terms of this ability to inhibit acetylcholinesterase.

The ability to inhibit acetylcholinesterase does not

appear to be a property common to all opioids since none of the other compounds of this class which were tested showed any marked activity. These results do not agree with those of Dewey et al (1969) who reported that high concentrations of morphine and pentazocine (50–500 μM) were effective in inhibiting acetylcholinesterase. However, these high concentrations are unlikely to have any relevance to the pharmacology of either compound in-vivo.

In conclusion, it has been shown that the (−)-enantiomer of meptazinol, possesses, in addition to its property as an opioid analgesic drug, the ability to inhibit acetylcholinesterase. This property of meptazinol may explain both the increase in the size of the electrically-evoked contraction of the guinea-pig ileum in-vitro and the signs of cholinergic stimulation which have been observed after administration of meptazinol in-vivo.

REFERENCES

Ambache, N., Freeman, M. A., Hobbiger, F. (1969) J. Physiol. London, 200: 50–51P

Bill, D. J., Hartley, J. E., Stephens, R. J., Thompson, A. M. (1983) Br. J. Pharmacol. 79: 191–200

Blurton, P. A., Fox, J., Wood, M. D., Wyllie, M. G. (1982) Ibid. 77: 508P

Cox, B., Stobart, G. (1972) J. Pharm. Pharmacol. 24: 828–829

Dewey, W. L., Harris, L. S., Howes, J. F., Kennedy, J. S., Nuite, K. A., Hayhurst, V. (1969) Arch. Int. Pharmacodyn. 182: 471–480

Duchesne, R. J., Goodall, J., Hughes, I. E. (1984) J. Pharm. Pharmacol. 36: 560–562

Paton, W. D. M., Zar, M. A. (1965) J. Physiol. London, 179: 85–86P

Rappaport, F., Fischl, J., Pinto, N. (1959) Clin. Chim. Acta 4: 227–230

Stephens, R. J., Waterfall, J. F., Franklin, R. A. (1978) Gen. Pharmacol. 9: 73–78

Wikberg, J. (1977) Acta. Phys. Scand. 99: 190–207