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Pharmacological effects of meptazinol and its enantiomers on guinea-pig ileum and mouse vas deferens

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Electrically induced twitch responses of mouse vas deferens and guinea-pig ileum were inhibited by morphine, normorphine and the peptide opioid agonist RX783006; naloxone blocked the effects of all three opioid agonists yielding K_e values which were not significantly different and which were within the range (1–4 nM) expected for μ-type receptors. At concentrations between 0·1 and 10 μM (+)-meptazinol inhibited the twitch response of the ileum while (−)-meptazinol produced potentiation. Naloxone (20 nM) completely blocked the effect of (+)-meptazinol and further increased the potentiation produced by (−)-meptazinol. In the mouse vas deferens preparation neither isomer affected the electrically induced twitch response at concentrations below 5 μM and higher concentrations (10–300 μM) produced potentiation. Naloxone (20 nM) did not modify this effect. It is concluded that both isomers are opioid agonists on the μ-receptors in guinea-pig ileum but not in mouse vas deferens and that a cholinergic component, which may contribute to the action of meptazinol in-vivo, is present to a smaller extent in the (+)-isomer.

The pharmacological profile of meptazinol in-vivo is similar in many respects to that of other opioid analgesics which have agonist and antagonist properties (Stevens et al 1978). In-vitro, however, there are some differences; whereas opioid analgesics generally inhibit the electrically induced twitch response of the guinea-pig isolated ileum, meptazinol produces potentiation. This may reflect a cholinergic component in the action of meptazinol which may contribute to the production of analgesia (Bensreti et al 1983; Bill et al 1983). Meptazinol also increases the release of noradrenaline from rat brain synaptosomes (Paciorek et al 1983), vas deferens and atria (Bill et al 1981). This may contribute to the ability of meptazinol to restore mean arterial blood pressure in endotoxic shock (Paciorek et al 1983) but, though adrenoceptor stimulation can produce an antinociceptive effect (Hayes et al 1984), any contribution to the analgesic action of meptazinol is unclear.

Meptazinol is a racemate and both enantiomers are said to possess analgesic activity (Goode & White 1971). It is possible however that all the above effects are not shown equally by both the (+)- and the (−)-isomers and this paper presents an investigation into this possibility in guinea-pig isolated ileum and mouse vas deferens.

Methods

Guinea-pig isolated field-stimulated ileum. Male guinea-pigs (400–600 g) were stunned and killed by cervical dislocation. A 2 cm portion of ileum taken from 10 cm above the ileo-caecal junction was removed, cleared of adherent tissue, and mounted between two 5 mm coils of platinum wire (one above and the other below the tissue) in physiological saline (NaCl 134, KCl 2·68, $CaCl_2$ 1·80, $MgSO_4$ 1·05, NaH_2PO_4 0·032, $NaHCO_3$ 11·9, glucose 5·5 mM; gassed with 5% carbon dioxide in oxygen and also containing mepyramine (0·1 μM) and hexamethonium (60 μM)). The temperature was maintained at 36 °C and changes in length of the tissue in response to electrical stimulation (40 V, 2 ms duration, 0·1 Hz) were recorded isotonically (load 0·5 g). Reproducible responses to electrical stimulation were established after about 1 h.

Mouse isolated field-stimulated vas deferens. Male mice (T.O. strain; 30–35 g) were stunned and killed by cervical dislocation. The whole vas deferens was removed, cleared of adherent tissue and mounted in physiological saline (NaCl 128, KCl 5·63, $CaCl_2$ 2·16, NaH_2PO_4 1·19, $NaHCO_3$ 25, glucose 11·1, sucrose 13·1 mM; gassed with 5% carbon dioxide in oxygen). Changes in length of the tissue in response to electrical stimulation (40 V, 2 ms duration 0·1 Hz) were recorded isotonically (load 150 mg). Reproducible responses were established after about 45 min.

Experimental procedure. Log_{10}-concentration-effect curves were established, allowing 15 min between additions of agonist which remained in contact with the tissue for 3 min. When appropriate, the antagonist naloxone was added to the physiological saline and allowed to remain in contact with the tissue for 20 min before agonists were added. IC50 values were taken as the molar concentration of agonist required to inhibit the response to electrical stimulation by 50%. K_e values (K_e = [antagonist concentration]/(dose ratio − 1)) were calculated as described by Kosterlitz & Watt (1968) and Hughes et al (1975).

Drugs used. Atropine sulphate (Sigma), hexamethonium bromide (Sigma), mepyramine maleate (Sigma), morphine sulphate (Boots), normorphine hydrochloride (Reckett & Colman) and naloxone hydrochloride (Sigma). (±)-Meptazinol hydrochloride and its enantiomers were supplied by Wyeth Pharmaceuticals. Tyr-D-Ala-Gly-MePhe-$NH(CH_2)_2OH$ (RX783006) was obtained from Cambridge Research Chemicals, dissolved in oxygen-free water and stored at −70 °C.

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Results

Guinea-pig isolated field-stimulated ileum. Exposure to the opioid agonists RX783006, morphine and normorphine inhibited the twitch response elicited by electrical stimulation and the concentrations required to produce a 50% inhibition are shown in Table 1. All three agonists were able to produce at least 90% inhibition of the twitch response at the highest concentrations tested. Naloxone (20 nM) produced the expected inhibition of this effect and yielded K_e values which were not significantly different whichever agonist was used ($P > 0{\cdot}4$).

Table 1. Showing IC50 values (nM) for 3 opioid agonists and K_e values (nM) for naloxone in mouse vas deferens (MVD) and guinea-pig ileum (GPI) responding to electrical stimulation. Values are means ± s.e. and the number of determination contributing to each mean is shown (n).

Agonist	Tissue	n	IC50 (nM)	K_e for naloxone (nM)
RX783006	GPI	4	4·93 ± 0·59	1·56 ± 0·53
	MVD	6	116·0 ± 15·8	2·46 ± 0·37
Morphine	GPI	4	2·50 ± 0·35	1·97 ± 0·63
	MVD	5	4·14 ± 0·50	1·31 ± 0·66
Normorphine	GPI	3	3·88 ± 1·07	2·30 ± 1·04
	MVD	3	1·47 ± 0·28	1·98 ± 0·84

(±)-Meptazinol (1–10 μM) did not affect basal resting tension in unstimulated or stimulated preparations but produced a potentiation of the twitch response which increased up to 2-fold over the size of the control twitch before the addition of (±)-meptazinol. The individual enantiomers produced very different effects. (+)-Meptazinol produced an inhibition of the twitch response which was completely abolished by the presence of naloxone (20 nM). In contrast, (−)-meptazinol potentiated the twitch response and the degree of potentiation was increased even further in the presence of naloxone (20 nM) (Fig. 1).

Mouse isolated field-stimulated vas deferens. Exposure to the opioid agonists RX783006, morphine and normorphine produced an inhibition of the twitch response elicited by electrical stimualtion and the concentrations required to produce a 50% inhibition of the twitch are shown in Table 1. All three agonists were able to produce at least 90% inhibition of the twitch repsonse. Naloxone (20 nM) produced the expected inhibition of this effect and yielded K_e values which were not significantly different whichever agonist was used ($P > 0{\cdot}2$). Neither were these values significantly different statistically from the corresponding values obtained from guinea-pig ileum ($P > 0{\cdot}4$).

(±)-Meptazinol (10–100 μM) produced a potentiation of the twitch response as did both enantiomers and this effect was not modified by the presence of naloxone (20 nM) (Fig. 2) or atropine (100 nM). Lower concentrations of meptazinol or its enantiomers produced no effect.

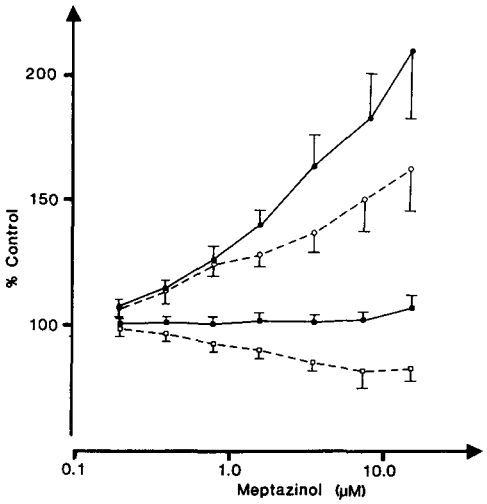

FIG. 1. Effect on the twitch response of the field-stimulated guinea-pig isolated ileum (percentage of control response) of (+) and (−)-meptazinol (■ and ● respectively) in the absence and presence (filled points) of naloxone (20 nM). Values are means (±s.e.); 9 determinations contributed to each point for (−)-meptazinol (10 for (+)-meptazinol).

Discussion

The high activity of the μ-selective agonists RX783006 and normorphine and the K_e values found for naloxone are compatible with the action of these agonists being mediated through μ-type opioid receptors. K_e values for naloxone at μ-receptors reported by other workers range from 1–4·2 nM (Kosterlitz & Watt 1968; Handa et al 1981; Shaw et al 1982) and the values reported above are well within this range. K_e values for naloxone at δ and κ-receptors are somewhat higher (typically

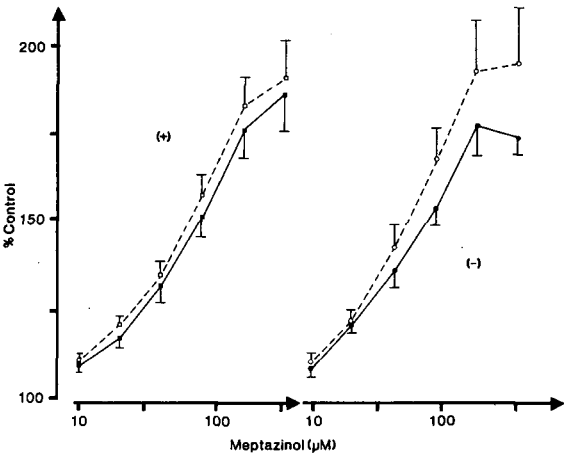

FIG. 2. Effect on the twitch response of the field-stimulated mouse vas deferens (percentage of control response) of (+)- and (−)-meptazinol (■ and ● respectively) in the absence or the presence (filled points) of naloxone (20 nM). Values are means (±s.e.) and 6 determinations contributed to each point.

14–25 nM; Shaw et al 1982; Ward et al 1983; Cotton et al 1984).

The (−)-isomer potentiated the response of the ileum to electrical stimulation and this effect is compatible with the cholinergic action of meptazinol reported elsewhere (Stephens et al 1978). In the presence of naloxone (20 nM) the potentiation was significantly increased indicating that an opioid agonist action was present and functionally antagonizing the potentiating effect. The (+)-isomer also appears to be an opioid agonist but has less cholinergic action since it produced inhibition of the twitch reponse and naloxone abolished the effect entirely. Comparison of the changes produced by naloxone in the concentration-effect curves suggests that both isomers are approximately equi-active as opioid agonists and this supports the observations of Goode & White (1971).

Mouse vas deferens may contain several types of opioid receptor and the strain used in these experiments clearly contains some μ-type receptors since the μ-selective agonists normorphine and RX783006 produced marked effects and the K_e values determined for naloxone were well within the range expected for μ-receptors.

(±)-Meptazinol and both isomers potentiated the response to electrical stimulation and the presence of neither naloxone nor atropine produced any alteration in this effect. Had any opioid agonist effect been present it would be expected that the potentiation would have been increased by naloxone but no such action was seen. Since the potentiation was unaltered by atropine, it cannot be due to a cholinergic effect such as has been suggested to account for the action on the ileum (Stephens et al 1978). It is more likely that the ability of meptazinol to increase the release of noradrenaline (Bill et al 1981) accounts for this action and unpublished data obtained in our laboratory indicates that the electrically evoked release of tritium from mouse vas deferens previously incubated with [^{3}H]noradrenaline is increased by meptazinol.

Meptazinol appears therefore to be an opioid agonist in guinea-pig ileum but not in mouse vas deferens and this suggests that the receptors at these sites may not be identical. Closer examination of the IC50 values for morphine, normorphine and RX783006 in the two tissues lends some support to this possibility; all three agonists appear to act on μ-type receptors as all yield very similar K_e values for naloxone typical of μ-receptors. If the receptors were identical it would be expected that the order of potency of the agonists (RX783006 > normorphine > morphine in guinea-pig ileum and RX783006 > morphine > normorphine in vas deferens) would be the same in both tissues but this is not so.

Evidence already exists that all μ-opioid receptors may not be identical and a division into μ_1 and μ_2 has been proposed (see Blurton et al 1984). Furthermore, Sayre et al (1983), using irreversible antagonists have produced evidence which suggests that the μ-receptors in guinea-pig ileum and mouse vas deferens are not identical and the data reported above support this possibility. The lack of difference between the K_e values for naloxone in the two tissues does not necessarily weigh against this as naloxone may fail to distinguish between the receptors.

It should be noted however that differences in receptor numbers, agonist access or biotransformation may contribute to differences between IC50 values in two tissues. (+)-Meptazinol is clearly a partial agonist in guinea-pig ileum as the concentration-effect curve is shallow and the maximal effect smaller than that produced by normorphine and RX783006. The sodium ratio of 1·4 for meptazinol (Lien et al 1979) suggests that meptazinol has a low efficacy and, if there are fewer spare receptors in mouse vas deferens this could account for the apparent lack of agonist action of (+)-meptazinol in this preparation.

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