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The effects of the selective κ-opioid agonist MR 2034 on the guinea-pig ileum

IAN M. COUPAR, MICHAEL J. QUINN, *Unit of Addictive Drug Research, Victorian College of Pharmacy, 381 Royal Parade, Parkville, Victoria 3052, Australia*

Abstract—The effects of the selective κ-opioid agonist MR 2034 on the guinea-pig ileum were compared with those produced by the μ-agonist morphine. MR 2034 induced acute tolerance and inhibited electrically evoked contractions to the same extent as morphine. The slope of the concentration-effect curve to MR 2034 was not significantly different from that of morphine. However, MR 2034 and morphine may act at their respective receptors since naloxone was 6·57 times more effective at inhibiting responses to morphine than MR 2034. MR 2034, like morphine, induced acute dependence as revealed by naloxone-induced contractions of tissues that had been incubated with MR 2034 for 5 h. The functional properties of κ-opoid receptors are the same as μ-receptors in the guinea-pig ileum.

The guinea-pig ileum contains populations of both μ- and κ-opioid receptors (Hutchinson et al 1975) and has been used extensively as a model of opioid action (Kosterlitz & Waterfield 1975; Collier et al 1981). The aims of the following experiments were to determine the effects of κ-receptor stimulation in the guinea-pig ileum compared with μ-receptor stimulation. Selective analgesic tests in rodents have established that MR 2034 is a highly selective κ-agonist (Tyers 1980). In the present study the potency, efficacy and acute tolerance and dependence-producing properties of MR 2034 have been measured and compared with the effects produced by the prototype μ-agonist morphine.

Materials and methods

Adult guinea-pigs of either sex were killed by stunning and exsanguination and a segment of ileum removed approximately 10 cm proximal to the ileo-caecal junction. Segments of ileum were attached to a tissue holder fitted with platinum electrodes for transmural stimulation, and bathed in Krebs-Henseleit solution (NaCl 118, KCl 4·3, $NaHCO_3$ 25, $MgSO_4$ 1·2, KH_2PO_4 1·2, $CaCl_2$ 2·4, dextrose 11·1 mmol L^{-1}). The Krebs contained added mepyramine (0·125 μmol L^{-1}) and hexamethonium (70 μmol L^{-1}) to block the effects of histamine released by morphine and to restrict the effects of the agonists to the final cholinergic neurones of the myenteric plexus, respectively (Collier et al 1981). The solution was maintained at 37°C and was gassed with 5% CO_2 in O_2. The resting tension of tissues (1·5 to 2 cm length) was adjusted to approximately 1 g and increases in tension representing increases in longitudinal muscle contraction were recorded by force displacement transducers (Grass FT03) connected to a multichannel pen recorder (Grass, Model 79). The tissues were stimulated electrically from a Grass S48 with square wave pulses of 1 ms duration, 0·1 Hz and supramaximal voltage.

The ilea were stimulated continuously and equilibrated for 1 h before starting experiments. Responses to the agonists were induced using a 15 s contact time between each increasing concentration. Further responses to the agonists were repeated 30 min after washout. These conditions avoided the development of acute tolerance.

The antagonist potency of naloxone was determined by measuring the responses of the agonists in the absence and presence of four increasing concentrations of naloxone (10 nmol L^{-1}–1 μmol L^{-1}, contact time 5 min). The resultant dose-ratios were used to estimate the pA_2 values (Arunlakshana & Schild 1959).

Naloxone was also used to reveal the degree of dependence induced by the agonists. Tissues were incubated in the presence of maximal concentrations of either morphine (1·6 μmol L^{-1}) or MR 2034 (160 nmol L^{-1}). The tissue baths were washed out and replaced with fresh Krebs/agonist mixture every h. At the end of 5 h the incubation solution was replaced with Krebs only and 5 min later the tissues were exposed to increasing concentrations of naloxone (contact time 1 min).

IC50 and pA_2 values were calculated using regression analysis (Tallarida & Murray 1981). These values and also the values of E_{max} and the slope of the pA_2s are means ± s.e.m. or 95% confidence interval.

MR 2034, (-)-(1*R*, 5*R*, 9*R*, 2′′*S*)-5,9-dimethyl-2′-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan D-tartrate, was obtained from Boehringer Ingelheim, morphine HCl from MacFarlane Smith, and naloxone HCl from Endo.

Results

Qualitative assessment of responses in pilot experiments showed that the maximal concentratioins of MR 2034, like morphine, induced variable degrees of acute tolerance. However, this could be avoided by ensuring that tissues were only exposed to maximal concentrations of the agonists for 15 s and allowing 30 min before re-establishing responses.

Maximally effective concentrations of both MR 2034 and morphine produced similar inhibition of the electrically evoked contractions. However, MR 2034 was approximately 20 times more potent than morphine.

Comparison of the pA_2 values showed that naloxone was 6·57 times more effective as a competitive antagonist of morphine than MR 2034. However, the difference in pA_2 values was only of borderline statistical significance (Table 1).

Table 1. Comparison of MR 2034 and morphine. E_{max} is percent inhibition of electrically evoked contractions. The slopes of the agonist concentration effect curves were not significantly different ($P > 0.05$) and the slopes of the Schild plots for naloxone were not significantly different from −1 ($P > 0.05$). $P = 0.11$ (two-tailed Student's unpaired *t*-test) for the difference in pA_2 values. Values given are means ± s.e.m. except for the IC50 values where the range is the 95% confidence interval. Numbers in brackets are the number of tissues used.

	MR 2034	Morphine
E_{max}	83·67 ± 3·52% (7)	82·8 ± 3·53% (7)
IC50 (nmol L^{-1})	10 (3·1 − 32·6) (6)	190 (81–442) (7)
pA_2 for naloxone	7·83 ± 0·16 (7)	8·65 ± 0·44 (7)
Slope	−0·89 ± 0·12 (7)	−1·08 ± 0·25 (7)

Naloxone produced no effect on ilea that had not been exposed to the agonists but produced strong concentration-related contractions of tissues that had been incubated with either MR 2034 or morphine. Naloxone produced larger responses in morphine- than MR 2034-treated ilea. High

Correspondence to: I. M. Coupar, Unit of Addictive Drug Research, Victorian College of Pharmacy, 381 Royal Parade, Parkville, Victoria 3052, Australia.

concentrations of naloxone (400 nmol L^{-1} in morphine- and 800 nmol L^{-1} in MR 2034-incubated ilea) induced spontaneous contractions, Fig. 1.

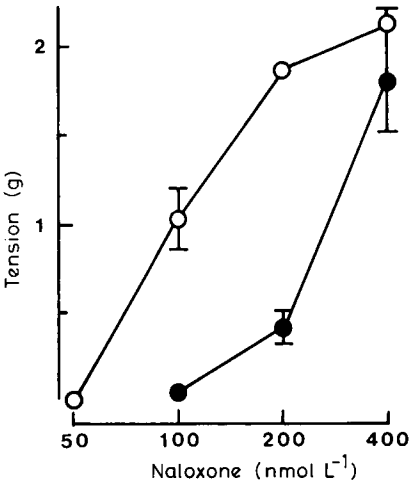

FIG. 1. Increase in tension induced by naloxone of tissues preincubated for 5 h in morphine (1·6 μmol L^{-1}, ○) and MR 2034 (160 nmol L^{-1}, ●). Bars represent s.e.m., n = 4.

Discussion

κ-Opioid agonists are distinguished from μ-agonists on the basis of their antinociceptive profiles. κ-Agonists are active against pressure as the noxious stimulus rather than heat whereas μ-agonists are effective against both heat and pressure (Martin et al 1976; Upton et al 1982). In addition, κ-agonists do not substitute for morphine in morphine-dependent animals (Swain & Seevers 1974; Wood 1982). In one study, MR 2034 was shown to be the most selective κ-agonist of a large series of analgesics tested (Tyers 1980). The guinea-pig ileum is assumed to contain a population of κ-receptors as well as clearly established μ-receptors. There are two main reasons for this assumption. One is based on the different tissue sensitivities to agonists where the guinea-pig ileum is more sensitive to benzomorphans, including MR 2034, than the mouse vas deferens (Hutchinson et al 1975). Secondly, naloxone displays a small degree of selectivity towards μ-compared with κ-agonists. For example, naloxone is approximately 5·5 times more potent at inhibiting responses to the μ-agonist normorphine than to MR 2034 (Hutchinson et al 1975).

The present results confirm that MR 2034 is an agonist in the guinea-pig ileum being approximately 20 times more potent than morphine. The E_{max} of MR 2034 was the same as morphine and the slope if its concentration-effect curve was not different to that of morphine. This could indicate that both agonists act on the same receptor. Consequently the different affinity of naloxone at μ- and κ-receptors was used in an attempt to resolve this uncertainty. Previous reports have shown that naloxone is a competitive antagonist of morphine giving Schild plots with slopes of −1. For instance, one published pA_2 value for naloxone was 8·5 (slope −1·06, Ward & Takemori 1976) which is similar to the value of 8·65 (slope −1·05) found in the present study. Naloxone has previously been established as a competitive antagonist of MR 2034 (Hutchinson et al 1975) and this is confirmed by the present results. In addition they show that the concentration of naloxone required to inhibit responses to MR 2034 is 6·6 times greater than to inhibit responses to morphine. This difference was of borderline statistical significance partly as a result of the low selectivity of naloxone towards μ-receptors. Until highly selective κ-antagonists are developed it should not be ruled out that MR 2034 may activate μ-receptors in the guinea-pig ileum. This has not been established although the reverse has: that is morphine can act as a κ-agonist in this tissue (Ward et al 1982).†

The guinea-pig ileum has been established as a model to explore the mechanism of opioid-induced tolerance and dependence. Morphine and normorphine have been used extensively in such experiments and have both been shown to induce dependence as indicated by withdrawal contracture on removal of the agonist and its replacement with naloxone. This sign of dependence occurs in the final cholinergic neurons of the myenteric plexus (see Collier et al 1981). It is not yet clear what contribution, if any, κ-receptors make towards the development of dependence. One report using a short incubation time of 2 min found that MR 2034 did not induce dependence in the guinea-pig ileum (Chahl 1986). However, the present results clearly show that MR 2034 does cause this characteristic effect of the opiates. Deperdence on MR 2034 developed over a period of 5 h in tissues incubated at 37°C which are optimal conditions for inducing dependence on morphine (Collier et al 1981).

The benzomorphans do not substitute for morphine in morphine-dependent monkeys (Swain & Seevers 1974). However, the present results using the guinea-pig ileum show that the effects of a highly selective κ-opioid agonist are similar to those produced by morphine. This indicates that the functional properties of both opioid receptor subtypes are similar in the guinea-pig ileum.

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†The recent development of two selective κ-agonists binaltorphimine and nor-binaltorphimine promises to clarify the existence and function of κ-receptors in the guinea-pig ileum (Portoghese et al 1987).

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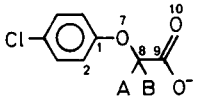
Letter to the Editor

"The influence of conformational factors on the metabolic conjugation of aryloxyacetates"—a comment

COLIN H. L. KENNARD, * GRAHAM SMITH, *Department of Chemistry, University of Queensland, Brisbane, Queensland 4067, * Department of Chemistry, Queensland Institute of Technology, Brisbane, Queensland 4000, Australia*

The crystal structure of three aryloxyacetates have torsion angles significantly different from those forecast by van de Waterbeemd et al (1986) using quantum mechanical PCILO calculations. However, these torsion angles are near a minimum in their published three dimensional conformational maps.

In developing a model for the binding site of glucuronyltransferase, van de Waterbeemd et al (1986) used quantum mechanical calculations to explain the differences between *p*-chlorophenoxyacetic acid, **I**, 2-(*p*-chlorophenoxy)propionic acid, **II**, (inert towards metabolic conjugation) and 1-(*p*-chlorophenoxy)-2-methylpropionic acid, **III**, (undergoes extensive glucuronidation). The three variable torsion angles are defined in Scheme I. Keeping $\tau 3$ constant, they plotted the conformational energy for varying values of $\tau 1$ and $\tau 2$, and said that all compounds had similar regions of very low energy 0–8 kJ mol^{-1} for $\tau 2 = 0 \pm 30^{\circ}$; $\tau 1 = 30$–90 and 210–300°.



Scheme I. $\tau 1$, 2–1–7–8; $\tau 2$, 1–7–8–9; $\tau 3$, 7–8–9–10.

In the region of low conformation energy (8–21kJ mol^{-1}), $\tau 2 = 0 \pm 90^{\circ}$ for compounds **I** and **II**, but $\tau 2 = 180 \pm 90^{\circ}$ for **III**. Although no suitable method is currently available to determine these values at the bonding site, their crystal structures are known (Kennard et al 1981, 1982), and they do differ from the values selected for these theoretical models (Table 1). Furthermore, the preferred solid state conformation for Type I unsubstituted and monosubstituted phenoxyacetic acids is planar and tional change), ca. 180° (Kennard & Smith 1979). A similar series of Type II (2-phenoxypropionic) and Type III (phenoxyisobutyric) acids are also extended with $\tau 2$ ca. 90°. However, in the solid state, the acids are dimers about a centre of inversion, and for **II** and **III** $\tau 3$ is not 0°. So far there is no evidence to suggest that the dimer formation in this type of acid has any influence on the torsion angles about $\tau 1$, $\tau 2$ and $\tau 3$. It should be noted that the crystallographic results are included as near a minimum in their three dimensional conformational maps. Therefore these calculations do agree with the experimental evidence in the solid state, in spite of the fact that $\tau 3$ was kept at 0°.

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Table 1. Comparison for the significant torsion angles (τ) in aryloxyacetes between quantum mechanical calculated values and those found from crystal structure determination.

			$\tau 1$		$\tau 2$		$\tau 3$	
	A	B	Found C(2)-C(1)-O(7)-C(8)	Calc	Found C(1)-O(7)-C(8)-C(9)	Calc	Found O(7)-C(8)-C(9)-O(10)	Assumed
I	H	H	+179·2	30–90 210–300	+178·5	0±30 0±90	−2·3	0
II	H	CH_3	+171·1	30–90 210–300	+73·8	0±30 0±90	+25·7	0
III	CH_3	CH_3	+130·4	30–90 210–300	+79·0	0±30 180±90	+35·9	0

Correspondence to: C. H. L. Kennard, Dept of Chemistry, University of Queensland, Brisbane, Queensland 4067, Australia.