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14-Methoxymetopon, a very potent μ-opioid receptor-selective analgesic with an unusual pharmacological profile

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Abstract

14-Methoxymetopon is a potent opioid analgesic. When given systemically, it is approximately 500-fold more active than morphine. However, this enhanced potency is markedly increased with either spinal or supraspinal administration, where its analgesic activity is more than a million-fold greater than morphine. It was μ-opioid receptor selective in binding assays and its analgesia was blocked only by μ-opioid receptor-selective antagonists. Yet, it had a different selectivity profile than either morphine or morphine-6β-glucuronide. Unlike morphine, 14-methoxymetopon was antagonized by 3-*O*-methylnaltrexone, it was sensitive to antisense probes targeting exons 1, 2 and 8 of the opioid receptor gene and was inactive both spinally and supraspinally in CXBK mice. Although it retarded gastrointestinal transit, it displayed a ceiling effect with no dose lowering transit by more than 65%, in contrast to the complete inhibition of transit by morphine. These finding demonstrate that 14-methoxymetopon is a highly potent μ-opioid with a pharmacological profile distinct from that of the traditional μ-opioid morphine.

Keywords: Opioid; Analgesia; Metopon; Morphine

1. Introduction

μ-Opioids represent the major class of strong analgesics used clinically and include agents such as morphine, methadone, fentanyl and oxycodone. Despite their profound utility in the management of pain, they have a number of adverse actions that impede their overall utility, including respiratory depression, sedation and constipation. Furthermore, they all are associated with tolerance and dependence with chronic use and many also are abused. It has been suggested that receptor mechanisms mediating some of these side effects might differ from those involved with analgesia (Heyman et al., 1988; Ling et al., 1984, 1985; Pasternak et al., 1980; Paul et al., 1989; Paul and Pasternak, 1988; Wolozin and Pasternak, 1981), raising the possibility of selective drugs lacking many of these side effects. One example was Tyr-D-Arg[2]-Phe-sarcosine, or TAPS, a peptide analgesic that produced respiratory stimulation instead of depression (Paakkari et al., 1992, 1993). 5,14-*O*-dimethyloxymorphone or 14-methoxymetopon (HS198) is quite potent, reportedly 300–20,000-fold more potent than morphine in a range of analgesic assays, including hot plate, tail electrical stimulation and tailflick in rats (Freye et al., 2000; Fürst et al., 1993; Schmidhammer et al., 1990a,b; Zernig et al., 2000). Yet, it elicits little respiratory depression and has significantly less hypotension and bradycardia than the highly potent μ-opioid, sufentanil (Freye et al., 2000). This dissociation between analgesia and respiratory depression, two μ-opioid receptor mediated events, is quite intriguing and sets 14-methoxymetopon apart from other μ-opioid drugs. In the current study, we have further characterized the μ-opioid receptor

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mechanisms of 14-methoxymetopon and compared them to traditional μ-opioid drugs.

2. Methods

Morphine sulfate, morphine-6β-glucuronide, naltrexone, β-funaltrexamine, norbinaltorphimine and 3-*O*-methylnaltrexone were gifts from the Research Technology Branch of the National Institute on Drug Abuse. 14-Methoxymetopon was synthesized as previously reported (Schmidhammer et al., 1990b). Naloxonazine was synthesized as described previously (Hahn et al., 1982). Naltrindole was purchased from Sigma-Research Biochemicals and halothane was purchased from Halocarbon Laboratory (Hackensack, NJ, USA). All radioligands were purchased from New England Nuclear (Boston, MA, USA), with the exception of [^{3}H]naloxone benzoylhydrazone, which was synthesized as previously described (Price et al., 1989).

2.1. Radiant heat tailflick assay

Male Crl:CD-1® (ICR) BR mice (20–25 g) (Charles Rivers Laboratories, Raleigh, NC, USA) and CXBK (Jackson Laboratory, Bar Harbor, ME, USA) mice were maintained on a 12-h light/dark cycle with food and water available ad libitum. All in vivo studies utilized these CD-1 mice, unless stated otherwise, and were carried out in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health, and all studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). Drugs were administered subcutaneously (s.c.), intracerebroventricularly (2–5 μl, i.c.v.) (Haley and McCormick, 1957) or intrathecally (2–5 μl, i.t.) (Hylden and Wilcox, 1980).

Antinociception, also termed analgesia in this study, was assessed using the radiant heat tailflick assay (Rossi et al., 1995), an assay that has been widely used and validated (Le Bars et al., 2001). Baseline latencies ranged from 2 to 3 s. A 10 s cutoff was imposed to minimize tissue damage. Analgesia was defined quantally as a doubling or greater of the baseline latency for the individual mouse. Since analgesic assays were evaluated quantally, group comparisons were performed with the Fischer exact test. All experiments were replicated at least twice with each group in each experiment containing at least 10 mice and the combined results of all replications presented. A computerized Litchfield and Wilcoxin method was used to determine effective dose ED_{50} values and 95% confidence limits on the quantal data (Tallarida and Murray, 1987).

2.2. Antisense mapping

Antisense mapping was first demonstrated with the δ-opioid receptor (Standifer et al., 1994) and extended to a variety of other proteins. It permits the downregulation of proteins by targeting specific exons within their mRNA, providing an opportunity to selectively target individual splice variants from a common gene. The MOR-1 antisense oligodeoxynucleotide sequences were based on the cloned mouse μ-opioid receptor sequence, as previously reported (Rossi et al., 1995). Oligodeoxynucleotides were synthesized by Midland Certified Reagent (Midland, TX, USA), purified in our laboratory and dissolved in 0.9% saline. The probes targeting exons 1 and 2 were CGCCCCAGCCTCTTCCTCT and TTGGTGGCAGTCTTCATTTTGG, respectively. The mismatch probe was based upon the exon 1 probe and differs only in the location of four bases (underlined) (CGCCCCGACCTCTTCCCTT). Mice received antisense (5 μg/2 μl, i.c.v.) on days 1, 3 and 5 and were tested on day 6, as previously described (Rossi et al., 1995; Standifer et al., 1994).

2.3. Gastrointestinal motility assay

Gastrointestinal transit was determined as previously described (Paul and Pasternak, 1988). In brief, after withholding food for 8 h, animals received the indicated drug and then were given a charcoal meal (0.2 ml; 10% of purified charcoal and 2.5% of gum tragacanth, w/v) by gavage and were sacrificed 30 min later. The distance traveled by the charcoal meal was then measured and reported in centimeters.

2.4. Receptor binding assays

Opioid receptor binding assays were performed as previously reported (Clark et al., 1989). In brief, membrane preparations were incubated in potassium phosphate (50 mM; pH 7.4) buffer at 25 °C for either 60 min (κ_1-opioid and κ_3-opioid binding) or 180 min (μ_1-opioid, μ_2-opioid and δ-opioid binding) with the indicated radioligand and various concentrations of unlabelled ligand to give a total assay volume of 2 ml. Non-specific binding was determined with levallorphan (1 μM). μ-Binding assays were performed using calf thalamus membrane preparations with either [^{3}H][D-Ala^2,D-Leu^5]enkephalin ([^{3}H]DADLE, 0.7 nM) in the presence of [D-Pen^2,D-Pen^5]enkephalin (DPDPE, 10 nM) for μ_1 binding or [^{3}H][D-Ala^2,MePhe^4,Gly(ol)^5]-enkephalin ([^{3}H]DAMGO, 1.0 nM) in the presence of [D-Ser^2,Leu^5]enkephalin-Thr^6 (DSLET, 5 nM) for μ_2 binding, with $MgCl_2$ (5 mM) in both membrane preparations. δ-Opioid receptor binding was determined using [^{3}H]DPDPE (1 nM) with calf frontal cortex membranes and κ_3-opioid binding with [^{3}H]naloxone benzoylhydrazone (1 nM) with calf striatal membrane preparations. K_1-opioid receptor binding was determined with [^{3}H]U69,593 ([(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-ly]-benzeneacetamide, 1 nM) in guinea pig cerebellar membrane preparations.

3. Results

3.1. Receptor binding studies

14-Methoxymetopon is a potent μ-opioid receptor selective analgesic (Zernig et al., 2000; Fürst et al., 1993). The current study confirmed its selectivity for μ-opioid binding sites (Table 1). It showed approximately 3-fold greater affinity for μ_1-opioid binding sites than μ_2-opioid sites. Its affinity for δ-opioid and κ_1-opioid binding sites was approximately 100-fold poorer. In view of its poor affinity for δ-opioid binding sites and insensitivity towards δ-opioid receptor selective antagonists (see below), we did not examine its affinity for δ-opioid receptor subtypes. Its inhibition of [^{3}H] naloxone benzoylhydrazone binding, however, was slightly more complex, as shown by its low Hill coefficient. This competition curve was best fit to two sites, with a higher affinity ($K_i = 1.5$ nM) and a lower affinity ($K_i = 45$ nM) component. Although 14-methoxymetopon competed the higher affinity component of κ_3-opioid binding reasonably well, its affinity still was 5-fold lower than against μ-opioid binding.

3.2. 14-Methoxymetopon analgesia

Behaviorally, 14-methoxymetopon is a potent analgesic, active in the hot plate and writhing assays (Fürst et al., 1993; Schmidhammer et al., 1990a,b). 14-Methoxymetopon also was an effective analgesic in the radiant heat tailflick assay (Fig. 1 and Table 2). Systemically, 14-methoxymetopon was approximately 500-fold more potent than morphine. However, it was interesting that its analgesic actions, as defined quantally, plateaued at approximately 80% and never achieved a complete analgesic response of 100%. This contrasted to the results with morphine, which easily elicited a full analgesic response in all animals at the higher doses. Furthermore, among the analgesic 14-methoxymetopon animals few had latencies that approached the cutoff value of 10 s, despite fulfilling the quantal criteria for analgesia. In contrast, most of the morphine-treated animals approached the cutoff value at higher drug doses. This suggested that 14-methoxymetopon might be a partial agonist.

Table 1
Competition of opioid receptor binding by 14-methoxymetopon

Binding assay	K_i value (nM)	Hill coefficient
μ_1	0.10 ± 0.02	0.92
μ_2	0.34 ± 0.11	1.08
δ	25.69 ± 5.92	0.99
κ_1	22.95 ± 7.25	0.91
κ_3	1.48 ± 0.39	0.60
	45.10 ± 12.37	

Competition studies were performed as described in Section 2. IC_{50} values were determined by nonlinear regression analysis and converted to K_i values using the Cheng and Prusoff (1973) approach. Values represent mean ± S.E.M. of at least three independent experiments, each of which was performed in triplicate.

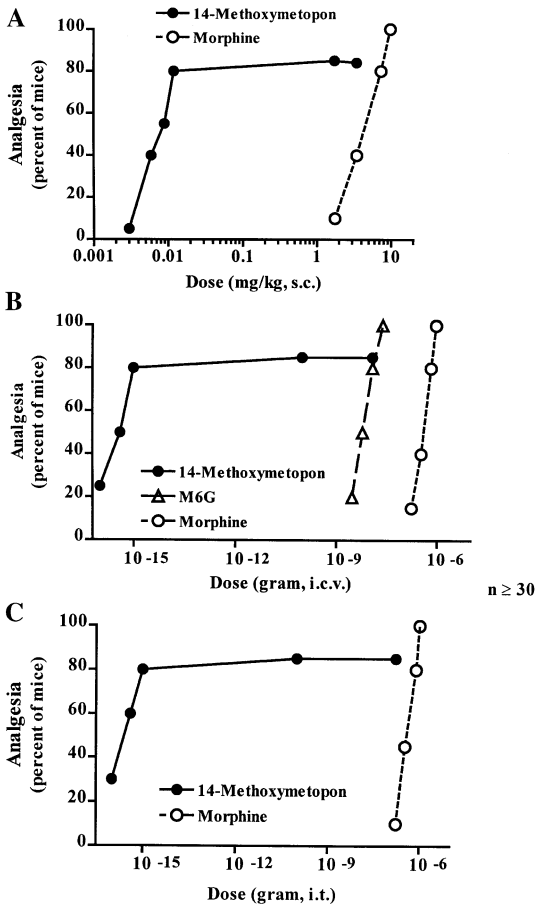

Fig. 1. Dose–response curves for 14-methoxymetopon and other opioids. Groups of mice received the indicated agonists (A) subcutaneously (s.c.; $n \geq 20$), (B) intracerebroventricularly (i.c.v.; $n \geq 20$) or (C) intrathecally (i.t.; $n \geq 30$) and analgesia was assessed 30 (s.c.), 15 (i.c.v.) and 5 min (i.t.) post injection. Results were combined from at least two independent replications. Dose–response curves were plotted and ED_{50}'s were calculated and presented in Table 1.

The relative potency of 14-methoxymetopon increased even further when it was administered centrally, where it was over a million-fold more active than morphine both supraspinally and spinally. Despite this extraordinary potency, 14-methoxymetopon analgesia still plateaued, failing to elicit an analgesic response greater than 85% despite using doses far beyond its ED_{50} value.

3.3. Pharmacological characterization of 14-methoxymetopon analgesia

Selective antagonists have been very useful in defining the actions of opioid analgesics. 14-Methoxymetopon analgesia was readily antagonized by the general opioid antagonist naltrexone (Fig. 2). We next examined a series of antagonists previously shown to selectively block the various subtypes. We chose a dose of 14-methoxymetopon that produced a response of approximately 80% to facilitate the assessment of the antagonist, which lowers responses. The μ-selective opioid antagonist β-funaltrexamine, which blocks

Table 2
14-Methoxymetopon analgesia in the radiant heat tailflick assay

Drug	Route of administration					
	Systemic (s.c.)		Supraspinal (i.c.v.)		Spinal (i.t.)	
	ED_{50} (95% CL)	Relative potency	ED_{50} (95% CL)	Relative potency	ED_{50} (95% CL)	Relative potency
Morphine	3.8 mg/kg (2.6–5.6)	1	367 ng (250–540)	1	330 ng (203–527)	1
Morphine-6β-glucuronide	3.65 mg/kg (2.6–5.1)	1.04	7.01 ng (5.36–9.16)	52	6.72 ng (5.71–8.33)	49
14-Methoxymetopon	7.6 μg/kg (6.2–9.3)	500	0.29 fg (0.17–0.51)	>1,000,000	0.31 fg (0.16–0.43)	>1,000,000

Dose–response curves were evaluated and ED_{50} values with 95% confidence limits determined on the quantal data, as decribed in Section 2. Results are from Fig. 1. Relative potencies were determined compared to morphine.

all μ-opioid receptor subtypes (Ward et al., 1981, 1982), also blocked both morphine and 14-methoxymetopon analgesia, as did naloxonazine, a μ_1-selective antagonist (Hahn et al., 1982; Ling et al., 1985, 1986). 3-0-Methylnaltrexone is an interesting naltrexone analog that can selectively block the analgesic actions of morphine-6β-glucuronide and heroin at doses that are ineffective against morphine (Brown et al., 1997; Walker et al., 1999). In the current study, 3-0-methylnaltrexone lacked activity against morphine, but effectively blocked 14-methoxymetopon analgesia, distinguishing between the two μ-opioids. The inactivity of the δ-selective opioid antagonist naltrindole and the κ_1-opioid receptor-selective drug norbinaltorphimine at doses that effectively blocked their own receptor-selective agonists established the μ selectivity of both analgesics.

CXBK mice reportedly are deficient in μ_1-opioid binding sites (Moskowitz and Goodman, 1985). Pharmacologically, CXBK mice are insensitive to morphine given either supraspinally or systemically (Pick et al., 1993), routes of adminsitration that conform to a μ_1-opioid receptor mechanism of action, as shown by their sensitivity to blockade by naloxonazine in traditional strains of mice (Ling et al., 1983; Pasternak et al., 1983; Paul et al., 1989). Yet, spinal morphine analgesia, an action that is not antagonized by naloxonazine (Heyman et al., 1988; Paul et al., 1989), retains its analgesic actions in CXBK mice. In the current study, morphine was inactive when administered supraspinally (Fig. 3A). In contrast, morphine-6β-glucuronide retained its analgesic activity, as previously reported (Rossi et al., 1996). In this study, 14-methoxymetopon also lacked analgesic activity when administered supraspinally, even at

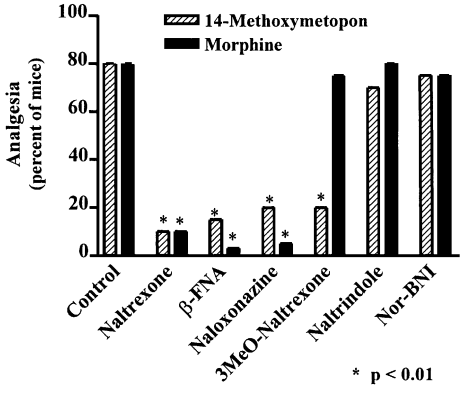

Fig. 2. Effects of selective opioid antagonists on supraspinal 14-methoxymetopon and morphine analgesia. Groups of mice ($n \geq 20$) were given saline, naltrexone (1 mg/kg, s.c.), 3-*O*-methylnaltrexone (0.25 mg/kg, s.c.), naltrindole (δ-opioid receptor antagonist, 20 mg/kg, s.c.) or norbinaltorphimine (10 mg/kg, s.c.) immediately before morphine (700 ng, i.c.v.) or 14-methoxymetopon (1 fg, i.c.v.) while β-funaltrexamine (40 mg/kg, s.c.) and naloxonazine (35 mg/kg, s.c.) were given 24 h earlier.

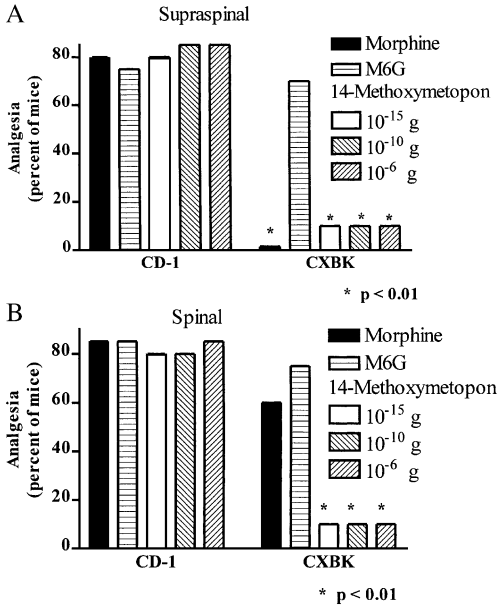

Fig. 3. 14-Methoxymetopon analgesia in CXBK mice. (A) Groups of CD-1 or CXBK mice ($n \geq 20$) received morphine (700 ng, i.c.v.), morphine-6β-glucuronide (12.5 ng, i.c.v.) or 14-methoxymetopon (1 fg, 0.1 ng or 1 μg, i.c.v.) and analgesia was assessed. Morphine and 14-methoxymetopon responses were significantly lower ($P < 0.01$) in the CXBK mice. (B) Groups of CD-1 or CXBK mice ($n \geq 20$) received morphine (700 ng, i.t.), morphine-6β-glucuronide (12.5 ng, i.t.) or 14-methoxymetopon (1 fg, 0.1 ng or 1 μg, i.t.) and analgesia was assessed. The 14-methoxymetopon response was signficantly lower ($P < 0.01$) in the CXBK mice.

doses far beyond its ED_{50} in CD-1 mice. Spinally, morphine and morphine-6β-glucuronide analgesia were retained in the CXBK mice (Fig. 3B). Unlike either of them, 14-methoxymetopon elicited little analgesia spinally, even at extremely high doses.

3.4. Antisense mapping 14-methoxymetopon analgesia

The μ-opioid receptor MOR-1 was cloned in 1993 (Chen et al., 1993; Eppler et al., 1993; Thompson et al., 1993; Wang et al., 1993). Since then, a number of variants have been identified (Bare et al., 1994; Pan et al., 1998, 2000, 2001; Zimprich et al., 1995). Antisense mapping has proven a valuable approach towards correlating cloned proteins and function, particularly in the evaluation of possible splice variants (Pasternak and Pan, 2000; Rossi et al., 1995, 1997; Standifer et al., 1994). Antisense probes based upon exons 1 or 8 downregulate morphine analgesia, but not that of morphine-6β-glucuronide (Pan et al., 1999; Rossi et al., 1997). Conversely, exon 2 antisense probes block morphine-6β-glucuronide, and not morphine, analgesia (Rossi et al., 1995). The pharmacological profile of 14-methoxymetopon analgesia differed from that of morphine or morphine-6β-glucuronide with regards to both selective μ-opioid antagonists and in CXBK mice. We therefore compared its sensitivity to antisense probes targeting MOR-1. The antisense mapping sensitivity profile of 14-methoxymetopon differed from that of either morphine or morphine-6β-glucuronide. As previously established, the exon 1 and the exon 8 antisense blocked morphine while the exon 2 antisense blocked morphine-6β-glucuronide. However, 14-methoxymetopon analgesia was decreased by all three antisense probes, a profile distinct from either of the two other μ-opioids. The inactivity of the mismatch oligodeoxynucleotide confirmed the specificity of the approach (Fig. 4).

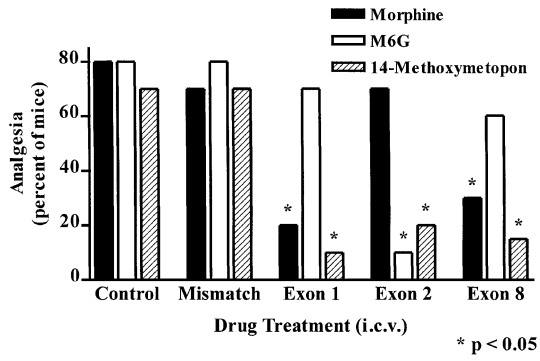

Fig. 4. Antisense mapping 14-methoxymetopon analgesia against MOR-1. Male CD-1 mice ($n \geq 20$) received i.c.v. injections of antisense or mismatch (5.0 μg/2.0 μl) or saline on days 1, 3, 5 and tested on day 6. Drugs were administered intracerebroventricularly (i.c.v.) and analgesia was assessed 15 min post injection. Antisense targeting exon 1 significantly reduced morphine (700 ng, i.c.v.) and 14-methoxymetopon (1 fg, i.c.v.) analgesia ($P < 0.05$). Downregulation of exon 2 significantly blocked morphine-6β-glucuronide (12.5 ng, i.c.v.) and 14-methoxymetopon (1 fg, i.c.v.) antinociception ($P < 0.05$). Saline and mismatch treatments had no effect.

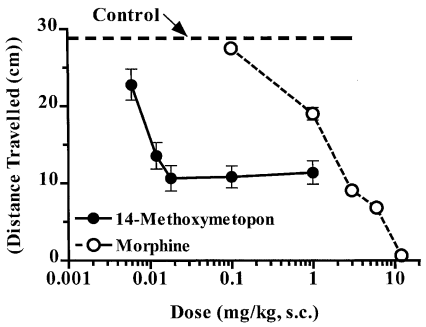

Fig. 5. Inhibition of gastrointestinal transit by 14-methoxymetopon and morphine. Groups of mice ($n \geq 20$) received the indicated doses of morphine or 14-methoxymetopon and GI motility was assessed 30 min later, as described in Section 2. Control transit was 28.3 cm. 14-Methoxymetopon significantly inhibited transit, as determined by analysis of variance ($P < 0.01$).

3.5. Modulation of gastrointestinal transit by 14-methoxymetopon and morphine

Morphine decreases gastrointestinal transit (Reisine and Pasternak, 1996). In our current study, morphine effectively slowed transit in a dose-dependent manner, with high doses effectively abolishing transit in the paradigm (Fig. 5). Although 14-methoxymetopon dose-dependently inhibited gastrointestinal motility, its actions plateaued and never eliminated transit.

4. Discussion

In the current study, 14-methoxymetopon was an extremely potent analgesic, confirming prior reports (Freye et al., 2000; Fürst et al., 1993; Schmidhammer et al., 1990a,b; Zernig et al., 2000). The receptor binding assays confirmed the μ selectivity of the agent, with a slightly greater affinity for μ_1 compared to μ_2 binding sites and revealed that 14-methoxymetopon competed κ_3-opioid receptor binding less potently. Moreover, it competed κ_3 binding in a biphasic manner, suggesting that its high affinity may be limited to a subpopulation of sites labeled within the κ_3-opioid binding assay. Pharmacologically, its actions appeared to be mediated through μ-opioid receptors, based upon its sensitivity towards the μ-opioid selective antagonists and the antisense mapping.

Prior work reported that 14-methoxymetopon is between 300–20,000-fold more potent than morphine, depending upon the assay used (Freye et al., 2000; Fürst et al., 1993; Schmidhammer et al., 1990a; Zernig et al., 2000). We observed a similar potency difference following systemic administration, where 14-methoxymetopon was 500-fold more active than morphine. However, our results with direct administration into the central nervous system were quite surprising. When given either spinally or supraspinally, the potency of 14-methoxymetopon was

extraordinary. Indeed, the potency of the drug centrally was more than a million-fold greater than morphine. Clearly, 14-methoxymetopon was a very potent analgesic. Yet, it was never able to achieve an overall analgesic response greater than 85%. The actual latencies in analgesic animals rarely approached the cut off level of 10 s, although they were greater than double baseline values. This contrasts markedly with morphine where responses in analgesic mice typically reached cut off values. A similar limited response was observed on gastrointestinal transit. 14-Methoxymetopon lowered gastrointestinal transit and was more potent than morphine, but its maximal inhibition was only approximately 65% whereas morphine effectively blocked transit completely in this paradigm. These observations would suggest that 14-methoxymetopon was a partial agonist in the gastrointestinal transit assay. Furthermore, this limited effect on gastrointestinal transit implies that it has a better therapeutic index between the inhibition of gastrointestinal transit and analgesia than morphine. This is further supported by its lack of respiratory depression (Freye et al., 2000).

Prior studies had concluded that 14-methoxymetopon was a highly selective μ-opioid. Our current studies support this classification. In vivo, its actions were readily reversed by the highly μ-selective opioid antagonists naloxonazine and β-funaltrexamine, but not by the δ- and κ_1-selective opioid drugs naltrindole and nor-binaltorphimine. In addition, antisense probes targeting the cloned μ-opioid receptor MOR-1 attenuated 14-methoxymetopon analgesia. However, we also observed pharmacological differences between 14-methoxymetopon and either morphine or morphine-6β-glucuronide with regards to its sensitivity to 3-*O*-methylnaltrexone or antisense mapping, implying a distinct action of this drug.

The sensitivity of CXBK mice to μ-opioids has proven quite interesting (Baron et al., 1975; Pick et al., 1993; Reith et al., 1981). Based upon naloxonazine sensitivity, supraspinal morphine analgesia has been classified as a μ_1-opioid receptor action while spinal analgesia has been suggested to be μ_2-opioid receptor mediated (Paul et al., 1989). Since CXBK mice are deficient in μ_1-opioid binding sites (Moskowitz and Goodman, 1985), morphine would be expected to be relatively inactive supraspinally in the CXBK mice and active spinally, results originally reported almost a decade ago (Pick et al., 1993) and confirmed in the current study. The inactivity of 14-methoxymetopon spinally in the CXBK mice suggests the absence of μ_2-opioid activity for the drug, a possibility consistent with its lack of respiratory depression (Freye et al., 2000), another μ_2-opioid receptor mediated action (Ling et al., 1983, 1985). Yet, 14-methoxymetopon demonstrated high affinity for μ_2-opioid receptors in receptor binding assays. The inhibition of gastrointestinal transit also involves μ_2-opioid mechanisms (Heyman et al., 1988; Paul and Pasternak, 1988). Here, 14-methoxymetopon appeared to be a partial agonist. If spinal analgesia required a greater efficacy than the inhibition of gastrointestinal transit, its analgesic inactivity spinally in the CXBK mice might reflect its limited intrinsic activity at μ_2-opioid receptors.

In conclusion, 14-methoxymetopon appears to be a very potent μ-opioid analgesic with an interesting pharmacology. Its classification as a μ-opioid is strongly supported by selective antagonists, antisense mapping studies and opioid receptor binding studies. Despite its potent analgesic activity, its effects on gastrointestinal transit, a potential measure of its ability to produce constipation clinically, were far less than those seen with morphine. Other studies have also documented a far lower potential to induce respiratory depression, sedation and cardiovascular effects (Freye et al., 2000), illustrating a pharmacological profile quite distinct from either morphine or morphine-6β-glucuronide. 14-Methoxymetopon is a unique opioid with potentially fewer problematic side-effects that opens interesting questions into the pharmacology of μ-opioids and may prove to be valuable in the clinical management of pain.

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