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European Journal of Pharmacology 294 (1995) 201–206

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Metopon and two unique derivatives: affinity and selectivity for the multiple opioid receptors

Jay P. McLaughlin [a], Deanne Nowak [b], Alice Sebastian [b], Arthur G. Schultz [b], Sydney Archer [b], Jean M. Bidlack [a,*]

[a] *Department of Pharmacology, The University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642, USA*
[b] *Department of Chemistry, Cogswell Laboratory, Rensselaer Polytechnic Institute, Troy, NY 12181, USA*

Received 24 April 1995; revised 25 July 1995; accepted 18 August 1995

Abstract

5β-Methyl-7,8-dihydromorphinone (metopon), an isomer [6a*S*-(6aα,9aα,10β)13a*S*]-1,10-methano-4-hydroxy-11-methyl-6,6a,8,9,10,11,12,13-octahydro-[1]-benzopyrano[4,3,e]isoquinoline-7-(9a*H*)-one (compound 1) derived from a photochemical rearrangement of 5β-methylmorphinone, and [6a*S*-(6aα,9aα,10β)13a*S*]-1,10-methano-4-hydroxy-11-methyl-6,6a,8,9, 10,11,12,13-octahydro-[1]-benzopyrano[4,3,e]-14β-(*p*-nitrocinnamoylamino) isoquinoline-7-(9a*H*)-one (compound 2) were characterized for opioid receptor affinity, selectivity and analgesic properties. In competition binding assays using bovine striatal membranes, the three compounds inhibited the binding of 0.25 nM [^{3}H][D-Ala^2,(Me)-Phe^4,Gly(ol)^5]enkephalin (DAMGO), a μ-selective peptide, with IC_{50} values less than 5 nM. All three compounds exhibited lower affinity for δ- and κ-opioid receptors. In the mouse 55°C warm-water tail-flick assay, both metopon and compound 1 displayed antinociception that lasted for 60 min after i.c.v. injection. Morphine sulfate, metopon and compound 1 produced 50% antinociception with i.c.v. doses of 0.83, 2.0 and 4.0 nmol, respectively. The μ-selective, irreversible opioid receptor antagonist β-funaltrexamine blocked antinociception induced by metopon and compound 1, while δ- and κ-opioid receptor selective antagonists did not effect antinociception. These findings demonstrate metopon and its isomer bound with high affinity to the μ-opioid receptor and produced antinociception through this receptor.

Keywords: Morphine derivative; Metopon; Cinnamoylamino group; β-Endorphin receptor; Analgesia; (Mouse)

1. Introduction

The development and characterization of selective opioid receptor agonists and antagonists is important for further understanding the pharmacological and biochemical properties of the multiple opioid receptors (Martin et al., 1976). One important opioid receptor agonist is 5β-methyl-7,8-dihydromorphinone (metopon) (Small et al., 1936; Stork and Bauer, 1953). Metopon was initially synthesized in an attempt to find oral substitutes for morphine (Small et al., 1936). It has been reported to be from 3 to 10 times more effective than morphine in producing morphine-like effects, such as analgesia and ileum contraction (Eddy, 1948; Keats and Beecher, 1952). A number of derivatives of metopon containing a 14β-cinnamoylamino group produced wash-resistant inhibition of μ-opioid receptor binding to bovine striatal membranes, and acted as long-term μ-opioid receptor selective antagonists devoid of any agonistic effects in the mouse warm-water tail-flick assay (Jiang et al., 1993, 1994; McLaughlin et al., 1994). However, little is known about the opioid receptor selectivity and affinity of metopon itself. In addition, [6a*S*-(6aα,9aα,10β)13a*S*]-1,10-methano-4-hydroxy-11-methyl-6,6a,8,9,10,11,12,13-octahydro-[1]-benzopyrano-[4,3,e]isoquinoline-7-(9a*H*)-one, an isomer of metopon, was synthesized. Compound 1 was derived from a photochemical rearrangement of 5β-methylmorphinone that incorporates the 5-methyl group into the benzopyran ring (Fig. 1). A derivative of this isomer containing a 14β-*p*-nitrocinnamoylamino group, [6a*S*-(6aα,9aα,10β)13a*S*]-1,10-methano-4-hydroxy-11-meth-

* Corresponding author. Department of Pharmacology, The University of Rochester, School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA. Tel.: (716) 275-5609; fax: (716) 244-9283.

SSDI 0014-2999(95)00536-6

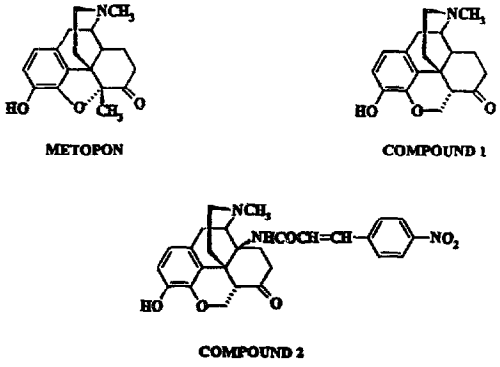

Fig. 1. Structures of metopon, compound 1 and compound 2. Compound 1 is an isomer of metopon with the methyl group in the C-5 position of metopon incorporated into the benzopyran ring. Compound 2 is a derivative of compound 1, containing a *p*-nitrocinnamoylamino group in the C-14β position.

yl-6,6a,8,9,10,11,12,13-octahydro-[1]-benzopyrano[4,3,e]-14β-(*p*-nitrocinnamoylamino)isoquinoline-7-(9a*H*)-one (compound 2) (Fig. 1), was also synthesized to determine if this derivative would produce wash-resistant inhibition of μ-opioid receptor binding like the 14β-*p*-nitrocinnamoylamino derivative of metopon, 5β-methyl-14β-(*p*-nitrocinnamoylamino)-7,8-dihydromorphinone (MET-CAMO) (Jiang et al., 1994). The present study characterized the affinity and selectivity of metopon, compound 1 and compound 2 for the μ-, δ- and κ-opioid receptors in competition binding experiments, as well as characterizing the antinociceptive properties produced by metopon and compound 1 in the mouse 55°C warm-water tail-flick assay.

2. Materials and methods

2.1. Synthesis of metopon, compound 1 and compound 2

Preparation of compound 1

To a solution of [6a*S*-(6aα,9aα,10β)13a*S*]-1,10-methano-4-hydroxy-11-methyl-6,6a,10,11,12,13-hexahydro-[1]-benzpyrano[4,3-e]isoquinoline-7-(9a*H*)-one (Schultz et al., 1994) (51.1 mg, 0.172 mmol) in ethyl acetate (10 ml) was added 5% rhodium on alumina (10 mg). The mixture was hydrogenated at 55 pounds per square inch for 17 h and then filtered through Celite under vacuum. Flash chromatography on alumina (1–10% methanol in chloroform) gave compound 1 (36.5 mg, 71% semicrystalline solid). ^{1}H Nuclear Magnetic Resonance (deuterochloroform, 500 MHz) δ 6.68 (d, 1 H, J = 7.8), 6.52 (d, 1 H, J = 8.3), 4.96 (d, 1 H, J = 11.2), 4.28 (dd, 1 H, J = 11.3, 3.5), 3.06 (d, 1 H, J = 14.2), 3.04 (s, 1 H), 2.60 (dd, 1 H, J = 12.2, 2.9), 2.53 (dd, 1 H, J = 18.6, 6.4), 2.44 (s, 3 H), 2.38 (m, 2 H), 2.28 (m, 1 H, J = 12.2, 3.9), 1.96 (m, 1 H, J = 12.2, 4.9), 1.88 (d, 1 H, J = 12.2), 1.80 (m, 1 H), 1.50 (m, 1 H).

Preparation of compound 2

To a solution of 5β-methyl-14β-[(*p*-nitrocinnamoyl)amino]codeinone (250 mg) in dry chloroform, cooled to −20°C, was added boron tribromide (235 ml) in chloroform dropwise with stirring. The reaction mixture was slowly brought to room temperature and stirred at that temperature for 2 h. To this reaction mixture, 1 ml of methanol and sodium bicarbonate solution was added and the organic layer was separated. The aqueous layer was extracted with chloroform : methanol (9 : 1) (3 × 30 ml) and the combined extracts were washed with brine, dried (sodium sulfate) and evaporated. The residue obtained was chromatographed on silica gel using chloroform : methanol (96 : 4) as the eluant. Compound 2 was obtained (20 mg) as the minor product, melting point 265–269°C decomposition, Infrared Spectroscopy (potassium bromide): 3450, 3350, 2900, 1690, 1650, 1620, 1590, 145 cm^{-1}; ^{1}H Nuclear Magnetic Resonance (deuterochloroform) δ 2.43 (3 H, s, NCH_3), 4.27–4.29, 5.01–5.03 (2 H, dd, d, OCH_2-C), 6.62–6.64 (1 H, d, Ar-H), 6.65–6.68 (1 H, d, NH-COCH =), 6.76–6.78 (1 H, d, Ar-H), 7.65–7.67 (2 H, d, Ar-H), 7.66–7.69 (1 H, d, = CH-Ar), 8.22–8.24 (2 H, d, Ar-H). The major product was MET-CAMO (Sebastian et al., 1993).

Preparation of metopon

Metopon was prepared by the method of Gates and co-workers (Boden et al., 1982).

2.2. Opioid receptor binding to bovine striatal membranes

Bovine striatal membranes were prepared as described previously (Jiang et al., 1994). The affinity and selectivity of metopon and compounds 1 and 2 for the multiple opioid receptors was determined by incubating the membranes with radiolabeled ligands and 12 different concentrations of metopon, compound 1 or compound 2 at 25°C in a final volume of 1 ml of 50 mM Tris-HCl, pH 7.5. Incubation times of 60 min were used for the μ-opioid receptor selective peptide [^{3}H][D-Ala^2,(Me)Phe^4,Gly(ol)^5]enkephalin (DAMGO) and the κ-opioid receptor selective ligand [^{3}H]U69,593, and a 4-h incubation was used with the δ-opioid receptor selective peptide [^{3}H][D-Pen^2,pCl-Phe^4,D-Pen^5]enkephalin (pCl-DPDPE). To determine the IC_{50} values for the inhibition of binding by the compounds, the final concentrations of [^{3}H]DAMGO, [^{3}H]pCl-DPDPE and [^{3}H]U69,593 were 0.25 nM, 0.2 nM and 1 nM, respectively. Nonspecific binding was measured by inclusion of 10 μM naloxone. Binding was terminated by filtering the samples through Schleicher and Schuell No. 32 glass fiber filters (Keene, NH, USA) using a Brandel 48-well cell harvester. Filters were soaked for at least 60 min in 0.25% polyethylenimine for [^{3}H]pCl-DPDPE and [^{3}H]U69,593 binding experiments. After filtration, filters were washed 3 times with 3 ml of cold

50 mM Tris-HCl, pH 7.5, and were counted in 2 ml of Ecoscint A scintillation fluid.

The 14β-*p*-nitrocinnamoylamino side chain of compound 2 could permit it to potentially bind covalently to the opioid receptor. Experiments measuring wash-resistant inhibition of opioid receptor binding were performed to detect potential covalent binding. To determine the concentration of compound 2 needed to obtain wash-resistant inhibition of opioid receptor binding, bovine striatal membranes, 10 mg of protein, were incubated with concentrations of compound 2 ranging from 20 to 2600 nM at 37°C for 15 min in a final volume of 2 ml. The contents of the tubes were then diluted to 40 ml with cold 50 mM Tris-HCl, pH 7.5 and centrifuged at $39000 \times g$ for 15 min at 4°C. The washing step was repeated for a total of 4 times. Finally, the membranes were resuspended in 2 ml of 50 mM Tris-HCl, pH 7.5, and opioid receptor binding to 0.2 ml of membranes was determined as described above. The isomers, compound 2 and MET-CAMO, were compared in the wash-resistant inhibition of [^{3}H]DAMGO binding experiments.

2.3. Animals

All antinociceptive experiments used male, ICR mice (20–24 g, Harlan Sprague Dawley; Indianapolis, IN, USA). Mice were kept in groups of eight in a temperature controlled room with a 12-h light-dark cycle. Food and water were available ad libitum until the time of the experiment.

2.4. Injection techniques

Intracerebroventricular (i.c.v.) injections were made directly into the lateral ventricle according to the modified method of Haley and McCormick (1957). The volume of all i.c.v. injections was 5 μl, using a 10-μl Hamilton microliter syringe. The mouse was lightly anesthetized with ether, an incision was made in the scalp, and the injection was made 2 mm lateral and 2 mm caudal to bregma at a depth of 3 mm.

2.5. Tail-flick assay

The thermal nociceptive stimulus was 55°C water, with the latency to tail flick or withdrawal taken as the endpoint (Vaught and Takemori, 1979). After determining control latencies, the mice received graded i.c.v. doses of opioid receptor agonists. Morphine sulfate, metopon and compound 1 were each given as single i.c.v. injections with antinociceptive effect measured 20 min after injection unless otherwise stated. In the receptor selectivity studies, either the κ-opioid receptor selective antagonist nor-binaltorphimine or the δ-opioid receptor selective antagonist ICI 174,864 were each given with the agonist in the same injection. β-Funaltrexamine, the μ-opioid receptor selective antagonist, was injected 24 h before agonist injection. A cut-off time of 15 s was used; if the mouse failed to display a tail flick in that time, the tail was removed from the water and the animal assigned a maximal antinociceptive score of 100%. Mice which showed no response within 5 s in the initial control test were eliminated from the experiment. At each time point, antinociception was calculated according to the following formula: % antinociception = $100 \times$ (test latency − control latency)/(15 − control latency).

2.6. Chemicals

[^{3}H]DAMGO (60 Ci/mmol) and [^{3}H]U69,593 (64 Ci/mmol) were purchased from Amersham (Arlington Heights, IL, USA). [^{3}H]pCl-DPDPE (48.6 Ci/mmol) was obtained from New England Nuclear (Boston, MA, USA). Morphine sulfate was purchased from Mallinckrodt Chemical Company (St. Louis, MO, USA). Nor-binaltorphimine, ICI 174,864 and β-funaltrexamine were purchased from Research Biochemicals International (Natick, MA, USA).

In the mouse tail-flick assay, metopon and compounds 1 and 2 were dissolved in 20% dimethylsulfoxide, a concentration that did not produce any detectable behavioral effect. β-Funaltrexamine, ICI 174,864 and nor-binaltorphimine were dissolved in distilled water.

2.7. Statistics

IC_{50} values were calculated by least squares fit to a logarithm-probit analysis. The K_i values of unlabeled compounds were calculated from the equation $K_i = IC_{50}/(1 + S)$, where S = (concentration of radioligand)/(K_D of radioligand) (Cheng and Prusoff, 1973). All dose-response lines were analyzed, using the regression methods described by Tallarida and Murray (1986). Regression lines, D_{50} (dose producing 50% antinociception) values and 95% confidence limits were determined with each individual data point using the computer program by Tallarida and Murray (1986). All data points shown are the means of 7–10 mice, with standard error of the mean represented by error bars.

3. Results

3.1. Affinity and selectivity of metopon and its derivatives for the multiple opioid receptors in bovine striatal membranes

The affinity of metopon, compound 1 and compound 2 for the multiple opioid receptors in mem-

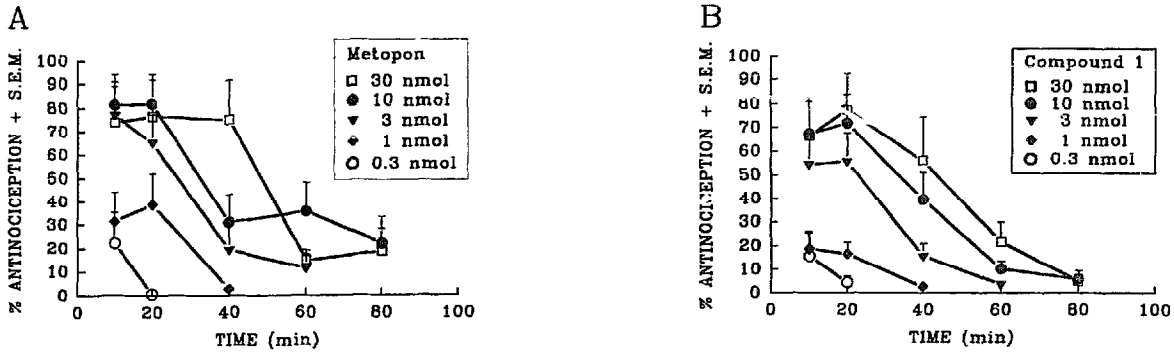

Fig. 2. Time-response lines for single graded i.c.v. doses of metopon (A) or compound 1 (B) in the mouse tail-flick assay.

branes was measured by determining the IC_{50} values for the inhibition of μ-, δ- and κ-opioid receptor binding (Table 1). As metopon and compound 1 lack the 14β-nitrocinnamoylamino group, they cannot bind covalently to the receptor, so respective K_i values are compared here. Because the 14β-nitrocinnamoylamino derivative has the potential to bind covalently to the receptor, it cannot be assumed to bind at equilibrium and therefore only IC_{50} values are reported for compound 2. Metopon showed the highest affinity for the μ-opioid receptor, inhibiting [^{3}H]DAMGO binding with a K_i value of 0.34 ± 0.03 nM. Metopon inhibited [^{3}H]pCl-DPDPE and [^{3}H]U69,593 binding with K_i values of 22.2 ± 1.5 nM and 24.3 ± 1.0 nM, respectively. Similarly, compound 1 exhibited the highest affinity for the μ-opioid receptor, although at approximately 5-fold greater concentrations than metopon, inhibiting [^{3}H]DAMGO binding with a K_i value of 1.47 ± 0.02 nM. Compound 1 inhibited binding to the δ- and κ-opioid receptors, with K_i values of 106 ± 14 nM and 168 ± 6 nM, respectively. Compound 2 demonstrated similar affinity and selectivity for the multiple opioid receptors as compound 1 (Table 1).

MET-CAMO has been shown to produce wash-resistant inhibition of [^{3}H]DAMGO binding, possibly through the covalent binding of the 14β-*p*-nitrocinnamoylamino constituent to the μ-opioid receptor (Jiang et al., 1994). Compound 2 is an isomer of MET-CAMO, with the 5-methyl group incorporated into the benzopyran ring. To detect possible covalent binding to the μ-opioid receptor by the 14β-*p*-nitrocinnamoylamino constituent of compound 2, experiments measuring wash-resistant inhibition of the binding of 0.25 nM [^{3}H]DAMGO were performed with compound 2 and MET-CAMO for comparison. At concentrations of 200 nM, MET-CAMO reduced the binding of 0.25 nM [^{3}H]DAMGO by $61 \pm 1\%$ in a wash-resistant manner, whereas compound 2 inhibited binding by only $16 \pm 2\%$. A 50% wash-resistant inhibition of binding by compound 2 was obtained at 1500 ± 160 nM. Taken together, these results strongly suggest compound 2 did not bind covalently to the μ-opioid receptor.

Table 1
IC_{50} values for the inhibition of μ-, δ- and κ-opioid receptor binding to bovine striatal membranes by metopon, compound 1 and compound 2

Radiolabeled ligands	IC_{50} (nM) ± S.E.M.		
	Metopon	Compound 1	Compound 2
[^{3}H]DAMGO (μ)	0.85 ± 0.07	3.64 ± 0.04	2.34 ± 0.11
[^{3}H]pCl-DPDPE (δ)	36.5 ± 2.5	174 ± 22	155 ± 19
[^{3}H]U69,593 (κ)	81.0 ± 3.3	558 ± 19	1090 ± 90

Bovine striatal membranes were incubated with 12 different concentrations of each opioid in the presence of 0.25 nM [^{3}H]DAMGO, 0.2 nM [^{3}H]pCl-DPDPE or 1 nM [^{3}H]U69,593 in 50 mM Tris-HCl, pH 7.5, at 25°C as described in Materials and methods. K_i values for metopon and compound 1 are reported in the text, whereas only IC_{50} values are reported for compound 2, because compound 2 has the potential to bind covalently. Data are listed as the mean IC_{50} values ± S.E.M. from at least three experiments, performed in triplicate.

3.2. Mouse tail-flick assay

Both metopon and compound 1 produced a time- and dose-dependent antinociception after i.c.v. administration in the mouse 55°C warm-water tail-flick assay (Figs. 2 and 3). Metopon and compound 1 produced maximal analgesic effects 20 min after i.c.v. administration of 3 nmol of metopon and 10 nmol of compound 1, and the antinociception lasted up to 60 min (Fig. 2).

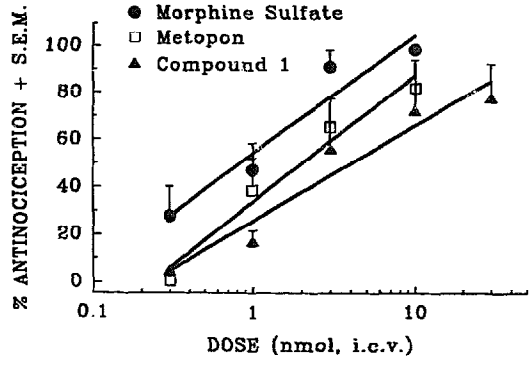

Fig. 3. Dose-response lines of morphine sulfate, metopon and compound 1 given by i.c.v. injection 20 min before testing in the mouse tail-flick assay.

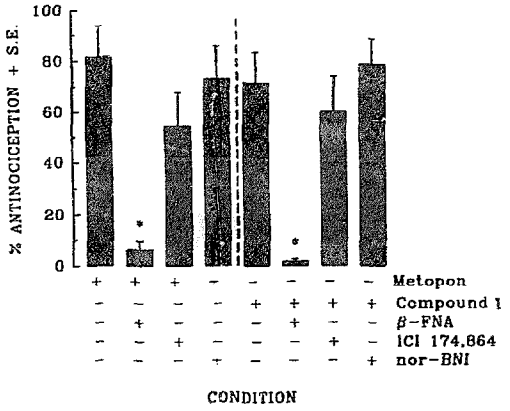

Fig. 4. Antinociceptive effects of i.c.v. metopon and compound 1 (10 nmol, −20 min) in mice with or without pretreatment with i.c.v. β-funaltrexamine (β-FNA)(20 nmol, −24 h) or co-administered with either ICI 174,864 (4 nmol, −20 min) or nor-binaltorphimine (nor-BNI) (0.6 nmol, −20 min) in the mouse tail-flick assay. * $P \leq 0.01$, significantly different from the corresponding compound alone.

The antinociceptive D_{50} values of metopon and compound 1 (and 95% confidence limits) were 2.0 (0.99–4.0) nmol and 4.0 (1.7 to 9.1) nmol, respectively (Fig. 3). In comparison, the antinociceptive D_{50} value of morphine sulfate was 0.83 (0.23–2.92) nmol.

Receptor selectivity of both metopon and compound 1 after i.c.v. administration with selective opioid receptor antagonists was tested in the mouse tail-flick assay, as shown in Fig. 4. Concentrations of the antagonists were chosen to insure inhibition of only one type of opioid receptor. Nor-binaltorphimine was used at a concentration of 1 nmol, a dose shown to inhibit only κ-opioid receptor binding (Horan et al., 1992), and a concentration of 4 nmol of ICI 174,864 was chosen for selective δ-opioid receptor antagonistic properties (Heyman et al., 1987). The μ-opioid receptor selective, irreversible antagonist β-funaltrexamine was injected as a single 20-nmol i.c.v. pretreatment 24 h before testing, a time and dose previously established as having the peak antagonistic action at μ-, but not δ- or κ-opioid receptors (Heyman et al., 1989; Jiang et al., 1990). This pretreatment of mice with β-funaltrexamine before injection of metopon or compound 1 blocked the antinociceptive effects of both compounds. Co-administration of the κ-opioid receptor selective antagonist nor-binaltorphimine and the δ-opioid receptor selective antagonist ICI 174,864 had no significant effect on antinociception produced by either compound. These results demonstrate both metopon and compound 1 produced antinociception through the μ-opioid receptor.

4. Discussion

The present study investigated the binding characteristics of metopon, an isomer of metopon and a 14β-nitrocinnamoylamino derivative of the isomer of metopon. All three compounds bound with high affinity to the μ-opioid binding site, as determined by their K_i and IC_{50} values for the inhibition of [^{3}H]DAMGO binding. Similar preferences for binding to the μ-opioid receptor were reported with the 14-alkoxymetopon derivatives 14-methoxymetopon, 14-ethoxymetopon and 14-methoxy-5-methylmorphinone (Fürst et al., 1993), suggesting the presence of a C-5 methyl group enhances selectivity for the μ-opioid receptor. Here, metopon had approximately 5-fold higher affinity for all three receptor types than compounds 1 and 2, with the 5-methyl group incorporated into the benzopyran ring.

Experiments testing the ability of compound 2 to produce wash-resistant inhibition of [^{3}H]DAMGO binding demonstrated that micromolar concentrations were required. This is in marked contrast to earlier work performed with an isomer of compound 2, MET-CAMO (Jiang et al., 1994). MET-CAMO produced a wash-resistant inhibition of the binding of 0.25 nM [^{3}H]DAMGO in a concentration-dependent manner, with a 50% wash-resistant inhibition of binding produced with 82 ± 33 nM MET-CAMO (Jiang et al., 1994). Because the only difference between these two compounds is the location of the methyl group in the C-5 position (MET-CAMO) or in the benzopyran ring (compound 2), these results suggest the presence of the 5β-methyl constituent is important to the development of wash-resistant inhibition of binding, itself suggestive of covalent binding.

The present study further characterized the activity and receptor selectivity of metopon and compound 1 in vivo, using the mouse 55°C warm-water tail-flick assay. Initial determination of analgesia in cats and respiratory depression in rabbits found metopon to be 10 times more effective than morphine when administered orally or parenterally (Eddy, 1948). Later, 74 post-operative patients given alternating subcutaneous injections of morphine and metopon to relieve pain reported 3.7 mg of metopon produced analgesia equivalent to 10 mg of morphine, suggesting metopon had only 3-fold greater efficacy than morphine (Keats and Beecher, 1952). Since the 95% confidence intervals for the D_{50} values for antinociception produced by metopon and morphine sulfate overlap, the present results suggested metopon was only as effective as morphine at producing an analgesic response when administered by i.c.v. injection in the mouse 55°C warm-water tail-flick assay. However, like morphine, metopon and compound 1 had the highest affinity for the μ-opioid receptor. Other derivatives of metopon, such as MET-CAMO, which contains a 14β-*p*-nitrocinnamoylamino group (Sebastian et al., 1993; Jiang et al., 1994), and MET-Cl-CAMO, which contains a 14β-*p*-chlorocinnamoylamino group (McLaughlin et al., 1994), have

shown similar preference for the μ-opioid receptor. Antinociception produced by the 14-alkoxymetopon derivatives in the mouse tail-flick test was antagonized by low doses of naloxone, suggesting the derivatives interacted with the μ-opioid receptor (Fürst et al., 1993). Furthermore, 14-methoxymetopon generated similar pA_2 values to morphine in mouse tail-flick tests in the presence of naloxone, suggesting the two compounds probably interact with the same μ-opioid receptor population (Fürst et al., 1993). All of this evidence suggests the presence of a C-5 methyl constituent increases selectivity for the μ-opioid receptor without significant increases in antinociceptive effect.

Acknowledgements

We thank Kevin P. Hill for excellent technical assistance. This work was supported by U.S. Public Health Service Grants DA03742, DA07232, DA01674, GM33061 and AI08751.

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