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Highly potent novel opioid receptor agonist in the 14-alkoxymetopon series

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The newly synthesized 14-alkoxymetopon derivatives, 14-methoxymetopon, 14-ethoxymetopon, 14-methoxy-5-methylmorphinone, exhibit high affinity for the naloxone binding sites in rat brain. A substantial decrease in affinity was observed, in the presence of NaCl indicating a high degree of agonist activity. All three 14-alkoxymetopon derivatives displayed high affinity for [^{3}H][D-Ala^2,(Me)Phe^4,Gly-ol^5]enkephalin ([^{3}H]DAMGO) binding sites, much less potency toward δ sites and were the least effective at κ sites. Isolated tissue studies using the guinea pig ileum preparation confirmed their high agonist potency. Following administration the new compounds produced naloxone reversible antinociceptive effects and were 130–300 times more potent than morphine in the acetic acid induced abdominal constriction model in the mouse, and the hot plate and tail flick tests in the rat. The compounds also produced dose-dependent muscle rigidity, and potentiated barbiturate-induced narcosis. The in vivo apparent pA_2 values for naloxone against 14-ethoxymetopon and morphine were similar in analgesia, suggesting an interaction with the same (μ) receptor site. The dependence liability of 14-alkoxymetopon derivatives in the withdrawal jumping test was less pronounced than that of morphine in either rats or mice, similar to tolerance to the their analgesic action. It is concluded that the 14-alkoxymetopon derivatives studied are selective and potent agonists at μ opioid receptors, with reduced dependence liability.

Opioid receptors; Analgesia; Physical dependence; 14-Alkoxymetopons; (Binding)

1. Introduction

Substitution in position 14 of N-methylmorphinan-6-ones has a significant role in opioid receptor agonist activity. Structure-activity relationship studies showed that although a 14-hydroxy group does not significantly alter antinociception (Jacobson et al., 1981; Schmidhammer et al., 1983), introduction of a 14-methoxy group leads to a dramatic increase in antinociceptive potency (Schmidhammer et al., 1984).

A similar observation was made with 14-methoxymetopon, which was found to possess much higher antinociceptive potency in the acetic acid writhing test in mice than its 14-hydroxy counterpart, 14-hydroxymetopon (Schmidhammer et al., 1990).

To evaluate the mechanism of action of these novel highly potent 14-alkoxymetopon derivatives, biochemical (in vitro opioid receptor binding assays) and further pharmacological experiments (e.g. analgesic assays, narcosis potentiation, catalepsy, testing of physical dependence and tolerance) were performed.

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2. Materials and methods

2.1. Materials

[^{3}H][D-Ala^2,(Me)Phe^4,Gly-ol^5]enkephalin ([^{3}H]DAMGO) (60 Ci/mmol),(5α,7α,8β)-(−)-N-methyl-N-(7-[pirrolidinyl]-1-oxaspiro-[4,5]dec-8yl)-benzenacetamyd ([^{3}H]U69.593) (44 Ci/mmol) were purchased from Amersham. [^{3}H][D-Ser^2,Leu^5,Thr^6]enkephalin ([^{3}H]DSLET) (41.5 Ci/mmol) was from New England Nuclear. [^{3}H]Naloxone (71.9 Ci/mmol) was prepared at the Isotope Laboratory of the Biological Research Center (Szeged, Hungary), as described earlier (Tóth et al., 1982). Morphine, normorphine and naloxone hydrochloride were kindly provided by Alkaloida, Chemical Works (Hungary). Inactin (ethyl-butyl-thiobarbiturate) was from Gulden (Germany). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Biochemical assays

2.2.1.1. Membrane preparation

A crude membrane fraction from rat (PVG/C strain) was prepared according to Simon et al. (1986). Briefly, the rats were decapitated and whole brains without cerebella were homogenized in 30 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) with a Teflon-glass homogenizer. After centrifugation for 20 min at 40000 $\times g$, the pellet was suspended in fresh buffer and incubated at 37°C for 30 min to remove endogenous opioids. The centrifugation step described above was repeated, the pellet was resuspended in a Tris buffer containing 320 mM sucrose and was stored at −70°C. Before use the membranes were thawed and washed to remove sucrose. Protein concentration was determined by the method of Bradford (1976) with bovine serum albumin as a standard.

2.2.1.2. In vitro receptor binding assays

Binding experiments were run in Tris-HCl buffer (pH 7.4) in a final volume of 1 ml containing 0.3–0.5 mg protein as described by Buzás et al. (1992). Briefly, incubation was carried out for 1 h at 0°C in the case of [^{3}H]naloxone, 45 min at 35°C in the case of [^{3}H] DAMGO, 30 min at 30°C in the case of [^{3}H]U69.593, and 45 min at 25°C in the case of [^{3}H]DSLET. Incubation was terminated by rapid filtration through Whatman glass fiber filters using a Brandel Cell Harvester, followed by washing twice with 10 ml of ice-cold Tris-HCl buffer. The radioactivity was measured in a toluene-based scintillation cocktail with a Beckman LS 5000TD scintillation spectrophotometer. All assays were performed in duplicate and repeated at least three times. The percentage of specific binding was 85, 50 and 60 for the μ, κ and δ and ligands respectively.

2.2.1.3. Data analysis

Displacement data were analyzed by means of the LIGAND computer program (Munson and Rodbard, 1983) utilizing a non-linear least squares fitting algorithm.

2.2.2. Pharmacological tests

2.2.2.1. Animals and procedures

Male and female Sprague-Dawley rats weighing 120–150 g and CFL-P (Carworth Eur. England) mice of both sexes weighing 20–25 g were used. They were allowed free access to standard laboratory diet and tap water, and were kept under artificial light for 12 h every day. The room temperature was 22 ± 2°C. All drugs were dissolved in physiological saline. The drugs were administered s.c. to six rats and ten mice, in a volume of 5 and 10 ml/kg of body weight, respectively, if not stated otherwise. Guinea pigs of 300–400 g body weight were also used.

2.2.2.2. Guinea pig longitudinal muscle myenteric plexus preparation (guinea pig ileum; GPI)

The myenteric plexus longitudinal muscle was prepared according to Paton and Vizi (1969). The strip was suspended in a 5-ml bath and was stimulated by an electrical field at 0.1 Hz, pulse duration 1 ms, and supramaximal voltage.

2.2.2.3. Analgesiometric assays

2.2.2.3.1. Hot plate test The original method of Wolf and McDonald was used (1944). Hot plate latencies were determined by placing each rat on a hot plate kept at 55 ± 1°C and observing the occurrence of nociceptive responses: licking of paw or jumping. Each animal served as its own control. The arbitrary cut-off time, taken as 100%, was 2.5 times the control reaction time.

2.2.2.3.2. Tail flick test The original method of D'Amour and Smith (1941) was used to determine analgesia in the rat by measuring the time required to respond to a radiating heat stimulus. A beam light was focused on the tip of the tail, and the latency required for the rat to remove its tail was determined before (baseline) and after drug administration, using an arbitrary cut off time of twice the control reaction time and expressed as a percentage.

The AD_{50} values are defined as the doses of naloxone required to reduce by 50% the effects of equianalgesic (ED_{90}) doses of test compounds.

2.2.2.3.3. Acetic acid-induced abdominal constriction test Mice were injected i.p. with 0.2 ml of a 0.6% acetic acid solution to produce the writhing reaction according to Hendershot and Forsaith (1959). The number of abdominal constrictions (writhing) per animal was counted during a 5 min period. Analgesic activity was expressed as % inhibition of the average number of writhes in control animals.

2.2.2.4. Narcosis potentiation

This effect was determined by measuring the sleeping times in groups of 10 male rats. Inactin was injected as 35 mg/kg doses into the tail vein. The times at which animals lost and regained their righting reflex were recorded.

2.2.2.5. Catalepsy

Experiments were carried out with rats according to the original method of Morpurgo (1962). The cataleptic effects of the drugs tested were estimated 30 min after s.c. administration to groups of 10 rats. The percent of animals leaving both hind and fore legs over a horizon-

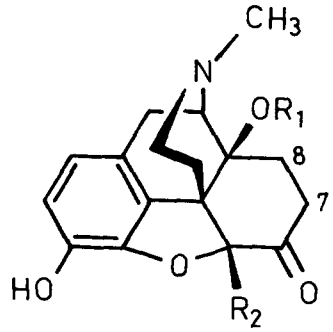

Fig. 1. The chemical structure of 14-alkoxymetopon derivatives. (1) $R_1 = R_2 = H$ (oxymorphone); (2) $R_1 = R_2 = CH_3$ (14-methoxymetopon); (3) $R_1 = C_2H_5$, $R_2 = CH_3$ (14-ethoxymetopon); (4) $R_1 = R_2 = CH_3$, Δ 7,8 (14-methoxy-5-methylmorphinon).

tal metal rod (4 cm above bench level) for longer than 45 s was recorded.

2.2.2.6. The effect of chronic administration

2.2.2.6.1. Testing physical dependence

2.2.2.6.1.1. In mice Physical dependence induction and precipitation of withdrawal were studied by means of the mouse naloxone-jumping method of Saelens et al. (1971). The effects of the tested drugs compared to those of morphine were studied in a two-day test: in the mice pretreated with seven equianalgesic doses of drugs, the number of jumps precipitated by 50 mg/kg naloxone injected i.p. was counted.

2.2.2.6.1.2. In rats Groups of five rats were injected twice daily at 9:00 and 15:30 h with morphine and test compounds (2), (3), (4) i.p. (fig. 1) in physiological saline. The initial doses on the first day were approximatively 10 times the antinociceptive ED_{50} values, measured in rat tail flick test: 20, 0.075, 0.120, 0.150 mg/kg for morphine and compounds (2), (3), (4) respectively. These doses were increased by daily increments, up to 100 times the analgesic ED_{50}: 170, 0.825, 1.380, 1.500 mg/kg for morphine and compounds (2), (3), (4), respectively, given twice a day, for 11 days. A group of five control rats received saline twice a day.

Quantitation of abstinence: rats were denied the 9:00 h injection of test drugs, and instead were given 3 mg/kg naloxone and the withdrawal syndrome precipitated was then observed for 120 min. The severity of abstinence was assessed by a point scoring technique according to Buckett (1964) slightly modified by us. The signs scored with the maximum possible score for each sign are shown in table 1.

Weight loss was also estimated, during the observation period and was taken as positive when it was > 3 g according to Cowan et al. (1988).

2.2.2.6.2. Quantitation of tolerance The analgesic activity (ED_{50}, mg/kg) was assessed using the tail flick test in rats treated according to the same protocol as used in the physical dependence test. The tolerance ratios (dose ratio: the ratio of ED_{50} in chronically treated animals to ED_{50} of saline-treated (control) animal) were calculated.

2.2.2.7. Estimation of the in vivo equivalent of pA_2: apparent pA_2

In our experiments the in vivo equivalents of pA_2 were determined for naloxone in the tail flick test (in the rat) according to Hayashi and Takemori (1971). The dose-response curves were made for the agonist alone and in combination with three different doses of naloxone, at the time of the peak effect of both drugs. The ratios of ED_{50}s in the presence of naloxone to the ED_{50}s of the drugs alone (dose ratio) were calculated for each shift in dose-response curve:

$$\text{Dose ratio} = \frac{ED_{50} \text{ in the presence of antagonist}}{ED_{50} \text{ of the drug alone}}$$

$-\log$ doses of naloxone, in mole per kg were plotted against log (dose ratio $- 1$), and fitted to a straight line. The intercept on the abscissa gives the apparent pA_2 value.

TABLE 1

Withdrawal signs monitored and maximum possible score for each sign.

Withdrawal sign	Score
Writhing	3
Squealing/rearing	2
Diarrhea	2
Teeth chatter/head shakes	1
Ptosis	1
Wet dog shakes	1

TABLE 2

Affinities of compounds (2), (3) and (4) to [7,8,19,20,^{3}H]naloxone binding sites in the absence and in the presence of 100 mM NaCl ($n = 3$).

	K_i (nM)		Index
	$-Na^+$	$+Na^+$	
(2)	0.87	36	41
(3)	0.75	100	133
(4)	0.89	98	110

TABLE 3

Inhibition of the binding of subtype-specific radioligands by compounds (2), (3) and (4) ($n = 3$).

	K_i (nM)			$S_{\mu/\delta}$	$S_{\mu/\kappa}$
	[^{3}H]DAMGO	[^{3}H]DSLET	[^{3}H]U69.593		
(2)	0.33 ± 0.05	13.7 ± 1.1	25.2 ± 6.8	41.5	76.6
(3)	0.46 ± 0.01	12.2 ± 1.8	43.2 ± 5.7	26.5	94.0
(4)	1.65 ± 0.03	14.4 ± 1.0	130.0 ± 22.0	8.7	78.8

TABLE 4

Relative affinity constants of compounds (2), (3) and(4) to μ, δ and κ receptors according to Kosterlitz and Paterson (1980).

Drug	Relative affinity		
	μ	δ	κ
(2)	96.4	2.3	1.3
(3)	95.4	3.6	1
(4)	88.7	10.1	1.2

TABLE 5

Agonist action on the isolated guinea pig longitudinal muscle preparation (n = 8).

Drug	Relative potency (normorphine = 1)
(2)	72.4
(3)	48.8
(4)	61.8

2.2.2.8. Data analysis

The ED_{50} and confidence limit determinations and regression and parallelism testing were done with the parallel line assay. The mean, S.E.M. and confidence limits were estimated for the individual values and Student's t-test was used for their comparison. A probability value of less than 5% was considered significant.

3. Results

3.1. Receptor binding

The agonist/antagonist characteristics and subtype specificity of compounds (2), (3) and (4) (fig. 1) (Schmidhammer et al., 1990) were investigated using in vitro receptor binding assays with the tritiated antagonist, naloxone, and subtype-specific selective agonists. In the competition studies all three compounds exhibited high affinity for the naloxone binding site and the estimated K_i values were under the nanomolar range (0.87, 0.75 and 0.89 nM respectively). When the experiments were carried out in the presence of 100 mM sodium, competition curves shifted to the right in each case and a substantial increase in K_i values was observed (table 2). An exceptionally high enhancement was found for compounds (2) and (3). Sodium indexes, reflecting the agonist character of the ligands, were estimated and found to be 41, 133 and 110 for compound (2), (3) and (4), respectively. These values indicate extremely high opioid agonist potencies of these drugs.

To establish the subtype specificity of the new compounds competition experiments were performed with highly selective radioligands (table 3). [^{3}H]DAMGO was used to label μ, [^{3}H]DSLET for δ, and [^{3}H]U69.593 for κ sites. All three 14-alkoxymetopon derivatives displayed high affinity for [^{3}H]DAMGO sites; K_i values were found to be 0.33, 0.46 and 1.65 nM for (2), (3) and (4), respectively. The compounds showed much less potency toward the δ sites (K_i values were about 12–14 nM), and were the least effective at κ sites (K_i values were 25.2, 43.2 and 130 nM, respectively).

The relative affinity constants of compounds (2), (3) and (4) were calculated (table 4) according to Kosterlitz and Paterson (1980), with the following equation

$$\text{Relative affinity} = \frac{K_{a,x}}{K_{a\mu} + K_{a\delta} + K_{a\kappa}} \quad (K_a = 1/K_i)$$

The estimated data suggest the strong μ preference of the ligands.

3.2. GPI

On the isolated, electrically stimulated GPI preparation, compounds (2), (3) and (4) induced a concentration-dependent, naloxone reversible (data not shown) inhibitory effect, 72.4, 61.8 and 48.8 times more potent, respectively, than normorphine (table 5).

3.3. Analgesic effects

As indicated in table 6, all the alkoxymetopon derivatives studied have remarkable antinociceptive ac-

TABLE 6

Antinociceptive effects of alkoxymetopon derivatives (2), (3), (4) compared to those of morphine in rats and mice. Antagonist effect of naloxone in the rat tail flick test.

Drug	Antinociceptive effects ED_{50}, mg/kg s.c. (95% confidence limits)			Naloxone antagonism AD_{50} mg/kg s.c. (95% confidence limits) Tail flick (rat)
	Hot plate (rat)	Tail flick (rat)	Abdominal constriction (mouse)	
(2)	0.015 (0.013–0.016)	0.0072 (0.005–0.0093)	0.007 (0.005–0.008)	0.032 (0.022–0.044)
(3)	0.017 (0.015–0.018)	0.011 (0.009–0.013)	0.015 (0.011–0.019)	0.017 (0.01–0.028)
(4)	0.015 (0.01–0.022)	0.014 (0.01–0.016)	0.015 (0.008–0.025)	0.056 (0.029–0.10)
Morphine	4.7 (3.6–6.11)	1.8 (1.28–2.52)	0.69 (0.49–0.96)	0.024 (0.018–0.031)

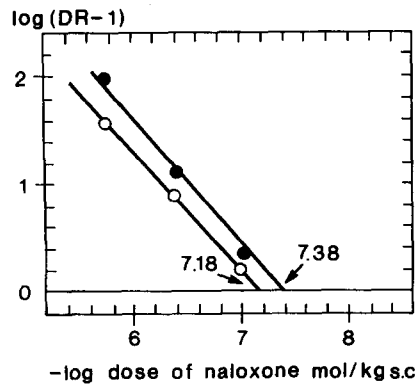

Fig. 2. Apparent pA_2 plots using naloxone and morphine (○) and naloxone and compound (2) (●) in the tail flick test. The −log dose of naloxone mol/kg is plotted against log (dose ratio − 1) (see text for details).

tion in several analgesic assays. The compounds were approximatively 300 times more potent, in the hot plate, 130–250 times more potent in the tail flick, and 50–100 times more active in the writhing test than morphine, when injected s.c.

3.3.1. Naloxone antagonism

The sensitivity of the compounds toward naloxone antagonism was demonstrated using the tail flick test (table 6). The AD_{50} values for naloxone were 0.032, 0.017 and 0.056 with compounds (2), (3) and (4), respectively, and 0.024 when morphine was used as agonist, demonstrating a high sensitivity of the alkoxymetopon derivatives for naloxone antagonism comparable to that of morphine.

The in vivo equivalent of the pA_2 (apparent pA_2) value was determined for naloxone vs. compound (2) and morphine (fig. 2). The apparent pA_2 values of naloxone, against morphine and compound (2) for tail flick analgesia were not significantly different: 7.18 and 7.38 respectively.

3.4. Sedative and cataleptic effect

Sedative and cataleptic effects are given in table 7. All three test compounds produced dose-dependent muscle rigidity by s.c. administration. The ratios between the ED_{50}s required to induce catalepsy and analgesia were 8.3, 2.0, 5.0 and 2.5 for compounds (2), (3), (4) and for morphine respectively. Compounds (2) and (4) potentiated barbiturate narcosis in their analgesic dose range, while higher doses were required in the case of compound (3).

TABLE 8

Development of physical dependence on compound (2) compared to morphine in mouse jumping test (Saelens et al., 1971).

Drug	Treatment i.p. (mg/kg)	n	Withdrawal jumping jumps No. jumped/treated	
			Mean ± S.E.M.	%
(2)	7×0.1	30	5.52 ± 1.7	30
	7×0.5	30	10.9 ± 2.4	70
	7×1.0	30	48.9 ± 5.2	90
Morphine	7×10.0	60	18.7 ± 0.9	78
	7×100.0	50	34.5 ± 1.4	100
Saline	7×5 ml/kg	10	0.0	0

3.5. Physical dependence

3.5.1. In mice

Naloxone elicited maximal jumping responses in morphine-dependent mice: compound (2) induced a lower degree of physical dependence, than morphine (38 and 78% showed jumping reaction, respectively) when animals were treated 7 times with doses equipotent to morphine (15 times their analgesic ED_{50} in mice: 0.1 and 10 mg/kg, respectively). Only the higher doses of compound (2) administered (7 × 1 mg/kg, 142 times ED_{50}) induced maximal jumping responses (table 8).

3.5.2. In rats

Naloxone induced abstinence scores obtained on the 11th day with each test agent are shown in table 9. Negligible syndromes (scores of < 2) were precipitated by naloxone in rats receiving saline and compounds (2), (3), (4), while a high abstinence score 7.2 was observed with rats receiving morphine: a significant weight loss was only associated with morphine.

TABLE 7

Comparison of the sedative (narcosis potentiation) cataleptic and analgesic (rat tail flick, RTF) effects (ED_{50}, mg/kg s.c. 95% confidence limits).

Drug	Catalepsy ED_{50} mg/kg s.c. (95% confidence limits)	Ratio of cataleptic vs. analgesic (RTF)ED_{50}	Sedative effects ED_{50} mg/kg s.c. (95% confidence limits)
(2)	0.06 (0.033–0.10)	8.3 [a]	0.0088 (0.006–0.012)
(3)	0.023 (0.016–0.032)	2.0	0.040 (0.025–0.064)
(4)	0.07 (0.058–0.084)	5.0 [a]	0.018 (0.010–0.03)
Morphine	4.5 (2.8–7.2)	2.5	0.7 (0.46–1.05)

[a] Significantly different from morphine $P < 0.05$.

TABLE 9

Abstinence scores precipitated by naloxone, weight loss, and the tolerance to antinociceptive action in rats receiving test agents twice daily for 11 days.

Compound	Abstinence score ± S.E. in the rat (max. = 10)	Weight loss (g) ± S.E.M.	Tolerance (rat tail flick)
Morphine	7.2 ± 1.3	12 ± 1.9	12.5 ± 0.5
(2)	1.4 ± 0.7 [a]	2.8 ± 1.5 [a]	1.8 ± 1.0 [b]
(3)	2.2 ± 1.1 [a]	3.5 ± 0.9 [b]	1.7 ± 0.9 [b]
(4)	1.5 ± 0.6 [b]	3.1 ± 1.4 [a]	2.2 ± 1.1 [a]

Significantly different from morphine [a] $P < 0.05$; [b] $P < 0.01$.

3.6. Tolerance

On day 11, the magnitude of tolerance was assessed in each group by establishing dose-response curves and ED_{50} values for morphine and test compounds. In morphine-treated animals, the morphine ED_{50} increased by a factor of 12.5 as compared to that for saline-treated animals, whereas the ED_{50} of compounds (2), (3), (4) shifted by a factor of approximatively 2, as indicated in table 9. Thus, a significantly greater shift (tolerance ratio) was observed in morphine-treated than in test compound-treated animals as compared to control animals. Therefore, in contrast to morphine, two treatments daily with increasing doses in groups treated by compounds (2), (3), (4) failed to induce significant tolerance to the antinociceptive action.

4. Discussion

Both biochemical and pharmacological assays provided further evidence for the strong opioid agonist effects of 14-alkoxymetopons.

The ligands exhibited high affinity for the [^{3}H]naloxone binding sites (K_i values under nanomolar range). Extremely high sodium indexes were found in each case, indicating the high agonist potency of these compounds.

Compounds (2), (3) and (4) have been shown to possess potent, naloxone-reversible antinociceptive properties. The 14-OCH_3- and 14-OC_2H_5-substituted compounds were remarkably more potent than morphine, by a factor of 130–300 in the rat and 50–100 in the mouse. Compounds (2), (3) and (4) all significantly prolonged barbiturate narcosis, similarly to morphine, close to their expected antinociceptive dose range, except for compound (3) which was active at a higher dose. All three test compounds produced dose-dependent catalepsy by s.c. administration. The ratio between the ED_{50} required to induce catalepsy and that for analgesia was 8.3 and 5.0 times higher for compound (2) and (4) than for morphine while an identical ratio was found for morphine, and compound (3): 2.5 and 2.0 respectively. Compounds (2), (3) and (4) exhibited a potent agonistic action on isolated electrically stimulated GPI preparations as well.

When receptor selectivity of the ligands was tested with tritiated DAMGO, DSLET and U69.593, the highest affinity was for the μ sites. DSLET competed with much less affinity whereas the ligands were least potent at the κ sites. The relative affinity constants for the μ sites estimated according to Kosterlitz and Paterson (1980) were found to be 96.4, 95.4, and 88.7 for compounds (2), (3) and (4), respectively. This means that the 14-alkoxymetopons tested, label the μ sites almost exclusively. Further proof for their μ selectivity was obtained when sensitivity versus naloxone antagonism was investigated. It was found that very low doses of naloxone (0.017–0.056 mg/kg) were able to antagonize their analgesic effect in the tail flick test. This suggests an interaction with the μ opioid receptor, since it is widely accepted that this subtype is the most sensitive to the antagonist action of naloxone (Millan et al., 1989; Stein et al., 1989).

Additional evidence for the supposed interaction with μ receptors can be assumed from the pA_2 plot. Our analgesic studies demonstrated that compound (2) and morphine might interact with the same receptor population, since similar pA_2 values could be estimated in the presence of naloxone. The actual value for morphine was similar to those reported from other studies (Tallarida et al., 1979; Fürst and Knoll, 1985).

On the other hand, the alkoxymetopon derivatives differ from morphine in their physical dependence capacity as estimated in the mouse withdrawal jumping test: a dose of naloxone as high as 50 mg/kg i.p. precipitates only a very low incidence of repetitive jumping in this species when (2) is run in the primary dependence test described by Saelens et al. (1971). There was no significant sign of withdrawal when dependence was elicited by the administration of the 15-fold analgesic dose of compound (2), while treatment with an equivalent dose of morphine produced significant withdrawal jumping (70% of the animals). Only treatment with 75 times or 142 times the ED_{50} dose of compound (2) evoked a similar degree of dependence.

Compounds (2), (3), (4) were found to induce minimal physical dependence producing liability only after 11 days of treatment with an increasing dose which was approximately 100–125 times more than their ED_{50} by Buckett's method (1964), in the rat also. The negligible syndromes in rats that received compounds (2), (3), (4) were characterised mainly by the μ-like signs, e.g. wet dog shakes (Cowan et al., 1988), but quantitatively different levels of abstinence might exist. These results are consistent with the hypothesis that the develop-

ment of dependence on opiates is associated with μ opioid receptors (Bhargava and Gulati, 1990).

The low degree of tolerance observed after prolonged treatment with these compounds, is consistent with the possibility of such quantitative differences. This assumption also might gain support from the proposal of Sosnowski and Yaksh (1990) that high affinity compounds, which occupy only a small fraction of receptors, will down-regulate fewer receptors and induce tolerance only after a longer period of treatment. In contrast, the low affinity compounds, e.g. morphine, have a smaller receptor reserve which in turn induces tolerance after a relatively short period of treatment.

In summary, our present findings demonstrate a favourable dissociation between analgesic activity and dependence liability and tolerance-inducing capacity of the 14-alkoxymetopon derivatives studied.

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References

Bhargava, H.N. and A. Gulati, 1990, Down-regulation of brain and spinal cord μ-opiate receptors in morphine tolerant-dependent rats, Eur. J. Pharmacol. 190, 305.

Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of microgramm quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248.

Buckett, W.P., 1964, A new test for morphine-like physical dependence (addiction liability) in rats, Psychopharmacologia 6, 410.

Buzás, B., G. Tóth, S. Cavagnero, V.J. Hruby and A. Borsodi, 1992, Synthesis and binding characteristics of the highly δ-specific new tritiated opioid peptide, [^{3}H]deltorphin II, Life Sci. 50, 75.

Cowan, A., X.Z. Zhu, H.I. Mosberg, J.R. Omnaas and F. Porreca, 1988, Direct dependence studies in rats with agents selective for different types of opioid receptor, J. Pharmacol. Exp. Ther. 246, 950.

D'Amour, F.E. and D.L. Smith, 1941, A method for determining loss of pain sensation, J. Pharmacol. Exp. Ther. 72, 74.

Fürst, S. and J. Knoll, 1985, Differentation of multiple analgesic opiate receptors, Int. J. Tissue Reac. (VII) 1, 69.

Hayashi, G. and A.E. Takemori, 1971, The type of analgesic-receptor interaction involved in certain analgesic assays, Eur. J. Pharmacol. 35, 63.

Hendershot, L. and J. Forsaith, 1959, Antagonism of the frequency of phenylbenzoquinone induced writhing in the mouse by weak analgesics and non-analgesics, J. Pharmacol. Exp. Ther. 125, 237.

Jacobson, A.E., F.L. Hui, M.D. Rozwadowska and H. Schmidhammer, 1981, Structure-activity relationships of oxygenated morphinans I.: 4-mono- and 3,4-dimethoxy-N-methylmorphinans and -N-methylmorphinon-6-ons with unusually high antinociceptive potency, Helv. Chim. Acta 64, 1298.

Kosterlitz, H.W. and S.J. Paterson, 1980, Characterization of opioid receptors in nervous tissues, Proc. R. Soc. London Ser. B. 210, 113.

Millan, M.J., A. Czlonkowski and A. Herz, 1989, Kappa opioid receptor mediated antinociception in the rat II.: Supraspinal in addition to spinal sites in action, J. Pharmacol. Exp. Ther. 251, 342.

Morpurgo, C., 1962, Effect of anti-parkinson drugs on phenotiazine induced catatonic reaction, Arch. Int. Pharmacodyn. 137, 84.

Munson, P.J. and D. Rodbard, 1980, LIGAND: a versatile computerized approach for characterization of ligand binding systems, Anal. Biochem. 107, 220.

Paton, W.D.M. and E.S. Vizi, 1969, The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea pig longitudinal muscle strips, Br. J. Pharmacol. 35, 10.

Saelens, J.K., F.R. Granat and W.K. Sawyer, 1971, The mouse jumping test – A simple screening method to estimate the physical dependence capacity of analgesics, Arch. Int. Pharmacodyn. 190, 213.

Schmidhammer, H., A.E. Jacobson and A. Brossi, 1983, Chemical and biological study of aromatic oxygenated 6-ketomorphinans, Med. Res. Rev. 3, 1.

Schmidhammer, H., L. Aeppli, L. Atwell, F. Fritsch, A.E. Jacobson, N. Nebuchla and G. Sperk, 1984, Synthesis and biological evaluation of 14-alkoxymorphinans. 1. Highly potent opioid agonists in the series of (−)-14-methoxy-N-methylmorphinan-6-ones, J. Med. Chem. 27, 1575.

Schmidhammer, H., A. Schratz and J. Mitterdorfer, 1990, Synthesis and biological evaluation of 14-alkoxymorphinans: 14-methoxymetopon, an extremely potent opioid agonist, Helv. Chim. Acta 73, 1784.

Simon, J., S. Benyhe, K. Abutidze, A. Borsodi, M. Szucs and M. Wollemann, 1986, Kinetics and physical parameters of rat brain opioid receptors solubilized by digitonin and CHAPS, J. Neurochem. 46, 695.

Sosnowski, M. and T.L. Yaksh, 1990, Differential cross tolerance between intrathecal morphine and sufentanil in the rat, Anesthesiology 73, 1141.

Stein, C., M.J. Millan, T.S. Shippenberg, K. Peter and A. Herz, 1989, Peripheral opioid receptors mediating antinociception in inflammation. Evidence for involvement of μ, δ and κ receptors, J. Pharmacol. Exp. Ther. 248, 1269.

Tallarida, R.J., A. Cowan and M.W. Adler 1979, pA_2 and receptor differentiation: a statistical analysis of competitive antagonism, Life Sci. 25, 637.

Tóth, G., M. Krámer, F. Sirokmán, A. Borsodi and A. Rónai, 1982, Preparation of (7,8,19,20-^{3}H)naloxone of high specific activity, J. Label. Comp. Radiopharm. 19, 1021.

Wolf, C. and A.D. McDonald, 1944, The evaluation of analgesic action of pethidine hydrochloride (Demerol), J. Pharmacol. Exp. Ther. 80, 300.