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Antagonism of heroin and morphine self-administration in rats by the morphine-6β-glucuronide antagonist 3-*O*-methylnaltrexone

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Abstract

In mice, 3-*O*-methylnaltrexone blocks the analgesic actions of morphine-6β-glucuronide and heroin at doses which are inactive against morphine. We found a similar selectivity in rats. 3-*O*-Methylnaltrexone antagonized the analgesic actions of 6-acetylmorphine in Sprague–Dawley rats and heroin in Wistar rats at doses that were inactive against morphine. Inclusion of a fixed dose of 3-*O*-methylnaltrexone significantly shifted the analgesic dose–response curves for 6-acetylmorphine and heroin without altering the morphine dose–response curves. In a self-administration model, 3-*O*-methylnaltrexone treatment significantly increased both heroin and morphine intake during the first hour, suggestive of an antagonist effect. This effect at doses of 3-*O*-methylnaltrexone which were inactive against morphine analgesia implied a role for the morphine-6β-glucuronide opioid receptor in the reinforcing properties of heroin and morphine.

Keywords: Heroin; Addiction; Self-administration; Morphine; Morphine-6-glucuronide; Drug abuse

1. Introduction

Understanding the receptor mechanisms responsible for the addictive potential of drugs is an essential component of research on this pressing social problem. Most abused opioids act through μ-opioid receptors. However, the demonstration of pharmacologically distinct subtypes of μ-opioid receptors (Pasternak, 1993), along with the cloning of a number of μ-opioid receptor splice variants (Bare et al., 1994; Zimprich et al., 1995; Pan et al., 1999), offers the possibility that the addictive actions of drugs may reside in a subset of the μ-opioid receptors. This hypothesis took on greater potential significance with the demonstration of pharmacological differences between morphine and heroin and the suggestion that heroin acts in large part through the morphine-6β-glucuronide opioid receptor (Pasternak and Standifer, 1995; Rossi et al., 1995b, 1996, 1997; Brown et al., 1997). Evidence for these receptor subtypes comes from a variety of sources. Morphine is inactive supraspinally in the CXBK strain of mice (Baron et al., 1975; Reith et al., 1981). Yet, heroin and its active metabolite 6-acetylmorphine retain their analgesic potency in these same mice (Rossi et al., 1996). At the molecular level, antisense mapping studies also readily distinguish between morphine and heroin analgesia. Antisense probes targeting exon 1 of the cloned μ-opioid receptor (*MOR-1*) diminish morphine analgesia, but not that of morphine-6β-glucuronide. Conversely, additional antisense probes based upon exon 2 lower morphine-6β-glucuronide analgesia without interfering with morphine. These same exon 2 probes also block the analgesic actions of heroin and its active metabolite 6-acetylmorphine (Rossi et al., 1995a,b, 1996, 1997). Finally, the discovery that 3-*O*-methylnaltrexone can antagonize morphine-6β-glucuronide analgesia at doses which are inactive against morphine (Brown et al., 1997) has provided another tool to address the question of receptor selectivity of drug action. Self-administration of drugs is a powerful tool to assess the reinforcing potential of compounds (Woolverton and Schuster, 1983). Both morphine and heroin are readily self-administered by rats

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(Weeks and Collins, 1964; Blakesley et al., 1972). Competitive opioid antagonists increase the rate of self-administration (decreasing the inter-injection interval) on doses of the descending limb of the dose–effect function, presumably to surmount the antagonism of opioid receptors (Ettenberg et al., 1982). In the current studies, we examined the effects of 3-*O*-methylnaltrexone on heroin and morphine self-administration to assess the receptor selectivity of the reinforcing actions of these drugs.

2. Materials and methods

Opioid naïve male Sprague–Dawley and Wistar rats (180–220 g; Charles River Laboratories, Kingston, NY) were used in the analgesic studies. All drugs were a generous gift from the Research Technology branch of the National Institute on Drug Abuse. Drugs were administered subcutaneously (s.c.) and analgesia assessed in the radiant heat tailflick assay, as previously reported (Rossi et al., 1993, 1995a). Baseline latencies ranged from 2 to 3 s. A maximal latency cutoff of 10 s was used to minimize any tissue damage to the tail. Antagonists were administered immediately prior to the agonist, which was given 30 min prior to tailflick testing. Results are the means ± S.E.M. of the tailflick latencies.

Eleven male Wistar rats (450–550 g at the start of the study) were used for the self-administration experiments. Rats were housed in groups of two per cage, and maintained on a 12-h light/dark cycle. All self-administration sessions were performed during the their active (dark) phase. Rats were trained to lever-press for food on a FR 1 time-out (TO) 20 s schedule prior to implantation of jugular catheters. Catheters were implanted as described (Ahmed and Koob, 1997) and rats were allowed to recover seven days before the start of self-administration sessions. During recovery rats were intravenously given the antibiotic Timentin© (10 mg ticarcillin/0.33 mg clavulanate in 0.1 ml; SmithKline Beecham Pharmaceuticals, Philadelphia, PA) daily. Catheters were flushed daily with heparinized saline (0.1 ml; 3 units heparin/ml) before and after the self-administration sessions. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Seven rats were used for heroin self-administration and four for the morphine studies. All rats had a history of heroin self-administration and three rats had a history of high levels of opioid exposure. These groups were combined because their baseline heroin (10 μg/infusion) self-administration intakes immediately before initiating these experiments for the high-opioid exposure rats (22.0 ± 1.4 infusions/h) and the others (21.9 ± 6.2) were not appreciably different. In addition, naloxone (0.03 mg/kg, s.c.) administration increased heroin self-administration to an equal degree for the low-exposure (180 ± 30.8% of s.c. saline) and high-exposure groups (187 ± 30.0% of s.c. saline), indicating that sensitivity to heroin was not altered by the history of opioid exposure.

In the heroin group, rats self-administered 10 μg/infusion on a FR1 TO 20 s schedule for 3 h/day. Self-administration behavior was stable after 5 days, with mean of three consecutive days varying only by approximately 10%. Rats were given each 3-*O*-methylnaltrexone dose (s.c.) in a Latin Square design immediately before starting the self-administration sessions. Two baseline days with s.c. saline injections were included between each dose of 3-*O*-methylnaltrexone.

In the morphine group, rats self-administered 300 μg/infusion in 3-h sessions. This dose was shown to have equivalent reinforcing efficacy to a 10-μg heroin dose based on an operant place conditioning procedure (Hutto and Crowder, 1997). Rats achieved baseline criteria after nine sessions with the mean of three consecutive days varying only by approximately 10%. Rats were each given 3-*O*-methylnaltrexone dose (s.c.) in a Latin square design immediately before the start of the self-administration sessions. Two baseline days with s.c. saline injections were included between each dose.

The number of infusions for the first hour of each self-administration session was compared between different drug treatments. Data was analyzed by one-way analysis of variance (ANOVA) with dose as the repeated measure. Post hoc analysis was made with the Student's *t*-test. ED_{50} and ID_{50} were calculated as graded dose–response curves, as described in Tallarida and Murray (1987).

3. Results

In mice, 3-*O*-methylnaltrexone reverses the analgesic actions of morphine-6-glucuronide and heroin more potently than those of morphine (Brown et al., 1997). First, we explored whether 3-*O*-methylnaltrexone had a similar selectivity in rats. The sensitivity of equianalgesic doses of morphine and heroin to 3-*O*-methylnaltrexone in Wistar rats differed by more than 10-fold (Fig. 1a). Heroin analgesia was reduced by doses of 3-*O*-methylnaltrexone under 0.1 mg/kg, s.c. and the response approached baseline latencies by 0.25 mg/kg, s.c. In contrast, the same 3-*O*-methylnaltrexone dose had minimal effects on the morphine response. We then performed morphine and heroin dose–response curves in the absence and presence of a fixed 3-*O*-methylnaltrexone dose (0.25 mg/kg, s.c.) (Fig. 1b). The inclusion of 3-*O*-methylnaltrexone significantly shifted the heroin dose–response curve about three-fold to the right, as expected for a competitive antagonist. The morphine analgesic dose–response curve was unaffected by the antagonist at this dose.

Sprague–Dawley rats revealed similar results comparing 6-acetylmorphine, the active component of heroin, and

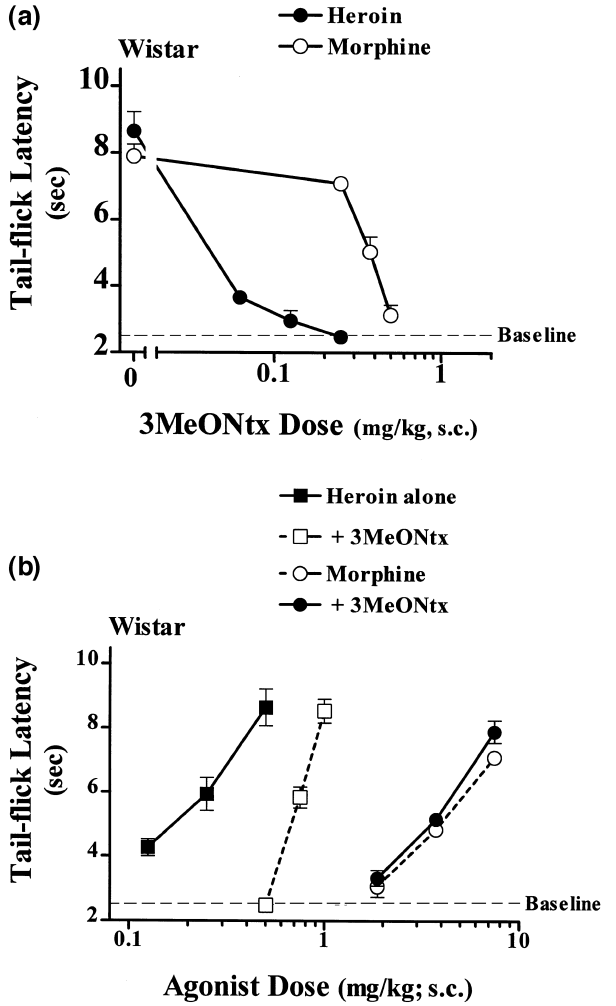

Fig. 1. Effect of 3-*O*-methylnaltrexone in Wistar rats. (a) Groups of Wistar rats ($n = 5$) were administered either morphine (7.5 mg/kg, s.c.) or heroin (0.5 mg/kg, s.c.) and the indicated dose of 3-*O*-methylnaltrexone (3MeONtx). Analgesia was assessed 30 min later. Results are presented as the means ± S.E.M. The ID_{50} for 3-*O*-methylnaltrexone was 0.33 mg/kg (0.21, 0.55) against morphine and 0.02 mg/kg (0.009, 0.08) against heroin. (b) Groups of Wistar rats ($n = 5$) were given the indicated doses of morphine or heroin alone or in combination with a fixed dose of 3-*O*-methylnaltrexone (0.25 mg/kg, s.c.). Results are presented as the means ± S.E.M. The morphine ED_{50} in was 4.3 mg/kg (1.9, 10.3) in control rats and 5.1 mg/kg (1.7, 16.2) in 3-*O*-methylnaltrexone-treated rats. The heroin ED_{50} was 0.32 mg/kg (0.10, 0.53) in control rats and 0.9 mg/kg (0.61, 1.4) in 3-*O*-methylnaltrexone-treated rats.

morphine (Fig. 2). At equianalgesic agonist doses, 3-*O*-methylnaltrexone lowered the analgesic response to 6-acetylmorphine almost 10-fold more effectively than morphine (Fig. 2a). Including a fixed dose of 3-*O*-methylnaltrexone (0.25 mg/kg, s.c.) significantly shifted the 6-acetylmorphine dose–response curve four-fold without affecting that of morphine (Fig. 2b).

In the self-administration studies, s.c. 3-*O*-methylnaltrexone dose-dependently increased heroin self-administration during the first hour ($F_{(3,18)} = 10.3$, $P < 0.001$). The lowest 3-*O*-methylnaltrexone dose showed a small increase in the number of infusions, although it did not reach statistical significance. However, the responses seen with the other two doses were significantly greater ($P < 0.01$, *t*-test) (Fig. 3a). Morphine self-administration was also dose-dependently increased by 3-*O*-methylnaltrexone during the first hour ($F_{(3,9)} = 12.1$, $P < 0.01$) with significant increases with higher two doses ($P < 0.01$, *t*-test). The effects of 3-*O*-methylnaltrexone on morphine were limited to the first of the sessions ($P < 0.05$, *t*-test) (Fig. 3b). The numbers of infusions in the second and third hour were similar to those seen in the saline group and were not

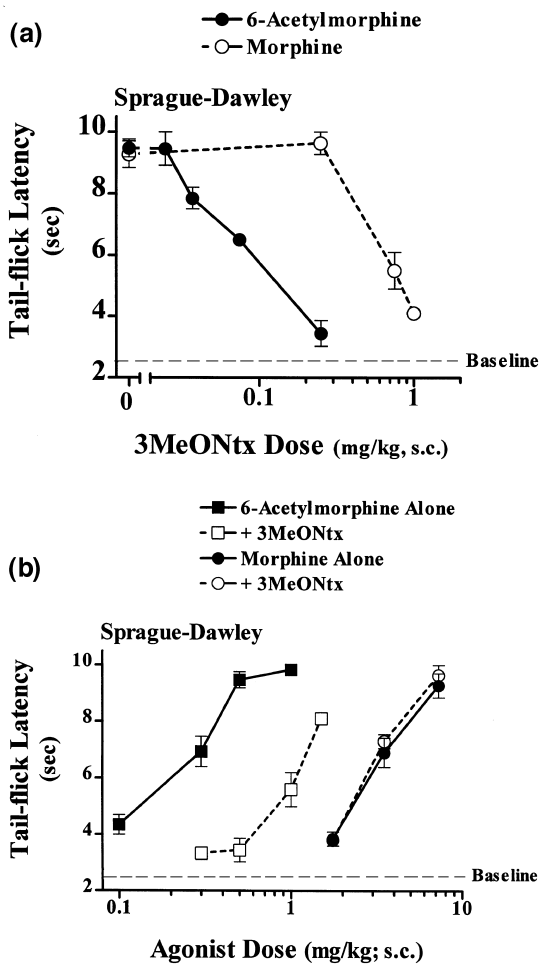

Fig. 2. Effect of 3-*O*-methylnaltrexone in Sprague–Dawley rats. (a) Groups of Sprague–Dawley rats ($n = 5$) were administered either morphine (7.5 mg/kg, s.c.) or 6-acetylmorphine (0.5 mg/kg, s.c.) and the indicated dose of 3-*O*-methylnaltrexone. Results are presented as the means ± S.E.M. The ID_{50} for 3-*O*-methylnaltrexone was 0.48 mg/kg (0.3, 0.7) against morphine and 0.05 mg/kg (0.02, 0.1) against heroin. (b) Groups of Sprague–Dawley rats ($n = 5$) were given the indicated doses of morphine or 6-acetylmorphine (0.5 mg/kg, s.c.) alone or in combination with a fixed dose of 3-*O*-methylnaltrexone (0.25 mg/kg, s.c.). Results are presented as the means ± S.E.M. The morphine ED_{50} in was 2.9 mg/kg (0.9, 9.1) in control rats and 2.5 mg/kg (0.3, 9.7) in 3-*O*-methylnaltrexone-treated rats. The 6-acetylmorphine ED_{50} in was 0.26 mg/kg (0.06, 0.85) in control rats and 1.1 mg/kg (1.0, 1.3) in 3-*O*-methylnaltrexone-treated rats.

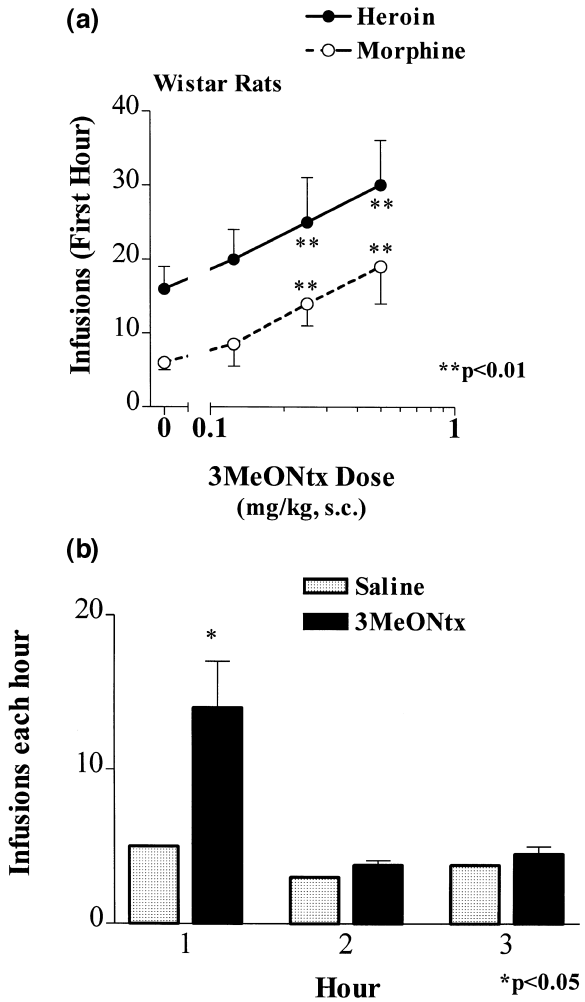

Fig. 3. Effect of 3-*O*-methylnaltrexone on heroin self-administration. (a) S.c. administration of 3-*O*-methylnaltrexone (saline vehicle, 0.125, 0.25, or 0.5 mg/kg) in a Latin Square design immediately before 3-h heroin (10 μg/infusion) or morphine (300 μg/infusion) sessions dose-dependently increased the number of heroin and morphine infusions self-administered during the first hour, $^{*}P < 0.01$ by post hoc Student's *t*-test. (b) S.c. administration of 3-*O*-methylnaltrexone (0.25 mg/kg, s.c.) immediately before a session increased the number of morphine self-infusions only during the first hour of a three self-administration session. The number of self-infusions during the first hour, the second hour, and the third hour were compared between saline vehicle and 3-*O*-methylnaltrexone-treated (0.25 mg/kg) rats, $^{*}P < 0.05$ by post hoc Student's *t*-test.

influenced by 3-*O*-methylnaltrexone. Similar results were seen with heroin self-administration. This may reflect a short duration of action of 3-*O*-methylnaltrexone.

4. Discussion

3-*O*-Methylnaltrexone is an unusual opioid antagonist. Unlike traditional antagonists, 3-*O*-methylnaltrexone is moderately selective for morphine-6β-glucuronide, heroin and 6-acetylmorphine analgesia. The original analgesic studies performed in mice have now been replicated in both Sprague–Dawley and Wistar rats. In both strains, 3-*O*-methylnaltrexone blocked the actions of heroin and 6-acetylmorphine far more effectively than morphine. Thus, 3-*O*-methylnaltrexone may provide a helpful tool in exploring the receptor mechanisms of opioid agonist action.

3-*O*-Methylnaltrexone also altered morphine and heroin self-administration, as seen by the increased heroin self-administration rate. At a dose which was ineffective against morphine analgesia (0.25 mg/kg), 3-*O*-methylnaltrexone significantly elevated the infusion rates. The activity of low 3-*O*-methylnaltrexone doses in the self-administration paradigm implied that the receptors involved were different from those involved with morphine analgesia and might correspond to those implicated in heroin and morphine-6β-glucuronide analgesia. More surprisingly, morphine reinforcement was as sensitive as heroin to the antagonist, implying that the morphine-6β-glucuronide sites were important for morphine reinforcement as well. Since mice and rats do not appreciably metabolize single doses of morphine into morphine-6-glucuronide (Inturrisi et al., 1996), morphine was probably interacting directly with the morphine-6β-glucuronide site in the current studies. However, the reinforcing properties of morphine clinically may involve both morphine and morphine-6β-glucuronide, which is a major morphine metabolite in humans (Sawe et al., 1985). This is particularly intriguing in view of the higher potency of morphine-6β-glucuronide in conditioned place preference, a measure of reinforcing properties (Abbott and Franklin, 1991).

The suggestion that the reinforcing actions of heroin and morphine are mediated through a receptor distinct from that responsible for morphine analgesia has major implications in our approach towards the development of novel analgesics and the treatments for drug abuse. 3-*O*-Methylnaltrexone is moderately selective for the morphine-6β-glucuronide site and can block reinforcement at doses which do not interfere with morphine analgesia. This raises the question of whether 3-*O*-methylnaltrexone or related agents might be able to block the reinforcing activity of the clinically used opioids. Furthermore, it suggests that it may be possible to design analgesics with far less reinforcing properties.

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References

Abbott, F.V., Franklin, K.B., 1991. Morphine-6-glucuronide contributes to rewarding effects of opiates. Life Sci. 48, 1157–1163.

Ahmed, S.H., Koob, G.F., 1997. Cocaine — but not food-seeking behavior is reinstated by stress after extinction. Psychopharmacology 132, 289–295.

Bare, L.A., Mansson, E., Yang, D., 1994. Expression of two variants of the human μ opioid receptor mRNA in SK-N-SH cells and human brain. FEBS Lett. 354, 213–216.

Baron, A., Shuster, L., Elefterhiou, B.E., Bailey, D.W., 1975. Opiate receptors in mice: genetic differences. Life Sci. 17, 633–640.

Blakesley, B.C., Dinneen, L.C., Elliott, R.D., Francis, D.L., 1972. Intravenous self-administration of heroin in the rat: experimental technique and computer analysis. Br. J. Pharmacol. 45, 181P–182P.

Brown, G.P., Yang, K., King, M.A., Rossi, G.C., Leventhal, L., Chang, A., Pasternak, G.W., 1997. 3-Methoxynaltrexone, a selective heroin/morphine-6β-glucuronide antagonist. FEBS Lett. 412, 35–38.

Ettenberg, A., Pettit, H.O., Bloom, F.E., Koob, G.F., 1982. Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacology 78, 204–209.

Hutto, C.W.J., Crowder, W.F., 1997. Dosage choices of rats for morphine, for heroin, and between morphine and heroin. Pharmacol. Biochem. Behav. 58, 133–140.

Inturrisi, C.E., Yoburn, B.C., Portenoy, R.K., Foley, K.M., 1996. Species dependent formation of morphine-6-glucuronide (M-6-G) from morphine (MOR). Comm. Probl. Drug Depend. 174, 157.

Pan, Y.-X., Xu, J., Bolan, E.A., Abbadie, C., Chang, A., Zuckerman, A., Rossi, G.C., Pasternak, G.W., 1999. Identification and characterization of three new alternatively spliced mu opioid receptor isoforms. Mol. Pharmacol. 56, 396–403.

Pasternak, G.W., 1993. Pharmacological mechanisms of opioid analgesics. Clin. Neuropharmacol. 16, 1–18.

Pasternak, G.W., Standifer, K.M., 1995. Mapping of opioid receptors using antisense oligodeoxynucleotides: correlating their molecular biology and pharmacology. Trends Pharmacol. Sci. 16, 344–350.

Reith, M.E.A., Sershen, H., Vadasz, C., Lajtha, A., 1981. Strain differences in opiate receptors in mouse brain. Eur. J. Pharmacol. 74, 377–380.

Rossi, G.C., Pasternak, G.W., Bodnar, R.J., 1993. Synergistic brainstem interactions for morphine analgesia. Brain Res. 624, 171–180.

Rossi, G.C., Standifer, K.M., Pasternak, G.W., 1995a. Differential blockade of morphine and morphine-6β-glucuronide analgesia by antisense oligodeoxynucleotides directed against MOR-1 and G-protein α subunits in rats. Neurosci. Lett. 198, 99–102.

Rossi, G.C., Pan, Y.-X., Brown, G.P., Pasternak, G.W., 1995b. Antisense mapping the MOR-1 opioid receptor: evidence for alternative splicing and a novel morphine-6β-glucuronide receptor. FEBS Lett. 369, 192–196.

Rossi, G.C., Brown, G.P., Leventhal, L., Yang, K., Pasternak, G.W., 1996. Novel receptor mechanisms for heroin and morphine-6β-glucuronide analgesia. Neurosci. Lett. 216, 1–4.

Rossi, G.C., Leventhal, L., Pan, Y.X., Cole, J., Su, W., Bodnar, R.J., Pasternak, G.W., 1997. Antisense mapping of MOR-1 in the rat: distinguishing between morphine and morphine-6β-glucuronide antinociception. J. Pharmacol. Exp. Ther. 281, 109–114.

Sawe, J., Kager, L., Svensson, B., Eng, J.O., Rane, A., 1985. Oral morphine in cancer patients: in vivo kinetics and in vitro hepatic glucuronidation. Br. J. Clin. Pharmacol. 19, 495–505.

Tallarida, R.J., Murray, R.B., 1987. Manual of Pharmacological Calculations with Computer Programs. Springer-Verlag, New York, p. 153.

Weeks, J.R., Collins, R.J., 1964. Factors affecting voluntary morphine intake in self-maintained addicted rats. Psychopharmacologia 14, 267–279.

Woolverton, W.L., Schuster, C.R., 1983. Behavioral and pharmacological aspects of opioid dependence: mixed agonist-antagonists. Pharmacol. Rev. 35, 33–52.

Zimprich, A., Simon, T., Hollt, V., 1995. Cloning and expression of an isoform of the rat μ opioid receptor (rMOR-1 B) which differs in agonist induced desensitization from rMOR-1. FEBS Lett. 359, 142–146.