ELSEVIER

European Journal of Pharmacology 284 (1995) 227–230

ejp

Short communication

# μ- and δ-opioid receptor antisense oligodeoxynucleotides antagonize morphine-induced growth hormone secretion in rats

Juhana J. Idänpään-Heikkilä [a,*], Pekka Rauhala [a], Pekka T. Männistö [b]

[a] *Institute of Biomedicine, Department of Pharmacology and Toxicology, University of Helsinki, Helsinki, Finland*
[b] *Department of Medical Pharmacology, University of Uppsala, Uppsala, Sweden*

Received 25 July 1995; accepted 28 July 1995

**Abstract**

Effects of i.c.v. pretreatment with antisense oligodeoxynucleotides (antisense oligos) targeted against either μ- or δ-opioid receptors on morphine-induced release of growth hormone (GH) and prolactin were studied in male rats. The stimulation of GH secretion by i.c.v. morphine was completely inhibited by the antisense oligo targeted against the cloned μ-opioid receptor and significantly reduced by the antisense oligo targeted against the cloned δ-opioid receptor. The antisense oligo targeted against the cloned μ-opioid receptor, but not that targeted against the cloned δ-opioid receptor, abolished the stimulatory effect of acute morphine on prolactin secretion. It is concluded that both the GH and prolactin secretion stimulating effect of morphine is mainly mediated by the cloned μ-opioid receptor. Further, the cloned δ-opioid receptor is involved in the morphine-induced stimulation of GH secretion.

*Keywords:* Morphine; Antisense oligodeoxynucleotide; μ-Opioid receptor; δ-Opioid receptor; Growth hormone; Prolactin

## 1. Introduction

The secretion of anterior pituitary hormones is regulated by opiates and endogenous opioid peptides acting on μ-, δ- and κ-opioid receptors (Van Wimersma Greidanus and Grossman, 1991). The morphine-induced release of prolactin is mediated through the μ-opioid receptor (Bruni et al., 1977), more accurately through the $\mu_1$ subtype (Spiegel et al., 1982; Koenig et al., 1984). The effect of morphine on the secretion of growth hormone (GH), however, is somewhat conflicting. It is stimulated by morphine and this stimulation is partially antagonized by naloxone (Bruni et al., 1977), but not by the $\mu_1$-opioid receptor antagonist naloxazone (Spiegel et al., 1982). Yet, in another study, this stimulation was not antagonized by naloxone or β-funaltrexamine, another μ-opioid receptor antagonist, but was blocked by the δ-opioid receptor antagonist ICI 154,129 (*N*,*N*-bisallyl-Tyr-Gly-Gly-ψ-($CH_2$S)-Phe-Leu-OH, Koenig et al., 1984).

Molecular cloning of μ-, δ- and κ-opioid receptors has enabled the use of antisense oligos to manipulate these receptor systems. An antisense oligodeoxynucleotide (antisense oligo) is a short piece of synthetic DNA with a nucleotide sequence that is the reverse of and complementary to a part of a mRNA. It therefore binds to mRNA, prevents the translation of the mRNA and inhibits the synthesis of the protein. Antisense oligos have already been proven to be useful pharmacological tools for studying neurotransmitter receptor activities both in vitro and in vivo (Wahlestedt, 1994).

An antisense oligo against the cloned μ-opioid receptor, injected into the periaqueductal gray of the rat, completely blocked the antinociceptive actions of morphine (Rossi et al., 1994). In a recent study, an antisense oligo against the μ-opioid receptor, administered i.c.v., selectively reduced the antinociceptive effect of a μ-opioid receptor agonist, but not that of a δ- or κ-opioid receptor agonist. In the same study, i.c.v. administration of the antisense oligo against the μ-opioid receptor also reduced the antinociceptive effect of morphine administered s.c. (Chen et al., 1995). Furthermore, an antisense oligo against the cloned δ-opioid receptor administered i.c.v., has inhibited the

* Corresponding author. Institute of Biomedicine, Department of Pharmacology and Toxicology, P.O. Box 8, FIN-00014 University of Helsinki, Finland. Tel. 358-0-191 8268, fax 358-0-191 8288.

Elsevier Science B.V.
*SSDI* 0014-2999(95)00462-9

antinociceptive response to a δ-opioid receptor agonist (Bilsky et al., 1994).

The aim of the present study was to explicitly solve, using antisense oligos which are highly selective and specific against the cloned opioid receptors, the role of the cloned μ-opioid receptor and the cloned δ-opioid receptor in the morphine-induced secretion of prolactin and especially in the discrepant secretion of GH.

## 2. Materials and methods

The study protocol was approved by the Ethics Committee for Animal Research of the Department of Biomedicine, University of Helsinki.

### 2.1. Animals and surgical procedures

Under chloral hydrate anaesthesia (350 mg/kg i.p.) permanent stainless steel guide cannulas were implanted into the 3rd ventricle of male Wistar rats (Han/Kuopio, Finland, weight 200–250 g). The surgical procedure and i.c.v. injection techniques have been described in detail earlier (Männistö et al., 1984).

### 2.2. Oligodeoxynucleotide synthesis and pretreatment procedures

Oligodeoxynucleotides were synthesized by The Estonian Biocentre, Tartu, Estonia. The sequences of the oligos were as follows: μ-antisense, 5′-CGC CCC AGC CTC TTC CTC T-3′; μ-mismatch, 5′-CGC CCC *GA*C CTC TTC C*CT* T-3′ (Rossi et al., 1994); δ-antisense, 5′-AGA GGG CAC CAG CTC CAT-3′; δ-mismatch, 5′-*C*GA G*C*G CA*A* CAG CT*G* CAT-3′ (Bilsky et al., 1994). In addition, we used metabolically more stable phosphorothioate analogues (thio-antisense oligos) (Wahlestedt, 1994).

Either saline or 30 μg of oligodeoxynucleotide was injected i.c.v. in a volume of 10 μl on days 1 (3rd postoperative day), 3 and 5 (Rossi et al., 1994).

### 2.3. Acute hormonal experiments and analytical

On day 6 the experiment was carried out between 12.00 h and 13.00 h, 24 h after the last pretreatment injection. Morphine (3 or 10 μg, pharmacy of the University of Helsinki, Finland) or saline was injected i.c.v. in a volume of 2 μl. Rats were killed by decapitation 30 min after the i.c.v. injection and trunk blood was collected. Serum GH and prolactin concentrations were determined by specific radioimmunoassays as described recently (Rauhala et al., 1995a,b).

### 2.4. Antinociceptive study

In a separate pilot study, to ascertain the efficacy of the μ-oligos, pretreatments were as above (excluding the δ-oligos) and the antinociceptive effect of morphine (10 μg) administered i.c.v. was assessed using the tail-flick test. The data on the tail-flick latencies were converted to maximal possible effects in percent (MPE%) using the following formula: MPE% = 100 × (postdrug latency − predrug latency)/(cut-off time − predrug latency). An Ugo Basile (Comerio, Italy) analgesia meter and a cut-off time of 8 s were used.

### 2.5. Statistical analysis

Data are presented as the mean ± S.E.M. Analysis of variance followed by Newman-Keuls test was used to evaluate statistically the differences between means. The level of significance was set at $P < 0.05$.

## 3. Results

In the pilot study, pretreatment with saline or μ-mismatch oligo did not modify the antinociceptive effect of morphine (10 μg i.c.v.) in the tail-flick test, but pretreatment with both μ-antisense and μ-thio-antisense oligos reduced the analgesic effect of morphine. The MPE% values at 20 min after morphine were 94%, 98 ± 2%, 33 ± 14% and 27 ± 6% for the groups ($n = 2–3$) pretreated with saline, μ-mismatch, μ-antisense and μ-thio-antisense oligos, respectively.

The secretion of GH was stimulated after 3 μg of morphine in the group pretreated with saline, μ-mismatch or δ-mismatch oligos (Fig. 1a). Pretreatment with δ-antisense and δ-thio-antisense oligos significantly reduced the stimulatory effect of morphine on GH secretion whereas pretreatment with μ-antisense and μ-thio-antisense oligos completely inhibited the effect of morphine (Fig. 1a). Morphine at 10 μg did not stimulate GH secretion in any of the pretreatment groups (data not shown).

In the saline pretreated rats, morphine at 3 μg stimulated the secretion of prolactin (Fig. 1b). Pretreatment with μ-antisense and μ-thio-antisense oligos abolished this effect, but pretreatment with μ-mismatch, δ-mismatch, δ-antisense or δ-thio-antisense oligos did not modify the prolactin response (Fig. 1b). Similarly, morphine at 10 μg stimulated prolactin secretion in the rats pretreated with saline and this response was inhibited in the rats pretreated with μ-antisense oligo but not in the rats pretreated with μ-mismatch, δ-mismatch or δ-antisense oligos (data not shown).

## 4. Discussion

We have shown that i.c.v. pretreatment with μ-antisense and μ-thio-antisense oligo abolishes the GH

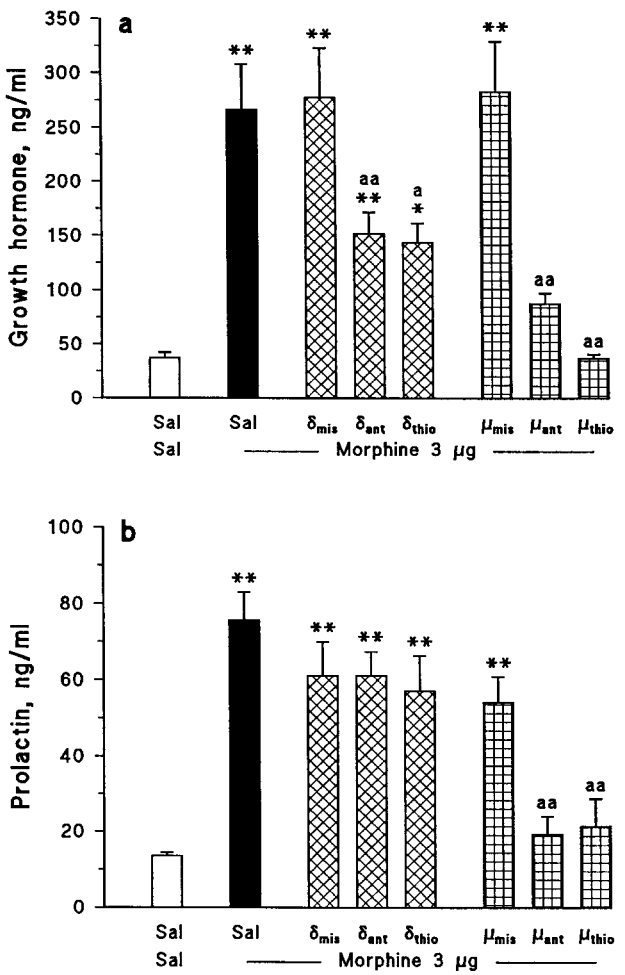


Fig. 1. The effect of pretreatment with saline (Sal), δ-mismatch ($\delta_{mis}$), δ-antisense ($\delta_{ant}$), δ-thio-antisense ($\delta_{thio}$), μ-mismatch ($\mu_{mis}$), μ-antisense ($\mu_{ant}$) or μ-thio-antisense ($\mu_{thio}$) oligodeoxynucleotide on morphine (3 μg i.c.v.)-induced increase in the serum levels of growth hormone (a) and prolactin (b). Mean ± S.E.M., $n = 6–8$ in each group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group (Sal, Sal), $^{a}P < 0.05$, $^{aa}P < 0.01$ compared with Sal + Morphine.

secretion stimulating effect of i.c.v. morphine, and that pretreatment with δ-antisense or δ-thio-antisense oligo strongly reduces this effect. The inactivity of both μ-mismatch and δ-mismatch antisense oligos supports the specificity of the responses. The μ-antisense oligo sequence used in our study is targeted against the cloned μ-opioid receptor gene and it has previously blocked morphine-induced antinociception (Rossi et al., 1994). Similarly, in our pilot study, the antinociceptive effect of i.c.v. morphine was blocked by the μ-antisense oligos. As $\mu_1$-opioid receptors mediate morphine analgesia, the cloned μ-opioid receptor gene may correspond to $\mu_1$-opioid receptor. However, alternative splicing in the processing of opioid receptor clones is possible. Therefore, the spliced mRNA species might contain the same sequence as the cloned μ-opioid receptor. Should this be true, these alternatively spliced mRNAs, possibly reflecting subtypes other than $\mu_1$-opioid receptor, also would be downregulated by the antisense oligos (Rossi et al., 1994). Therefore, no definite conclusions about the possible μ-opioid receptor subtype involved can be made.

According to our results the enhanced secretion of GH after morphine is partially mediated by the cloned δ-opioid receptor. The δ-antisense oligo sequence, used in our study, has previously been shown to block antinociception induced by i.c.v. $\delta_2$-opioid receptor agonists (Bilsky et al., 1994). These results therefore suggest that the cloned δ-opioid receptor, against which the antisense oligo is targeted, can be related to that pharmacologically classified as the $\delta_2$ subtype. As we used the same sequence, the enhanced secretion of GH after morphine may similarly be mediated by the $\delta_2$ subtype.

In the present study, the high dose of morphine did not stimulate GH secretion. This finding is consistent with the previous reports in which increasing peripheral doses of morphine have resulted in gradually decreasing secretion of GH (Simon et al., 1975; Pechnic et al., 1985; Rauhala et al., 1995a,b). Since serum GH levels are known to decrease in the rat following stress, a possible explanation for this observation is that the higher dose of mophine was stressful to the rats. High doses of morphine can also activate κ-opioid receptors which are known to inhibit GH secretion (Krulich et al., 1986). Further studies with κ-opioid receptor antisense oligos are obviously needed to clarify this issue.

Our results suggest that the cloned μ-opioid receptor mediates the morphine-induced release of prolactin. Previous studies with μ-opioid receptor antagonists suggest that the prolactin stimulating effect of morphine is mediated by μ-opioid receptors, and more precisely by the $\mu_1$ subtype (Bruni et al., 1977; Spiegel et al., 1982; Koenig et al., 1984). The cloned μ-opioid receptor may thus correspond to the $\mu_1$ subtype.

The treatment schedule used in this study, i.e. three injections over 5 days, was based on the report that the turnover rate of opioid receptors is 3–5 days (Standifer et al., 1994). The same parameters of antisense treatment have been used successfully in blocking the functions of NPY Y1, μ-, δ- and κ-opioid receptors (Chien et al., 1994; Rossi et al., 1994; Standifer et al., 1994; Wahlestedt, 1994; Chen et al., 1995).

In conclusion, we have shown using highly selective and specific antisense oligos, that the cloned μ-opioid receptor is an important mediator of the morphine-induced secretion of both prolactin and GH. In addition to the cloned μ-opioid receptor, the cloned δ-opioid receptor is involved in the morphine-induced secretion of GH. Further, the natural oligos are similarly useful as the thio-antisense oligos, at least when i.c.v. administration is used.

## Acknowledgements

The gift of the rat prolactin and growth hormone kits from Dr. A.F. Parlow and the National Institute of Diabetes and Digestive and Kidney Disease are greatly acknowledged. We appreciate the excellent technical help of Ms. Satu Mäkinen. This study was supported by the Academy of Finland (P.T.M.); the Finnish Medical Foundation (J.J.I.-H.); the Pharmacal Research Foundation, Finland (J.J.I.-H.); the Research and Science Foundation of Farmos, Finland (J.J.I.-H.) and the Sigrid Jusélius Foundation, Finland (P.T.M.).

## References

Bilsky, E.J., R.N. Bernstein, G.W. Pasternak, V.J. Hruby, D. Patel, F. Porreca and J. Lai, 1994, Selective inhibition of [D-Ala$^2$,Glu$^4$]deltorphin antinociception by supraspinal, but not spinal, administration of an antisense oligodeoxynucleotide to an opioid delta receptor, Life Sci. 55, PL37.

Bruni, J.F., D. Van Vugt, S. Marshall and J. Meites, 1977, Effects of naloxone, morphine and methionine enkephalin on serum prolactin, luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and growth hormone, Life Sci. 21, 461.

Chen, X.-H., J.U. Adams, E.B. Geller, J.K. DeRiel, M.W. Adler and L.-Y. Liu-Chen, 1995, An antisense ologodeoxynucleotide to $\mu$-opioid receptors inhibits $\mu$-opioid receptor agonist-induced analgesia in rats, Eur. J. Pharmacol. 275, 105.

Chien, C.-C., G. Brown, Y.-X. Pan and G.W. Pasternak, 1994, Blockade of U50,488H analgesia by antisense oligodeoxynucleotides to a $\kappa$-opioid receptor, Eur. J. Pharmacol. 253, R7.

Koenig, J.I., M.A. Mayfield, S.M. McCann and L. Krulich, 1984, Differential role of the opioid $\mu$ and $\delta$ receptors in the activation of prolactin (PRL) and growth hormone (GH) secretion by morphine in the male rat, Life Sci. 34, 1829.

Krulich, L., J.I. Koenig, S. Conway, S.M. McCann and M.A. Mayfield, 1986, Opioid $\kappa$ receptors and secretion of prolactin (PRL) and growth hormone (GH) in the rat. I. Effects of opioid $\kappa$ receptor agonists bremazocine and U-50,488 on secretion of PRL and GH: comparison with morphine, Neuroendocrinology 42, 75.

Männistö, P.T., P. Rauhala, R.K. Tuominen and J. Mattila, 1984, Dual action of morphine on cold-stimulated thyrotropin secretion in male rats, Life Sci. 35, 1101.

Pechnic, R., R. George and R.E. Poland, 1985, Identification of multiple opiate receptors through neuroendocrine responses. I. Effects of agonists, J. Pharmacol. Exp. Ther. 232, 163.

Rauhala, P., J.J. Idänpään-Heikkilä, A. Lang, R.K. Tuominen and P.T. Männistö, 1995a, Cold exposure attenuates effect of secretagogues on serum prolactin and growth hormone levels in male rats, Am. J. Physiol. 268, E758.

Rauhala, P., J.J. Idänpään-Heikkilä, R.K. Tuominen and P.T. Männistö, 1995b, Differential disappearence of tolerance to thermal, hormonal and locomotor effects of morphine in the male rat, Eur. J. Pharmacol. (in press).

Rossi, G., Y.-X. Pan, J. Cheng and G.W. Pasternak, 1994, Blockade of morphine analgesia by an antisense oligodeoxynucleotide against the mu receptor, Life Sci. 54, PL 375.

Simon, M., R. George and J. Garcia, 1975, Acute morphine effects on regonal brain amines, growth hormone and corticotropin, Eur. J. Pharmacol. 34, 21.

Spiegel, K., I.A. Kourides and G.W. Pasternak, 1982, Prolactin and growth hormone release by morphine in the rat: different receptor mechanisms, Science 217, 745.

Standifer, K.M., C.-C. Chien, C. Wahlestedt, G.P. Brown and G.W. Pasternak, 1994, Selective loss of $\delta$ opioid analgesia and binding by antisense oligonucleotides to a $\delta$ opioid receptor, Neuron 12, 805.

Van Wimersma Greidanus, T.B. and A.B. Grossman, 1991, Opioid regulation of pituitary function, Progr. Sens. Physiol. 12, 1.

Wahlestedt, C., 1994, Antisense oligonucleotide strategies in neuropharmacology, Trends Pharmacol. Sci. 15, 42.