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Morphine and Fentanyl Differently Affect MOP and NOP Gene Expression in Human Neuroblastoma SH-SY5Y Cells

Francesca Felicia Caputi · Francesca Lattanzio · Donatella Carretta · Daniela Mercatelli · Sanzio Candeletti · Patrizia Romualdi

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Abstract Morphine is widely used for the treatment of severe acute and chronic pain, but long-term therapy rapidly leads to tolerance. Morphine effects are mediated by μ opioid receptor (MOP) activation as well as for fentanyl that, in contrast to morphine, induces less tolerance to analgesia. The mechanisms underlying opioid tolerance involve complex processes, such as MOP desensitization, internalization, and/or changes of gene expression. The development of morphine tolerance also involves adaptive changes of the anti-opioid nociceptin/orphanin FQ–nociceptin receptor system, as suggested by the reduction of morphine tolerance in nociceptin opioid receptor (NOP) knockout mice. The aim of the present study was to investigate the MOP and NOP gene expression in the SH-SY5Y cells following morphine and fentanyl exposure. Results showed that cell exposure to 10 μM morphine for 5 h induced a significant decrease of MOP and NOP gene expression and that the MOP downregulation was reverted by the pretreatment with naloxone. Conversely, SH-SY5Y cells exposed to 0.1 and 1 μM fentanyl for 5 and 72 h showed a significant MOP upregulation, also reverted by naloxone pretreatment. Fentanyl induced no changes of NOP gene expression. The present findings showed a different effect by morphine and fentanyl on MOP mRNA levels that contributes to define the role of MOP gene expression changes in the mechanisms underlying the tolerance. Morphine also triggers an altered NOP-related signaling confirming that the nociceptin/orphanin FQ–nociceptin receptor system also plays a significant role in the development of morphine tolerance.

Keywords Morphine · Fentanyl · MOP · NOP · Opioid gene expression

Introduction

Opioid drugs are widely used for the treatment of severe acute and chronic pain. The effectiveness of long-term opioid therapy is affected by the development of tolerance to the analgesic effects. Despite several reports approached the study of mechanisms underlying opioid tolerance, this phenomenon is still largely unknown so that its elucidation has been compared to the "search of the Holy Grail" (Kieffer and Evans 2002). Morphine, the prototype of opioids, is broadly employed in many clinical settings; however, its chronic administration rapidly induces tolerance leading to the increase of doses to maintain the required antinociceptive effects.

The morphine-induced analgesia and tolerance are primarily mediated by μ opioid receptor (MOP) activation as demonstrated by studies on MOP knockout mice (Matthes et al. 1996). Fentanyl also exerts its antinociceptive effect predominantly through MOP (Narita et al. 2002), but it induces less tolerance to analgesia, compared to morphine, following long-term administration of equieffective doses (Emmet-Oglesby et al. 1988; Paronis and Holtzman 1992). These findings suggest that the MOP activation by morphine and fentanyl triggers different cellular and/or molecular events downstream.

Francesca Felicia Caputi and Francesca Lattanzio contributed equally to this work.

F. F. Caputi · F. Lattanzio · D. Carretta · D. Mercatelli · S. Candeletti · P. Romualdi (✉)
Department of Pharmacy and Biotechnology, Alma Mater Studiorum, University of Bologna, Via Irnerio, 48, 40126 Bologna, Italy
e-mail: patrizia.romualdi@unibo.it

The mechanism underlying the tolerance phenomenon is likely a multifaceted process involving adaptive changes at the molecular, cellular, and neural system levels (Williams et al. 2013). The cellular and molecular counteradaptations occurring in opioid tolerance probably involve MOP desensitization, internalization/endocytosis, and/or changes of gene expression (Martini and Whistler 2007). In particular, as to MOP gene expression changes, it has been demonstrated that fentanyl, but not morphine, induces a MOP upregulation in the rat pheochromocytoma PC12 cells (Yoshikawa et al. 2000).

The development of morphine tolerance and dependence also involves adaptive changes of the anti-opioid nociceptin/orphanin FQ–nociceptin receptor (N/OFQ-NOP) system. It has been demonstrated that NOP knockout mice show a partial loss of morphine tolerance (Ueda et al. 1997). Moreover, in normal mice, the chronic morphine treatment induces an increase of NOP gene expression in the spinal cord, and the administration of a N/OFQ antagonist prevents the development of morphine tolerance (Ueda et al. 2000; Chung et al. 2006).

The aim of the present study was to investigate the MOP and NOP gene expression in the human neuroblastoma-derived SK-N-SH cell line (SH-SY5Y cells) following morphine and fentanyl exposure; cells were exposed to different doses and time points.

Material and Methods

Cell Culture and Treatments

Human SH-SY5Y neuroblastoma cells, purchased from ICLC-IST (Genoa, Italy), were cultured in Dulbecco's modified Eagle medium, supplemented with 10 % (*v*/*v*) fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine. Cells were incubated at 37 °C in a humidified atmosphere containing 5 % CO_2. In all experiments, cells were allowed to reach 80 % confluence before starting treatments.

Before cell exposure, growth medium was changed and replaced with a fresh growth medium at 2 % FBS. Cells were exposed to 10 and 100 μM morphine or to 0.1 and 1 μM fentanyl for 5, 24, or 72 h. The growth medium was removed daily and replaced by fresh growth medium containing the appropriate concentrations of morphine and fentanyl. Control cells were maintained in a treatment-free growth medium and received the same schedule of growth medium changes.

Additional experiments were performed to determine the specificity of morphine and fentanyl effects on MOP receptors; cells were preincubated with 100 μM naloxone, a MOP antagonist, for 30 min and then exposed to morphine and fentanyl as described above.

Real-Time Quantitative Reverse Transcription PCR

The cell treatment was ended by aspiration of the medium, and cells were then washed twice with ice-cold phosphate buffer saline, in order to remove the drugs.

Total RNA was prepared according to the method of Chomczynski and Sacchi (1987). Briefly, RNA was extracted from cultured cells by homogenizing in a mixture of acid guanidinium/thiocyanate/phenol, adding 0.2 ml chloroform/1 ml of homogenate, and centrifuging the suspension at 12.000×*g* for 15 min at 4 °C. The aqueous phase was transferred to a fresh tube, an equal volume of isopropanol was added and incubated for 15 min at 4 °C, and the RNA pellet was isolated by centrifugation at 12.000×*g* for 15 min at 4 °C. The pellet was washed with 75 % ethanol, briefly air dried, and then resuspended in RNase-free water. Total RNA was digested with DNase RNase-free enzyme to eliminate genomic DNA content. The integrity of RNA and the absence of contaminating genomic DNA were checked by 1 % agarose gel electrophoresis. RNA concentrations were measured by spectrophotometry and adjusted accordingly. Only RNA samples with an optical density $(OD)_{260}/OD_{280}$ ratio >2 were used, subjected to DNase treatment, and converted to cDNA with the GeneAmp RNA PCR kit (Applied Biosystems, Foster City, CA, USA) by using random hexamers (0.50 μg of total RNA in a final reaction volume of 20 μl).

The cDNAs were subsequently diluted three times. Relative abundance of each mRNA species was assessed by real-time reverse transcription (RT)-PCR, employing 2 μl of the diluted samples in a final volume of 20 μl using the iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) on the Applied Biosystems Step One Real-Time PCR System. To provide precise quantification of the initial target in each PCR reaction, the amplification plot was examined, and the point of early log phase of product accumulation was defined by assigning a fluorescence threshold above the background, defined as the threshold cycle (Ct) number. Differences in Ct number were used to quantify the relative amount of PCR target contained within each sample. Relative expression of different gene transcripts was calculated by the delta-delta Ct (DDCt) method and converted to relative expression ratio (2^{-DDCt}) for statistical analysis (Livak and Schmittgen 2001). All data were normalized to the endogenous reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. After PCR, a dissociation curve (melting curve) was constructed in the range of 60 to 95 °C to evaluate the specificity of the amplification products (Lyon 2001). The

primers used for PCR amplification were designed using the Primer3 (Rozen and Skaletsky 2000) and are shown in Table 1.

Statistical Analysis

Gene expression data were statistically analyzed by one-way ANOVA followed by Dunnett's test using the GraphPad Prism software version 5 (GraphPad Software, San Diego, CA, USA). Data are reported as the mean values±standard error of the mean (SEM) of at least three independent experiments. A value of $p<0.05$ was considered significant.

Results

The cell exposure to 10 μM morphine for 5 h induced a significant decrease of MOP (0.50±0.08 vs control 1.00±0.13, $p<0.01$) and NOP gene expression (0.69±0.11 vs control 1.00±0.03, $p<0.05$) (Fig. 1a, b). No changes of gene expression were observed after cell exposure to 100 μM morphine for 5 h and to both doses of morphine (10 and 100 μM) at 24- and 72-h time points (Table 2).

The preincubation with 100 μM naloxone for 30 min followed by 10 μM morphine for 5 h blocked the MOP mRNA downregulation induced by morphine and restored mRNA levels to those comparable to control cells (1.00±0.04 vs control 1.00±0.13, Fig. 1a).

The cell exposure to 100 μM naloxone alone for 5 h did not significantly affect MOP mRNA levels compared to untreated control cells (0.91±0.09 vs control 1.00±0.11).

SH-SY5Y cells exposed to 0.1 and 1 μM fentanyl for 5 h showed a significant increase of MOP gene expression (1.69±0.15, $p<0.01$ and 2.10±0.37, $p<0.05$, respectively, vs control 1.00±0.11, Fig. 2a).

The pretreatment of cells with 100 μM naloxone for 30 min followed by 0.1 and 1 μM fentanyl for 5 h reverted the MOP upregulation, resulting in mRNA levels comparable to those of control unexposed cells (1.00±0.07 and 0.99±0.04 vs control 1.00±0.11, respectively, Fig. 2a).

Cell exposure to fentanyl for 72 h also significantly increased MOP mRNA levels (1.87±0.25, $p<0.05$ and 1.69±0.10, $p<0.01$ vs control 1.00±0.11, following 0.1 and 1 μM fentanyl, respectively, Fig. 2b).

The pretreatment of cells with 100 μM naloxone for 30 min followed by 0.1 and 1 μM fentanyl for 72 h reverted the MOP upregulation, resulting in mRNA levels not significantly different from those of control unexposed cells (0.80±0.12 and 0.87±0.08 vs control 1.00±0.12, respectively, Fig. 2b).

Cell exposure to 0.1 and 1 μM fentanyl for 24 h induced no changes of MOP gene expression (Table 2).

Finally, fentanyl treatment showed no modifications of NOP mRNA levels at any dose and at any time points examined (Table 2).

Discussion

The present study first demonstrated selective changes of MOP and NOP gene expression (measured by the real-time quantitative RT-PCR) following SH-SY5Y cell exposure to morphine and fentanyl. Data show that morphine induces MOP and NOP downregulation after 5 h of drug exposure, whereas fentanyl triggers MOP upregulation following 5 and 72 h of cell exposure with no changes on NOP gene expression.

The effect of morphine and fentanyl treatment on MOP gene expression might be likely mediated by MOP, as the pretreatment with the opioid antagonist naloxone is able to revert the effect of morphine and fentanyl, restoring MOP mRNA levels to those comparable with unexposed cells.

The MOP downregulation induced by morphine substantially confirms previous studies reported in the literature in the same neuroblastoma cells and in other cell lines. Our data differ in part from those observed in other in vitro investigations as for comparisons of doses and time of cell exposure to morphine. For example, Yu and coworkers (2003) reported a decrease of MOP mRNA levels following 24 h of SH-SY5Y cell exposure to 10 μM morphine, whereas our data failed to detect gene expression changes at this time point. This discrepancy could be due to the different techniques used to reveal gene expression modifications; in the present study, we used a real-time PCR method with the GAPDH as housekeeping gene, whereas Yu and coworkers (2003) developed a quantitative, competitive reverse transcriptase PCR technique with beta-actin gene as internal standard. In contrast, a recent study showed no changes of MOP gene expression following an exposure of 10 μM morphine for 24 h in SH-SY5Y cells revealed by the real-time quantitative RT-PCR analysis, accordingly with our data; this study was able to reveal MOP downregulation only in all-trans retinoic acid-differentiated SH-SY5Y human neuroblastoma cells (Prenus et al. 2012).

The present data of MOP downregulation after a 5-h exposure of morphine do not fit with a recent study showing no morphine-induced effect after 6 h of cell treatment (Mohan et al. 2010). It is possible that this mismatch is due to the different cell types used; in the present study, we used the SH-SY5Y cells that are a subclone of the SK-N-SH cell line used by Mohan and coworkers (2010). It is likely that these different cells react differently to biochemical stimuli.

Concerning other cell lines, it has previously been demonstrated that morphine also decreases MOP mRNA levels

Table 1 Primer sequences used for gene expression studies

Primer	Forward (5′–3′)	Reverse (3′–5′)	BP
MOP	ATCACGATCATGGCCCTCTACTCC	TGGTGGCAGTCTTCATCTTGGTGT	106
NOP	GGCCTCTGTTGTCGGTGTTC	GTAGCAGACAGAGATGACGAGCAC	175
GAPDH	GGTCGGAGTCAACGGATTT	TGGACTCCACGACGTACTCA	281

in MCF-7 human breast adenocarcinoma cells, but not in rat pheochromocytoma PC12 cells (Yoshikawa et al. 2000; Gach et al. 2008).

Overall, our data are in agreement with previous studies notwithstanding some mismatches probably due to the different schedules of cell exposure and the cell line and techniques used.

In the present study, morphine induces effect on MOP gene expression following 5-h cell exposure, whereas no changes of mRNA levels were observed after 24 and 72 h of treatment. These findings could be explained taking into account the ability of morphine to induce tolerance in vivo. Therefore, since changes of gene expression likely represent one of mechanisms underlying the tolerance development, it is plausible to speculate that, in our experimental conditions, morphine induces changes of MOP gene expression when cells are acutely exposed and that this effect disappears when the drug is chronically administered, as result of tolerance to the MOP gene expression alterations.

The present data of decreased mRNA levels in the SH-SY5Y cells exposed to morphine are in accord to a recent in vivo study showing a MOP downregulation in the hypothalamus following the intracerebroventricular administration of morphine (Zhu et al. 2012).

Unlike morphine, fentanyl induces a MOP upregulation accordingly to previous data showing, with a quantitative reverse transcription PCR method, increased mRNA levels in rat pheochromocytoma PC12 cells following fentanyl exposure (Yoshikawa et al. 2000). The time course of MOP gene expression changes by fentanyl observed in our data is not completely comparable to that reported by Yoshikawa and coworkers (2000) showing an increased MOP mRNA levels after a 6-h exposure of fentanyl that reached a peak at 24 h and then gradually declined until 72 h. We observed a comparable MOP upregulation at 5 and 72 h, but we fail to detect MOP gene expression changes after 24 h of fentanyl exposure. These discrepancies are likely due to the different experimental conditions used in the two studies (different cell line, PCR method, internal standard, and dose of fentanyl).

The lack of MOP mRNA level modifications observed at 24 h could represent a transitory tolerance-like cell mechanism, since after 72 h of exposure, a fentanyl-induced MOP upregulation still occurs, according to the property of this drug to induce less tolerance in vivo, in comparison with morphine, when chronically administered.

The different effects induced by morphine and fentanyl on MOP gene expression reported in the present study may

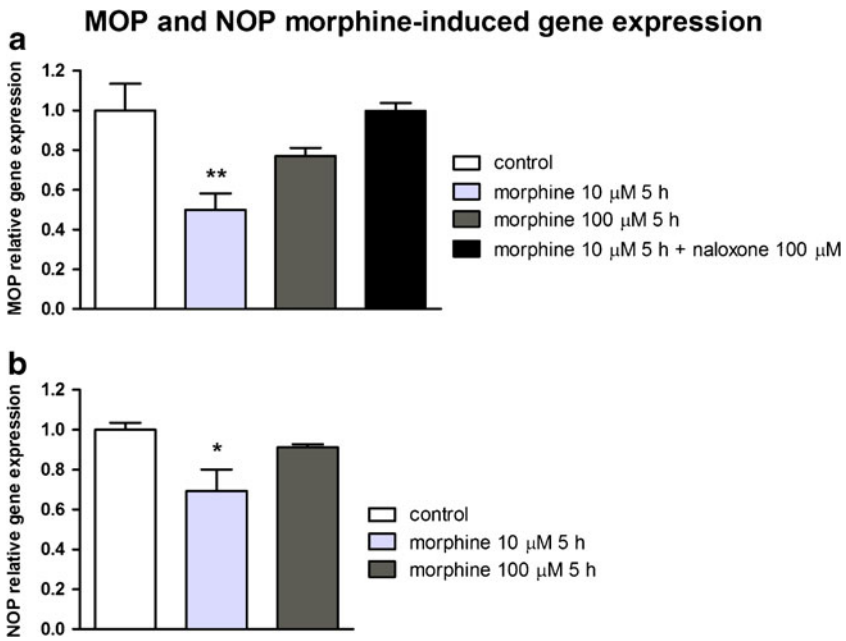

Fig. 1 Relative gene expression of MOP (**a**) and NOP (**b**) in SH-SY5Y neuroblastoma cells following exposure to morphine (10 and 100 μM) for 5 h. *Bars* represent 2^{-DDCt} values calculated by using the DDCt method. Gene expression was normalized to GAPDH. Data are expressed as mean±SEM percent of control untreated cells for three independent experiments (*$p<0.05$; **$p<0.01$ vs controls; ANOVA and Dunnett's test)

Table 2 Relative MOP and NOP gene expression in SH-SY5Y cells exposed to morphine and fentanyl at different time points

Treatment	MOP			NOP		
	5 h	24 h	72 h	5 h	24 h	72 h
Control	1.00±0.13	1.00±0.12	1.00±0.01	1.00±0.03	1.00±0.10	1.00±0.06
Morphine 10 μM	**0.50±0.08****	1.04±0.05	0.96±0.02	**0.69±0.11***	0.99±0.03	1.04±0.16
Morphine 100 μM	0.77±0.04	1.10±0.06	1.09±0.06	0.91±0.02	0.94±0.06	1.32±0.07
Control	1.00±0.11	1.00±0.11	1.00±0.11	1.00±0.12	1.00±0.12	1.00±0.12
Fentanyl 0.1 μM	**1.69±0.15****	1.07±0.14	**1.87±0.25***	1.45±0.13	1.46±0.10	0.97±0.11
Fentanyl 1 μM	**2.10±0.37***	1.13±0.13	**1.69±0.10****	1.45±0.20	1.38±0.21	0.88±0.12

Quantitative real-time PCR results. Gene expression was normalized to GAPDH, and mRNA levels are expressed relative to control cells as means±SEM (* $p<0.05$; ** $p<0.01$ vs controls; ANOVA and Dunnett's test)

contribute to explain the different degrees of tolerance that these drugs display in the clinical settings. Thus, the molecular adaptive changes occurring in opioid tolerance probably also involve alterations of gene expression in addition to the MOP desensitization and internalization/endocytosis (Martini and Whistler 2007). Concerning these latter phenomena, it has been demonstrated that the different degrees of opioid analgesic ability to induce internalization and downregulation of MOP appear to be inversely correlated with the aptitude of these drugs to induce opioid tolerance (Whistler et al. 1999; Kock and Höllt 2008). Thus, relative activity/versus endocytosis (RA/VE) value has been proposed as a parameter to predict/establish the degree of tolerance; opioids with low RA/VE values, like DAMGO or methadone, induce less tolerance than those with high RA/VE values, such as morphine that is unable to promote receptor desensitization and endocytosis (Martini and Whistler 2007).

The present findings might also be useful and used in terms of effectiveness of morphine and fentanyl coadministration in order to reduce the severity of morphine tolerance. Previous study already demonstrated that the coadministration of morphine and a MOP agonist with high internalization capacity is able to increase the morphine's ability to induce MOP internalization. In particular, the coadministration of a sub-analgesic dose of fentanyl promotes morphine-induced MOP internalization, together with the decrease of RA/VE value, leading to the increase of analgesic effect of morphine (Hashimoto et al. 2006). The different changes of MOP gene expression induced by morphine and fentanyl here reported could also account to the different degrees of tolerance by the two drugs, likely due to intracellular events involving the regulation of MOP synthesis. Moreover, the synergistic analgesic effects of morphine and fentanyl coadministration might be due to the

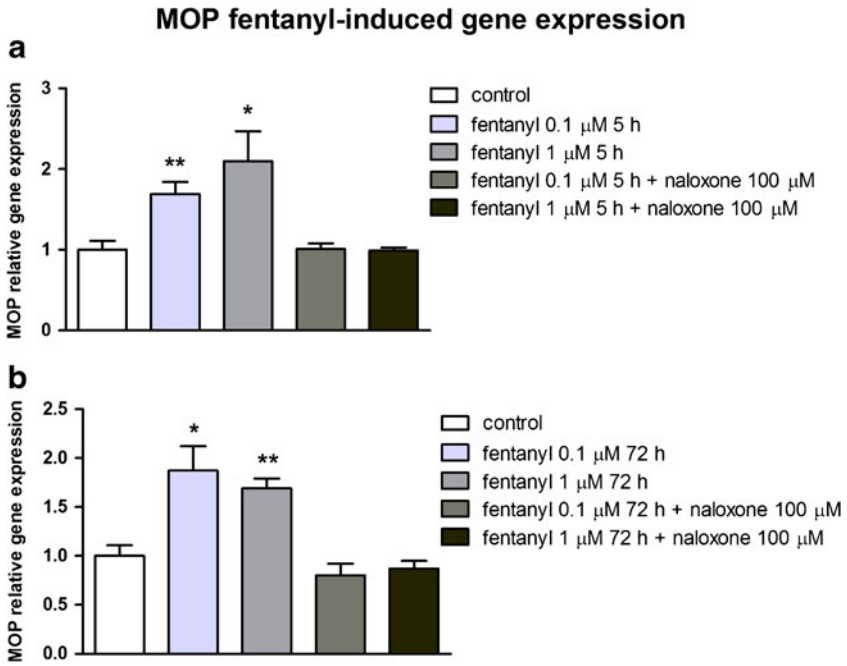

Fig. 2 Relative gene expression of MOP in SH-SY5Y neuroblastoma cells following exposure to fentanyl (0.1 and 1 μM) for 5 h (**a**) and 72 h (**b**). *Bars* represent 2^{-DDCt} values calculated by using the DDCt method. Gene expression was normalized to GAPDH. Data are expressed as mean± SEM percent of control untreated cells for three independent experiments (*$p<0.05$; **$p<0.01$ vs controls; ANOVA and Dunnett's test)

different molecular consequences downstream the MOP activation and involving the gene transcription.

Finally, the present study showed that morphine, but not fentanyl, induces a NOP downregulation following 5-h cell exposure. It has been already demonstrated that the N/OFQ-NOP system plays a role in morphine-related tolerance mechanisms; the NOP knockout mice and the N/OFQ antagonist prevent the development of morphine tolerance (Ueda et al. 1997, 2000; Chung et al. 2006; Scoto et al. 2010).

The NOP downregulation observed in the present study is quite surprising, since the N/OFQ-NOP system is considered an anti-opioid system so that we would have expected an increase of morphine-induced NOP gene expression. In fact, in mice, the chronic administration of morphine induces an increase of NOP gene expression in the spinal cord, indicating a role of this system in the counteradaptation to tolerance phenomena (Ueda et al. 2000).

However, an in vitro study showed that morphine induces a prepro-nociceptin (ppN)/OFQ upregulation in cultured astrocytes, but not neurons, suggesting that astrocytes also play a role in the neuronal plasticity induced by morphine (Takayama and Ueda 2005). The increase of ppN/OFQ mRNA levels was the maximum after 6 h of cell exposure to morphine, with a time point that matches with the 5-h morphine cell exposure triggering the NOP downregulation in our study. The NOP data in neuroblastoma cells here reported could be in part explained from this study. It is possible to speculate that the increase of ppN/OFQ gene expression occurring in astrocytes induces in neurons a reactive downregulation of its receptor NOP, suggesting that in both astrocytes and neuronal cells, the N/OFQ-NOP system components likely play an important role in neuronal plasticity induced by chronic morphine treatments.

Moreover, the morphine-induced ppN/OFQ upregulation in cultured astrocytes is consistent with the reported increase of N/OFQ peptide and ppN/OFQ mRNA levels in rat brain, following repeated morphine treatments (Yuan et al. 1999; Romualdi et al. 2002).

The present findings contribute to define the role of MOP gene expression changes by two different opioids known to induce a different degree of clinical tolerance to analgesic effects. Morphine also triggers an altered NOP-related signaling, confirming that the N/OFQ-NOP system also plays a significant role in the development of morphine tolerance.

References

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate–phenolchloroform extraction. Anal Biochem 162:156–159

Chung S, Pohl S, Zeng J, Civelli O, Reinscheid RK (2006) Endogenous orphanin FQ/nociceptin is involved in the development of morphine tolerance. J Pharmacol Exp Ther 318:262–267

Emmet-Oglesby MW, Shippenberg TS, Herz A (1988) Tolerance and cross-tolerance to the discriminative stimulus properties of fentanyl and morphine. J Pharmacol Exp Ther 245:17–23

Gach K, Piestrzeniewicz M, Fichna J, Stefanska B, Szemraj J, Janecka A (2008) Opioid-induced regulation of mu-opioid receptor gene expression in the MCF-7 breast cancer cell line. Biochem Cell Biol 86:217–226

Hashimoto T, Saito Y, Yamada K, Hara N, Kirihara Y, Tsuchiya M (2006) Enhancement of morphine analgesic effect with induction of μ-opioid receptor endocytosis in rats. Anesthesiology 105:574–580

Kieffer BL, Evans CJ (2002) Opioid tolerance—in search of the holy grail. Cell 108:587–590

Koch T, Höllt V (2008) Role of receptor internalization in opioid tolerance and dependence. Pharmacol Ther 117:199–206

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−delta delta C(T)) method. Methods 25:402–408

Lyon E (2001) Mutation detection using fluorescent hybridization probes and melting curve analysis. Expert Rev Mol Diagn 1:92–101

Martini L, Whistler JL (2007) The role of mu opioid receptor desensitization and endocytosis in morphine tolerance and dependence. Curr Opin Neurobiol 17:556–564

Matthes HW, Maldonado R, Simonin F et al (1996) Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature (Lond) 383:819–823

Mohan S, Davis RL, DeSilva U, Stevens CW (2010) Dual regulation of mu opioid receptors in SK-N-SH neuroblastoma cells by morphine and interleukin-1β: evidence for opioid-immune crosstalk. J Neuroimmunol 227:26–34

Narita M, Imai S, Itou Y, Yajima Y, Suzuki T (2002) Possible involvement of mu1-opioid receptors in the fentanyl- or morphine-induced antinociception at supraspinal and spinal sites. Life Sci 70:2341–2354

Paronis CA, Holtzman SG (1992) Development of tolerance to the analgesic activity of mu agonists after continuous infusion of morphine, meperidine or fentanyl in rats. J Pharmacol Exp Ther 262:1–9

Prenus RV, Luscar E, Zhu ZP, Badisa RB, Goodman CB (2012) Regulation of mammalian MOR-1 gene expression after chronic treatment with morphine. Int J Mol Med 30:1493–1497

Romualdi P, Landuzzi D, D'Addario C, Candeletti S (2002) Modulation of proorphaninFQ/N gene expression by morphine in the rat mesocorticolimbic system. Neuroreport 13:645–648

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386

Scoto GM, Aricò G, Iemolo A, Ronsisvalle G, Parenti C (2010) Selective inhibition of the NOP receptor in the ventrolateral periaqueductal gray attenuates the development and the expression of tolerance to morphine-induced antinociception in rats. Peptides 31:696–700

Takayama N, Ueda H (2005) Morphine-induced overexpression of prepro-nociceptin/orphanin FQ in cultured astrocytes. Peptides 26:2513–2517

Ueda H, Inoue M, Takeshima H, Iwasawa Y (2000) Enhanced spinal nociceptin receptor expression develops morphine tolerance and dependence. J Neurosci 20:7640–7647

Ueda H, Yamaguchi T, Tokuyama S, Inoue M, Nishi M, Takeshima H (1997) Partial loss of tolerance liability to morphine analgesia in mice lacking the nociceptin receptor gene. Neurosci Lett 237:136–138

Whistler JL, Chuang HH, Chu P, Jan LY, von Zastrow M (1999) Functional dissociation of mu opioid receptor signalling and endocytosis: implications for the biology of tolerance and addiction. Neuron 23:737–746

Williams JT, Ingram SL, Henderson G et al (2013) Regulation of μ-opioid receptors: desensitization, phosphorylation, internalization, and tolerance. Pharmacol Rev 15:223–254

Yoshikawa M, Nakayama H, Ueno S, Hirano M, Hatanaka H, Furuya H (2000) Chronic fentanyl treatments induce the up-regulation of μ opioid receptor mRNA in rat pheochromocytoma cells. Brain Research 859:217–223

Yu X, Mao X, Blake AD, Li WX, Chang SL (2003) Morphine and endomorphins differentially regulate mu-opioid receptor mRNA in SHSY-5Y human neuroblastoma cells. J Pharmacol Exp Ther 306:447–454

Yuan L, Han Z, Chang JK, Han JS (1999) Accelerated release and production of orphanin FQ in brain of chronic morphine tolerant rats. Brain Res 826:330–334

Zhu ZP, Badisa RB, Palm DE, Goodman CB (2012) Regulation of rat MOR-1 gene expression after chronic intracerebroventricular administration of morphine. Mol Med Rep 5:513–516