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Involvement of Activator Protein-1 in Transcriptional Regulation of the Human μ-Opioid Receptor Gene

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ABSTRACT

μ-Opioid receptors mediate such opioid effects as analgesia, euphoria, and immunomodulation. Gene expression of μ-opioid receptors can be modulated by various substances, including cytokines, hormones, and drugs. Some of these stimuli (e.g., IL-1β and cocaine) have been shown to activate members of the AP-1 transcription factor family. In addition, transcription of the μ-opioid receptor gene is induced by the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), an activator of protein kinase C, which in turn is an activator of AP-1 transcription factors. This indicates that signaling pathways involving protein kinase C and activator protein 1 (AP-1) transcription factors are important for the specific expression pattern of the μ-opioid receptor gene. In this report, we show that TPA activates AP-1 as well as the transcription factor nuclear factor κB (NFκB) in the μ-opioid receptor expressing neuroblastoma cell line SH SY5Y. In transfection experiments performed in these cells, both factors *trans*-activate expression of reporter gene constructs containing the human μ-opioid receptor gene promoter. By excluding the effects of TPA on NFκB with the specific NFκB inhibitor sulfasalazine, AP-1 regulatory elements were localized. Two AP-1 elements, which differ in one nucleotide each from the classic AP-1 binding site, were delineated to positions −2388 and −1434 of the promoter. Independent of their orientation, these elements conferred TPA responsiveness on the heterologous thymidine kinase promoter. AP-1 binding to these elements was confirmed using electrophoretic mobility shift and immunoshift assays.

μ-Opioid receptors interact with endogenous peptide ligands and opioid alkaloids to mediate their characteristic effects, such as the depression of respiratory and gastrointestinal functions, euphoria, and, most important clinically, analgesia (Reisine and Brownstein, 1994; Kieffer, 1995). Furthermore, opioids possess various immunomodulatory properties, which also involve μ-opioid receptors (Gaveriaux-Ruff et al., 1998; Roy et al., 1999; Sacerdote et al., 2000). μ-Opioid receptor gene transcription is regulated by various extracellular signals. Among these are cytokines, such as IL-1β (Ruzicka et al., 1996; Vidal et al., 1998), IL-4 (Kraus et al., 2001), and IL-6 (Bianchi et al., 1999); hormones, such as estrogen (Quinones-Jenab et al., 1997); and drugs, such as cocaine (Azaryan et al., 1996). Furthermore, the phorbol ester TPA had been shown to up-regulate μ-opioid receptor mRNA in the neuroblastoma cell line SH SY5Y (Zadina et al., 1994; Kraus et al., 1995). By mimicking the second messenger diacyl glycerol, TPA is an activator of PKC. This indicates that signals converging on PKC may regulate the gene's expression. PKC in turn can activate members of the AP-1 transcription factor family (Latchman, 1990). In the past, the *cis*-acting promoter elements with the consensus sequence 5′-TGA(C/G)TCA-3′ that bind AP-1 transcription factors were termed "TPA-responsive elements" (Angel et al., 1987). In this article, the term "AP-1 element" or "AP-1 binding site" is preferred because TPA also is a potent activator of the transcription factor NFκB and, therefore, NFκB binding sites are "TPA-responsive" as well (O'Neill and Kaltschmidt, 1997). The most prominent members of the AP-1 family are Jun and Fos proteins. They are activated/induced throughout the brain in response to a broad range of extracellular stimuli (Persico and Uhl, 1996; Herdegen and Leah, 1998). These include such stimuli that had been reported to induce μ-opioid receptor transcription, suggesting regulatory inter-relations.

Recently, a number of naturally occurring allelic variations have been located within the human μ-opioid receptor gene (Hoehe et al., 2000). Studying such polymorphisms may help to comprehend individual differences in vulnerability to drug abuse and opiate-mediated analgesia. One of the allelic variations described for the gene is located adjacent to a putative

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ABBREVIATIONS: IL, interleukin; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; PKC, protein kinase C; AP-1, activator protein-1; NFκB, nuclear factor κB; FCS, fetal calf serum; TNF-α, tumor necrosis factor-α; nt, nucleotides; tk, thymidine kinase; CAT, chloramphenicol acetyl transferase; HSV, herpes simplex virus.

AP-1 site within the promoter and thus may influence gene regulation.

To better understand transcriptional regulation of the human μ-opioid receptor gene and to discuss the impact of the polymorphism mentioned above, we determined the activation of transcription factors after the stimulation of SH SY5Y cells and identified *cis*-active AP-1 transcription factor-binding elements on the human μ-opioid receptor gene promoter.

Materials and Methods

Cell Culture and Transfection. SH SY5Y cells were cultivated in Dulbecco's modified Eagle's medium (PAA Laboratories, Linz, Austria) supplemented with 15% FCS (PAA Laboratories, Linz, Austria) and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin). Plasmid DNA used for transfection was isolated using Plasmid Kits (QIAGEN, Hilden, Germany). Before transfection, cells received fresh medium containing 1% FCS. The transfection of SH SY5Y cells and reporter gene assay has been described previously (Chen and Okayama, 1987; Kraus et al., 1995). After transfection, cells were allowed to grow for an additional 48 h in a 1% FCS medium containing 100 nM TPA (Sigma, Schnelldorf, Germany) or vehicle (ethanol, maximally 1% vol), or 100 U/ml TNF-α (R & D Systems, Wiesbaden, Germany) dissolved in phosphate-buffered saline. Sulfasalazine (Sigma, Schnelldorf, Germany) was dissolved in cell culture medium and added at a concentration of 1 mM 30 min before the other stimuli.

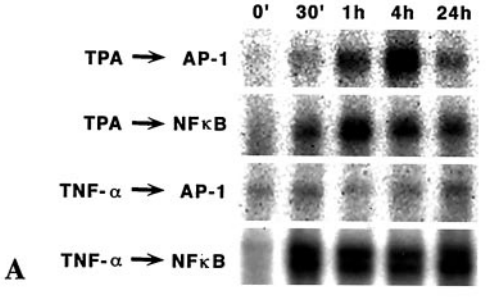
Construction of Reporter Plasmids. All reporter plasmids are from the pBLCAT2/pBLCAT3 vector system containing the CAT reporter gene (Luckow and Schutz, 1987). For cloning of the basic human μ-promoter reporter plasmid (phMOR-2624), a fragment ranging from nt −2624 to nt −165, which contains the prominent transcription initiation sites (Wendel and Hoehe, 1998), was inserted into the *Bam*HI and *Bgl*II sites of the vector pBLCAT2. Simultaneously, the HSV-tk promoter located between these vector sites was excised by this procedure. Constructs phMOR−2229 and phMOR−1372 were generated by cutting the basic construct with *Sau*I (nt −2229) and *Bgl*II (nt −1372), respectively, and *Hin*dIII (vector), filling the ends and ligating again. Construct phMOR−1702 was obtained by a 5′ deletion method using DNase as described previously (Lin et al., 1985). Construct phMOR−2229Δ-1372/−254 was generated by cutting out a *Bgl*II and *Pst*I (nt −254) fragment and then blunting and ligating. The internal deletions in the plasmids phMOR−2229Δ-1666/−1068 and phMOR−2541Δ-2349/−1125 were made with enzyme Bal31 after opening the plasmids with *Bgl*II and *Acc*I (nt −1854), respectively. For construction of phMOR−2478/−2287 and phMOR−1854/−1227, a *Sau*3A and an *Acc*I-*Hpa*I fragment, respectively, were cloned into pBLCAT2 in front of the tk promoter. Oligonucleotides with transcription factor binding sites were inserted in pBLCAT2 in front of the HSV-tk promoter into the vector's *Bam*HI site according to a method described previously (Kang and Inouye, 1993). The sequences of the classic AP-1 and NFκB binding sites and of M1 and M5, which were used in these experiments, are described in the next section, because the same oligonucleotides were used for cloning.

Extraction of Nuclear Protein and Electrophoretic Mobility Shift Assay. Twenty-four hours before stimulation with TPA (100 nM) and TNF-α (100 U/ml), SH SY5Y cells received fresh medium containing 1% FCS. For time kinetics experiments, cells were stimulated for different periods of time. For the electrophoretic mobility shift experiments defining AP-1 binding sites on the human μ-opioid receptor promoter, stimulation with TPA occurred for 4 h before nuclear protein extraction. The extraction procedure for nuclear proteins used in these assays has been described in detail in an earlier publication (Wöltje et al., 1998). Synthetic double-stranded oligonucleotides (Metabion, Martinsried, Germany) carrying putative AP-1 binding sites of the human μ-opioid receptor promoters and a classic AP-1 element were labeled with [γ-^{32}P]ATP (Amersham Biosciences, Braunschweig, Germany) according to standard methods. For each reaction, 5000 cpm-labeled probe DNA were incubated with 3 μl of SH SY5Y cell nuclear extract for 15 min at room temperature under conditions described previously (Wöltje et al., 1998). For immunoshift experiments, 4 μg of c-Fos antibody (K-25; Santa Cruz Biotechnology, Heidelberg, Germany) was added after the reaction and was incubated for an additional 60 min.

Sequences of the oligonucleotides were: classic AP-1: 5′-CGATTGACTCAGTACTGAGTCAATCG-3′; classic NFκB: 5′-AAAGTTGAGGGGACTTTCCCAGGCCT-3′; M1: 5′-AAACATATGATTCACCAGGCA-3′; M2: 5′-CCTAAGGAGAGTCAAGAGAAC-3′; M3: 5′-ACTGAAAGGACTCAGAACTAC-3′; M4: 5′-AAATGATTGACTCCAAGGTCA-3′; M5: 5′-TTACCTATGAGTTATCTGTTT-3′; M6: 5′-GGAAAATTGAGTGATGTTAGC-3′.

Results

Activation of AP-1 and NFκB Transcription Factors by TPA and TNF-α in SH SY5Y Cells. μ-Opioid receptors are expressed endogenously in the neuroblastoma cell line SH SY5Y, which therefore serves as a good model for studying μ-opioid receptor transcription (Zadina et al., 1993). Given the fact that AP-1 and NFκB transcription factors are activated by both TPA and TNF-α in several cell systems (Latchman, 1990; O'Neill and Kaltschmidt, 1997), we first investigated whether this is also true when stimulating the neuroblastoma cell line SH SY5Y with these agents (Fig. 1). The time kinetic of the induction of transcription factors was



	Co	TPA	TNF-α	sul + TPA	sul + TNF-α
AP-1 tk CAT	1.0 ±1	4.8 ±3***	1.1 ±1	4.7 ±3***	1.1 ±1
NFκB tk CAT	1.0 ±1	2.9 ±3***	2.7 ±4**	1.1 ±1	1.0 ±1
tk CAT	1.0 ±1	1.3 ±1	1.1 ±1	1.0 ±2	0.9 ±1
-2624 human MOR CAT	1.0 ±1	8.2 ±6**	3.6 ±3***	8.6 ±3***	1.1 ±2

B

Fig. 1. Effects of TPA and TNF-α on the activation of transcription factors AP-1 and NFκB and on the human μ-opioid receptor promoter in SH SY5Y cells. A, electrophoretic mobility shift assays reveal the activation/induction of transcription factors. SH SY5Y cells were stimulated with 100 nM TPA or 100 U/ml TNF-α for different periods of time, followed by nuclear extract preparation and mobility shift experiment. Only the specific bands that were identified previously by supershift experiments (data not shown) are plotted. As probes (indicated left), classic binding motifs of transcription factors AP-1 and NFκB were used. B, *trans*-activation of classic transcription factor response elements and the human μ-opioid receptor promoter. Results of transient transfection of SH SY5Y cells with CAT reporter gene constructs containing classic binding motifs for AP-1 and NFκB in front of the HSV-tk promoter, the cloning vector pBLCAT2 and a construct containing sequences up to nt −2624 of the human μ-opioid receptor promoter are given as fold induction compared with unstimulated control sites (Co). Sul, sulfasalazine.

determined by electrophoretic mobility shift assay (Fig. 1A). The experiments revealed that TPA induced both AP-1 and NFκB with a maximal induction after stimulation for 4 h and 1 h, respectively. In contrast, TNF-α induced NFκB (maximally after 30 min of stimulation) but not AP-1 in these cells. Next, *trans*-activation of reporter genes was studied in SH SY5Y cells testing constructs containing either a classic AP-1 binding site or a classic NFκB element in front of the HSV-tk promoter (Fig. 1B). Consistent with the data obtained by the electrophoretic mobility shift experiments, TPA induced the CAT reporter gene activity of both constructs, whereas TNF-α induced only CAT activity of the NFκB construct. To distinguish between AP-1 and NFκB effects after TPA stimulation, we used the specific NFκB inhibitor sulfasalazine (Wahl et al., 1998; Weber et al., 2000). The addition of 1 mM sulfasalazine 30 min before stimulation completely inhibited TPA-mediated *trans*-activation of the NFκB construct but not TPA-mediated AP-1 activation. As expected, *trans*-activation of the NFκB construct by TNF-α was also abolished with sulfasalazine. The cloning vector pBLCAT2 was not affected by any of these agents.

TPA and TNF-α Stimulate μ-Opioid Receptor Transcription. Figure 1B shows further responsiveness of human μ-opioid receptor promoter sequences to both TPA and TNF-α. For these experiments, sequences spanning from nt −2624 to nt −165, which contain the prominent transcription initiation sites of the human μ-opioid receptor gene, were tested in front of the CAT reporter gene (construct phMOR−2624). Incubation of transfected cells with TPA and sulfasalazine also led to a significant induction. This indicated that the μ-opioid receptor promoter contains at least one AP-1 element. Addition of sulfasalazine before stimulation with TNF-α completely abolished the TNF-α effect, indicating that the promoter contains at least one NFκB element as well.

The Human μ-Opioid Receptor Promoter Contains Two TPA-Responsive AP-1 Sites. To characterize molecular mechanisms of TPA-induced transcription, AP-1 binding sites on the μ-opioid receptor gene promoter were localized. Sequence comparison with the classic binding motif for AP-1 suggested that AP-1 factors might bind to six putative elements on the μ-opioid receptor promoter (Table 1). However, none of these motifs (termed M1 through M6) fits exactly to the classic AP-1 sequence, each containing one mismatch. Sequences with more mismatches were not taken into consideration. Figure 2A shows the transfection experiments to localize functional AP-1 elements on the μ-opioid receptor promoter. Because of additional NFκB binding sites on the promoter, it was necessary to use sulfasalazine in all the following transfection experiments. Consecutive 5′ end deletion of the longest construct indicated that the TPA responsive sequences are located upstream of nt −1372, excluding M6 as a functional AP-1 site (constructs −2624, lane 1, through −1372, lane 4). Inducibility of construct phMOR−2541Δ-2349/−1125 suggested that M1 may be a functional AP-1 element (lane 5). Transfection of construct phMOR−2229Δ-1666/−1068 demonstrated that M2, M3, and M4, which are contained on this construct, are not responsive to TPA (lane 6). However, construct phMOR−2229Δ−1372/−254 was TPA-inducible, indicating that M5 may be a second functional AP-1 site (lane 7). Next it was tested whether 5′ upstream sequences conferred TPA responsiveness on the heterologous HSV-tk promoter. Both constructs, phMOR−2478/−2287 containing M1 (lane 8) and phMOR−1854/−1227 containing M5 (lane 9), were responsive to TPA. In conclusion, transfections of various parts of the human μ-opioid receptor promoter suggested that M1 and M5 are likely to be functional AP-1 elements. To further test this hypothesis, oligonucleotides containing the two elements were cloned in front of the tk promoter to assay TPA-inducible CAT reporter gene expression (Fig. 2B). Indeed, oligonucleotides containing M1 and M5 were sufficient to mediate the TPA effect independent of their orientation.

TABLE 1

Sequences of putative AP-1 elements of the human μ-opioid receptor gene promoter (M1–M6) compared with the classic AP-1 site. S stands for either C or G.

AP-1	5′-TGASTCA-3′	
M1	5′-TGATTCA-3′	nt−2388
M2	5′-AGAGTCA-3′	nt−2224
M3	5′-GGACTCA-3′	nt−1994
M4	5′-TGACTCC-3′	nt−1699
M5	5′-TGAGTTA-3′	nt−1434
M6	5′-TGAGTGA-3′	nt −478

Transcription Factor Binding to the μ-Opioid Receptor AP-1 Elements. Electrophoretic mobility shift assays were performed to investigate the binding of AP-1 transcription factors to the putative AP-1 sites of the μ-opioid receptor gene promoter (Fig. 3). First, oligonucleotides containing M1 through M6 were tested for their ability to compete for AP-1 binding to a classic AP-1 element. This was obviously restricted to M1 and M5 (Fig. 3A), indicating that these elements are indeed AP-1 sites, which is in good accordance with the transfection experiments described above. Next, M1 and M5 oligonucleotides were used as probes and were tested for AP-1 binding. They were confirmed as AP-1 binding elements by competition experiments with classic AP-1 oligonucleotides as well as by supershift experiments with a specific antibody against c-Fos (Fig. 3B). Using M4 and M6 as probes, retarded complexes were seen as double bands that migrated below the AP-1 band (Fig. 3B). The similar patterns for M4 and M6 suggest that the sequences most probably bind similar nuclear factor(s), which are, however, clearly distinct from AP-1. The competition experiments (Fig. 3A) and immunoshift experiments (Fig. 3B) confirmed that factors different from AP-1 bind to M4 and M6. No nuclear factors bound to M2 and M3 probes (Fig. 3B).

Discussion

Earlier studies revealed that the μ-opioid receptor gene is induced by the phorbol ester TPA, an activator of PKC (Zadina et al., 1994; Kraus et al., 1995). PKC is a central player in signal transduction, with several pathways converging on this kinase. This study aimed to identify regulatory elements on the human μ-opioid receptor gene promoter that bind AP-1 transcription factors, through which PKC-mediated up-regulation of the gene may be achieved (Angel et al., 1987). The promoter contains a distal AP-1 element located at nt −2388 (corresponding to M1) and a proximal site located at nt −1434 (corresponding to M5). The region containing the proximal element is conserved also in the rat μ-opioid receptor gene promoter with a similar element (5′-TGAGTAA-3′) at position −1367 (Kraus et al., 1995). Electrophoretic mobility shift assays performed in this laboratory

demonstrated AP-1 binding to the rat element as well (data not shown). The sequences corresponding to the distal element are not yet known for the rat gene.

AP-1–controlled transcriptional regulation can be of considerable importance for fine-tuning gene expression in response to various stimuli. Our findings present a possible molecular link between μ-opioid receptor gene induction by defined stimuli on the one hand and AP-1 induction/activation by such stimuli on the other hand. In many investigations, it was demonstrated that members of the AP-1 family, in particular Fos, are highly regulated in brain by various drugs, hormones, and neurotransmitters (Persico and Uhl, 1996; Herdegen and Leah, 1998). For example, addictive drugs such as cocaine and morphine were shown to induce the c-fos gene in areas of the limbic system, including the nucleus accumbens (Erdtmann-Vourliotis et al., 1999). In the same area, up-regulation of μ-opioid receptor mRNA was observed after long-term cocaine treatment (Azaryan et al., 1996), suggesting a possible relationship. Regulation of μ-opioid receptor expression in brain areas involved in the perception of pleasure may contribute to explaining the euphoric effects of drugs.

The molecular mechanisms for the induction of μ-opioid receptor mRNA by the cytokine IL-1β could involve AP-1 as well. Several reports showed that IL-1β potently induces c-fos expression in various brain regions and astroglial cells (Brady et al., 1994; Fredholm and Altiok, 1994). In parallel, in primary astrocyte-enriched cultures of different brain regions (Ruzicka and Akil, 1997) and in neural microvascular endothelial cells (Vidal et al., 1998), μ-opioid receptor mRNA was found to be inducible by IL-1β. Alternatively, IL-1β–regulated μ-opioid receptor transcription could involve NFκB, which is also known to be activated in response to IL-1β (O'Neill and Kaltschmidt, 1997).

AP-1 transcription factors are probably also involved in autoregulatory mechanisms of μ-opioid receptor gene expression. Thus, ligand-induced μ-opioid receptor stimulation is known to activate PKC via the phospholipase C system (Zimprich et al., 1995). A possible autoregulatory scenario could be that the loss of receptor proteins, which is caused by the degradation processes after ligand binding (Koch et al., 2001), is compensated by PKC/AP-1–dependent resynthesis.

This study additionally revealed that the proinflammatory cytokine TNF-α regulates μ-opioid receptor gene expression via NFκB in SH SY5Y cells. Such a regulation in peripheral neuronal cells could be an important mechanism for inflammation-induced activation of the opioid system, which had repeatedly been observed (Parsons et al., 1990; Stein et al., 1990; Czlonkowski et al., 1993; Ji et al., 1995). A detailed investigation unraveling the regulation of μ-opioid receptor transcription in neural and immune cells by TNF-α will be the topic of another publication.

The μ-opioid receptor gene is being discussed as one of the candidates in search of a physiological basis of drug abuse (LaForge et al., 2000; Mayer and Höllt, 2001). Indeed, it was reported that the phenotypical consequences of naturally

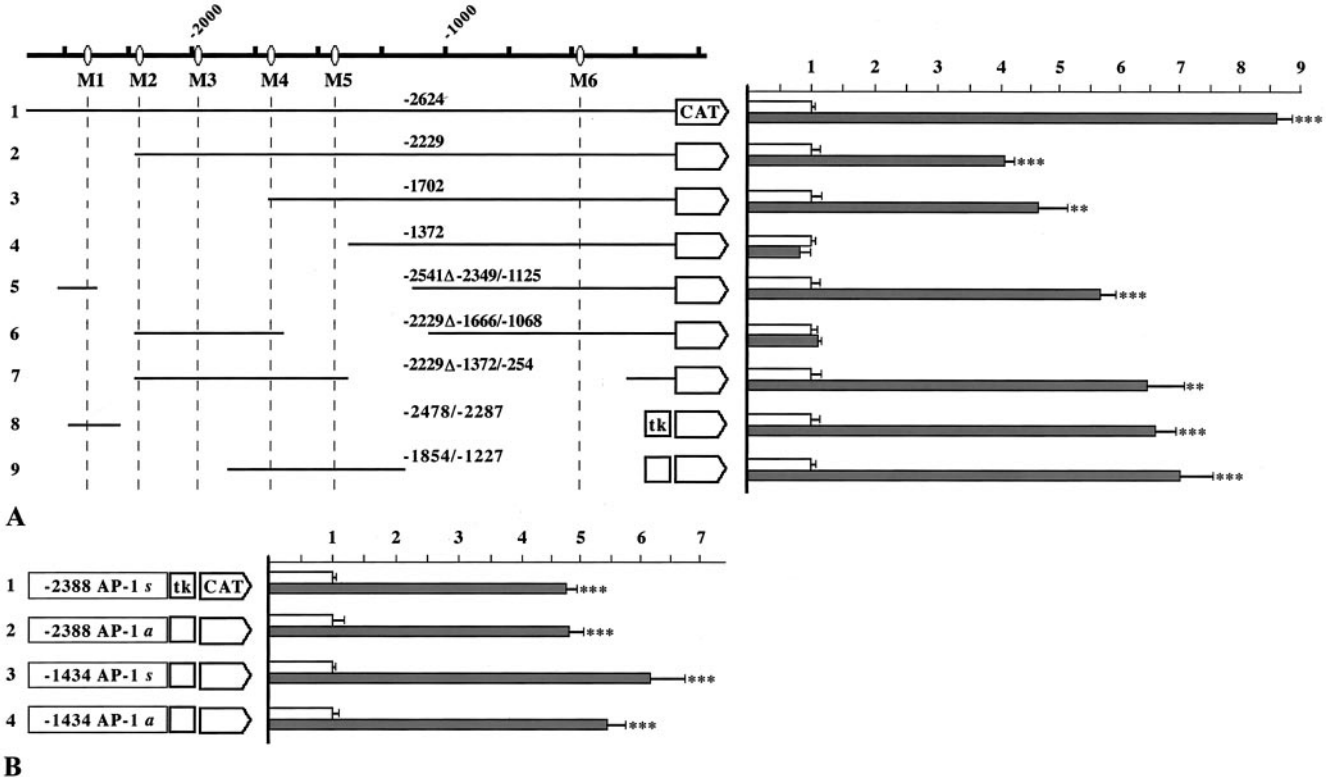


Fig. 2. Transfection experiments to identify AP-1 elements on the human μ-opioid receptor promoter. CAT reporter gene activities for various human μ-opioid receptor promoter constructs obtained after transfection of SH SY5Y cells are shown as fold induction (□, unstimulated controls; ■, TPA- and sulfasalazine-treated transfectants). Vertical dashed lines indicate the locations of six putative AP-1 binding sites (M1-M6, oval symbols). Reporter gene transcription was under the control of the homologous μ-opioid receptor promoter (lanes 1–7) or the HSV-tk promoter (lanes 8 and 9). B, transfection of constructs with oligonucleotides containing AP-1 elements M1 (nt −2388) and M5 (nt −1434) in front of the HSV-tk promoter. CAT activities are reported as fold inductions as described above. Both elements were tested in sense (s) and antisense (a) orientation. The results of at least three independent transfection experiments performed in triplicate plus S.E.M. are plotted.

occurring mutations of the gene include increased substance dependence (Hoehe et al., 2000). In general, polymorphisms located in a gene's promoter region may affect—positively or negatively—its transcription rate and/or its responsiveness to stimuli with dramatic consequences. We recently characterized an allelic variation within the IL-4–responsive element/STAT6 binding site of the human μ-opioid receptor promoter which reduces responsiveness of the gene to IL-4 to approximately 50% (Kraus et al., 2001). Another polymorphism within the μ-opioid receptor gene promoter is immediately adjacent to the M4 motif (Hoehe et al., 2000). In the variant genome, a T residue is inserted at the asterisk-marked position of the wild-type sequence 5′-ATGAT*TGACTCCAAGGT-3′ (the M4 motif is underlined). The results presented in this study exclude the possibility that this variation affects an AP-1 site, because neither Fos proteins nor other AP-1 site-specific factors bind to this sequence. It may yet be an interesting polymorphism, because mobility shift experiments suggested that another nuclear factor binds to the M4 wild-type motif. Further studies will be necessary to characterize this nuclear factor, as well as the influence of the mutation.

In conclusion, these findings contribute to the understanding of the regulation of μ-opioid receptors, which mediate various effects of widely used drugs in humans. Knowledge of regulatory mechanisms of pharmacologically important genes might become of more than academic interest if, for example, it is possible in the future to achieve a higher efficacy of drugs by specifically manipulating the expression of drug receptors.

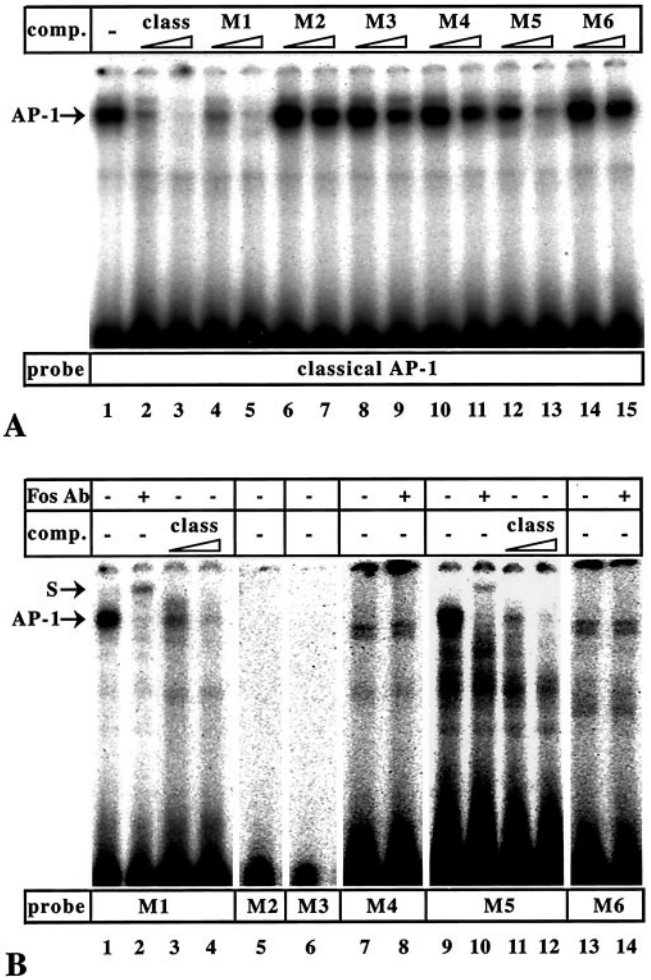


Fig. 3. Electrophoretic mobility shift assays demonstrating the binding of AP-1 to human μ-opioid receptor promoter elements. A, mobility shift experiments using the classic AP-1 probe incubated with nuclear extract of TPA-stimulated SH SY5Y cells. Lanes 2 through 15 show competition experiments (5- and 50-fold molar excess, as indicated by a triangle) with unlabeled homologous oligonucleotides and oligonucleotides containing the putative μ-opioid receptor AP-1 sites M1 through M6. An arrow indicates the AP-1 complex. B, electrophoretic mobility shift assay using M1 through M6 as probes (indicated at the bottom). The addition of classic AP-1 competitor DNA and addition of c-FOS antibody is indicated above. An arrow indicates the location of the immunoshifted band (S).

References

Angel P, Imagawa M, Chiu R, Stein B, Imbra RJ, Rahmsdorf HJ, Jonat C, Herrlich P, and Karin M (1987) Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated *trans*-acting factor. *Cell* **49:**729–739.

Azaryan AV, Coughlin LJ, Buzas B, Clock BJ, and Cox BM (1996) Effect of chronic cocaine treatment on mu- and delta-opioid receptor mRNA levels in dopaminergically innervated brain regions. *J Neurochem* **66:**443–448.

Bianchi M, Maggi R, Pimpinelli F, Rubino T, Parolaro D, Poli V, Ciliberto G, Panerai AE, and Sacerdote P (1999) Presence of a reduced opioid response in interleukin-6 knock out mice. *Eur J Neurosci* **11:**1501–1507.

Brady LS, Lynn AB, Herkenham M, and Gottesfeld Z (1994) Systemic interleukin-1 induces early and late patterns of c-fos mRNA expression in brain. *J Neurosci* **14:**4951–4964.

Chen C and Okayama H (1987) High-efficiency transformation of mammalian cells by plasmid DNA. *Mol Cell Biol* **7:**2745–2752.

Czlonkowski A, Stein C, and Herz A (1993) Peripheral mechanisms of opioid antinociception in inflammation: involvement of cytokines. *Eur J Pharmacol* **242:**229–235.

Erdtmann-Vourliotis M, Mayer P, Riechert U, and Höllt V (1999) Acute injection of drugs with low addictive potential (Δ^9- tetrahydrocannabinol, 3,4-methylenedioxymethamphetamine, lysergic acid diamide) causes a much higher c-fos expression in limbic brain areas than highly addicting drugs (cocaine and morphine). *Brain Res Mol Brain Res* **71:**313–324.

Fredholm BB and Altiok N (1994) Adenosine A2B receptor signalling is altered by stimulation of bradykinin or interleukin receptors in astroglioma cells. *Neurochem Int* **25:**99–102.

Gaveriaux-Ruff C, Matthes HW, Peluso J, and Kieffer BL (1998) Abolition of morphine-immunosuppression in mice lacking the mu-opioid receptor gene. *Proc Natl Acad Sci USA* **95:**6326–6330.

Herdegen T and Leah JD (1998) Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. *Brain Res Brain Res Rev* **28:**370–490.

Hoehe MR, Köpke K, Wendel B, Rohde K, Flachmeier C, Kidd KK, Berrettini WH, and Church GM (2000) Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. *Hum Mol Genet* **9:**2895–2908.

Ji RR, Zhang Q, Law PY, Loh HH, Elde R, and Hokfelt T (1995) Expression of mu-, delta-, and kappa-opioid receptor-like immunoreactivities in rat dorsal root ganglia after carrageenan-induced inflammation. *J Neurosci* **15:**8156–8166.

Kang C and Inouye M (1993) One-step insertion of oligonucleotide linkers or adaptors to DNA using unphosphorylated oligonucleotides. *Biotechniques* **15:**659–668.

Kieffer BL (1995) Recent advances in molecular recognition and signal transduction of active peptides: receptors for opioid peptides. *Cell Mol Neurobiol* **15:**615–635.

Koch T, Schulz S, Pfeiffer M, Klutzny M, Schroder H, Kahl E, and Höllt V (2001) C-terminal splice variants of the mouse mu-opioid receptor differ in morphine-induced internalization and receptor resensitization. *J Biol Chem* **276:**31408–31414.

Kraus J, Börner C, Giannini E, Hickfang K, Braun H, Mayer P, Hoehe MR, Ambrosch A, König W, and Höllt V (2001) Regulation of mu-opioid receptor gene transcription by interleukin-4 and influence of an allelic variation within a STAT6 transcription factor binding site. *J Biol Chem* **276:**43901–43908.

Kraus J, Horn G, Zimprich A, Simon T, Mayer P, and Höllt V (1995) Molecular cloning and functional analysis of the rat mu opioid receptor gene promoter. *Biochem Biophys Res Commun* **215:**591–597.

LaForge KS, Yuferov V, and Kreek MJ (2000) Opioid receptor and peptide gene polymorphisms: potential implications for addictions. *Eur J Pharmacol* **410:**249–268.

Latchman DS (1990) Eukaryotic transcription factors. *Biochem J* **270:**281–289.

Lin HC, Lei SP, and Wilcox G (1985) An improved DNA sequencing strategy. *Anal Biochem* **147:**114–119.

Luckow B and Schutz G (1987) CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. *Nucleic Acids Res* **15:**5490.

Mayer P and Höllt V (2001) Allelic and somatic variations in the endogenous opioid system of humans. *Pharmacol Ther* **91:**167–177.

O'Neill LA and Kaltschmidt C (1997) NF-kappa B: a crucial transcription factor for glial and neuronal cell function. *Trends Neurosci* **20:**252–258.

Parsons CG, Czlonkowski A, Stein C, and Herz A (1990) Peripheral opioid receptors mediating antinociception in inflammation. Activation by endogenous opioids and role of the pituitary-adrenal axis. *Pain* **41:**81–93.

Persico AM and Uhl GR (1996) Transcription factors: potential roles in drug-induced neuroplasticity. *Rev Neurosci* **7:**233–275.
Quinones-Jenab V, Jenab S, Ogawa S, Inturrisi C, and Pfaff DW (1997) Estrogen regulation of mu-opioid receptor mRNA in the forebrain of female rats. *Brain Res Mol Brain Res* **47:**134–138.
Reisine T and Brownstein MJ (1994) Opioid and cannabinoid receptors. *Curr Opin Neurobiol* **4:**406–412.
Roy S, Charboneau RG, and Barke RA (1999) Morphine synergizes with lipopolysaccharide in a chronic endotoxemia model. *J Neuroimmunol* **95:**107–114.
Ruzicka BB and Akil H (1997) The interleukin-1beta-mediated regulation of proenkephalin and opioid receptor messenger RNA in primary astrocyte-enriched cultures. *Neuroscience* **79:**517–524.
Sacerdote P, Manfredi B, Gaspani L, and Panerai AE (2000) The opioid antagonist naloxone induces a shift from type 2 to type 1 cytokine pattern in BALB/cJ mice. *Blood* **95:**2031–2036.
Stein C, Hassan AH, Przewlocki R, Gramsch C, Peter K, and Herz A (1990) Opioids from immunocytes interact with receptors on sensory nerves to inhibit nociception in inflammation. *Proc Natl Acad Sci USA* **87:**5935–5939.
Vidal EL, Patel NA, Wu G, Fiala M, and Chang SL (1998) Interleukin-1 induces the expression of mu opioid receptors in endothelial cells. *Immunopharmacology* **38:** 261–266.
Wahl C, Liptay S, Adler G, and Schmid RM (1998) Sulfasalazine: a potent and specific inhibitor of nuclear factor kappa B. *J Clin Invest* **101:**1163–1174.
Weber CK, Liptay S, Wirth T, Adler G, and Schmid RM (2000) Suppression of NF-kappaB activity by sulfasalazine is mediated by direct inhibition of IkappaB kinases alpha and beta. *Gastroenterology* **119:**1209–1218.
Wendel B and Hoehe MR (1998) The human mu opioid receptor gene: 5′ regulatory and intronic sequences. *J Mol Med* **76:**525–532.
Wöltje M, Kraus J, and Höllt V (1998) Identification of a functional 3′,5′-cyclic adenosine monophosphate response element within the second promoter of the mouse somatostatin receptor type 2 gene. *FEBS Lett* **440:**107–110.
Zadina JE, Chang SL, Ge LJ, and Kastin AJ (1993) Mu opiate receptor down-regulation by morphine and up-regulation by naloxone in SH-SY5Y human neuroblastoma cells. *J Pharmacol Exp Ther* **265:**254–262.
Zadina JE, Harrison LM, Ge LJ, Kastin AJ, and Chang SL (1994) Differential regulation of μ and δ opiate receptors by morphine, selective agonists and antagonists and differentiating agents in SH- SY5Y human neuroblastoma cells. *J Pharmacol Exp Ther* **270:**1086–1096.
Zimprich A, Simon T, and Höllt V (1995) Transfected rat mu opioid receptors (rMOR1 and rMOR1B) stimulate phospholipase C and Ca^{2+} mobilization. *Neuroreport* **7:**54–56.

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