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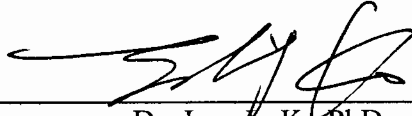
Identifying the Functional Interaction of PCBP and Sp Proteins in Regulation of mu-Opioid Receptor Gene Expression

Aida Rivera-Gines

Submitted in partial fulfillment of the requirements for the Degree of Master of Science in Microbiology from the Department of Biology of Seton Hall University August 2006

Approved By:



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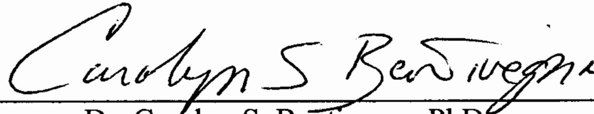

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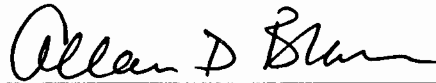

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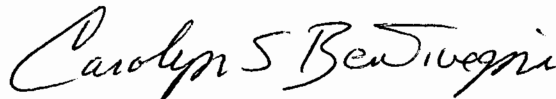

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Table of Contents

Section:	Page:
Abstract	1
Introduction	2
Methods and Materials	8
Results	11
Discussion	20
Conclusion	22
References	23

<u>**List of Figures**</u>

Figure 1	Page 13
Figure 2	Page 15
Figure 3	Page 16
Figure 4	Page 18
Figure 5	Page 19

Abstract

The mu opioid receptor (MOR) mediates analgesia and is responsible for the addictive effects of opiate drugs such as heroin and morphine. Cloning of the MOR gene has led to the identification of at least three promoters. Among these promoters, the proximal promoter is foremost in directing MOR 1 gene expression in brain and during development. Previous studies have demonstrated that Sp1 and Sp3 bound to the proximal promoter are involved in the regulation of MOR expression. Recently, the poly C binding protein 1 (PCBP) has also been shown to participate in the regulation of the MOR gene by binding to the single stranded DNA element within the proximal promoter. We therefore examined the functional interplay between the Sps and PCBP here. The functional interaction between PCBP and Sp proteins was examined using the cotransfection of the MOR promoter with Sp1, Sp3, and PCBP expression vectors into Drosophila SL-2 cells. Our results revealed that Sp1 exerted a greater trans-activation effect than Sp3 or PCBP alone on the MOR gene expression, and PCBP displayed the least transactivation effect. Stimulation of MOR gene expression by the combination of PCBP and Sps was additive, with Sp1 and Sp3 at 500ng level as well as with Sp1 at 100ng level. Interestingly, PCBP acted synergistically in stimulating MOR gene expression with Sp3 at 100ng level. These data indicated that the interaction between PCBP and Sp3 at low concentrations may differ from its interaction with Sp1 and Sp3 at higher concentrations. Overall these studies suggest a functional interaction between Sps and PCBP and further indicate that the ratio of Sps (Sp1 and Sp3) and PCBP within a cell may affect MOR gene expression.

Introduction

Opioids are the major class of analgesics used in the management of moderate to severe pain. Repeat administration of these analgesics, such as morphine, may cause the development of tolerance and physical dependence (1). Tolerance is an adaptive response to opioids, and it is defined as a loss of analgesic efficacy following continued use of opioid drugs, such that a higher dose is required for equivalent effect (2). Physical dependence is the term used to describe the physiological adaptation of the body when an opioid is abruptly discontinued or an opioid antagonist is administered (3). In morphine dependent subjects the nature of the symptoms depends on the species studied. In rodents such as rats and mice, physical dependence is typically manifested as the appearance of ptosis, teeth chattering, wet dog shakes, jumping and diarrhea (4). Opioid receptor agonists also exert effects on mood and motivation. They produce euphoria in humans and function as a positive reinforcer in a variety of species (1). The rewarding effects of these drugs become the primary stimuli, and thus the process of compulsive drug seeking and addiction ensues (5, 6).

These effects are mediated through opioid receptors, which interact with exogenous or endogenous opioid ligands. Using pharmacological studies and molecular cloning these receptors have been classified into three major types: μ, κ, and δ (7). All three opioid receptors have been shown to belong to the G protein coupled receptor superfamily (8). They are about 60% identical to each other with the greatest homology found in the transmembrane domains and intracellular loops; the greatest divergent areas were found in the N terminus, extracellular loops, and C terminus (9). These opioid receptors coupled to G proteins are known to inhibit adenylyl cyclases (10) and Ca^{2+} channels (11,12), as well as stimulate K^{+} channels (13) and increase

intracellular Ca^{2+} levels (14). In addition, opioid receptors have been shown to regulate the mitogen-activated protein (MAP) kinase cascade (15-17).

Among the receptors, the mu opioid receptor (MOR) is known to be the main site of interaction for major clinical analgesics, particularly morphine. Its critical role in morphine induced analgesia, tolerance, and physical dependence has been confirmed by MOR gene knockout studies (18-19). Further analysis reveals that the analgesic effects of MOR activation are at least partially attributed to the Gβγ dimer released from Gi/o, which activates G protein activated inwardly rectifying channels (20) and inhibits voltage dependent calcium channels (VDCCs) (21). The overall effect of hyperpolarization, caused by the increased potassium conductance, is the suppression of cellular activities. Therefore reducing neuronal excitability and causing the analgesic effects induced by opioids such as morphine.

Tolerance to the analgesic and rewarding properties of opioid drugs occur through activation of MOR (22). Like many other G protein coupled receptors, MOR can undergo rapid desensitization and internalization following exposure to an agonist (23-25). MOR desensitization and internalization begins with phosphorylation of activated receptors by G protein coupled receptor kinases, followed by arrestin binding (26). MOR desensitization is likely to be the event preceding reduced response to an opioid agonist by MOR expressing neurons (26). However, it is unknown why morphine causes rapid desensitization in some cell systems and not in others (27). It may be a result of varying intracellular environment of different cells, raising the possibility that opioid receptor desensitization is mediated by different processes (28).

The MOR is mainly expressed in the central nervous system, with receptors varying in densities in different regions and playing different roles (29). Deciphering the structural

organization of the MOR gene has enabled investigators to study the mechanisms responsible for gene regulation and expression. The mouse MOR gene is over 100 Kb and consists of several exons (30-31). The presence of dual TATA less promoters, referred to as the distal and the proximal promoter, are known to locate within 1 Kb from the transcription initiation site (32-33). The distal promoter initiates MOR transcription from a single transcription initiation site, positioned 794 bp upstream of the translation start site (34). The proximal promoter initiates MOR transcription from four major transcription sites, all positioned within a region ranging from 291 to 268 bp upstream of the translation site (30). Subsequent studies have confirmed that the proximal promoter is the principal regulatory promoter of MOR gene transcription in mouse adult brain (32) and during mouse development (35).

Within the MOR proximal promoter region several Sp factor binding sites critical to promoter activity have been identified (36). These sites are a morAP-2 like element (37), a MOR iGA motif (36), and an Sp1 binding site (38). All three elements form complexes with Sp factors (Sp1 and/or Sp3) and are essential for MOR proximal promoter activity.

Sp1 and Sp3 belong to the Sp family of transcription factors and are ubiquitous zinc finger proteins that contain similar DNA binding domains (39). Sp1 and Sp3 may compete for common target sequences; their effect depends on promoter structure and cellular environment (40), as well as their potential interaction with other transcription factors (41). Therefore Sp1 and Sp3 can differ in their capacity to regulate transcription. For example, Sp1 can activate transcription of a large number of regulated and constitutively expressed genes whether the promoter contains a TATA box or not (42). It has been shown that Sp1 can transactivate many TATA less promoters by interacting with general transcription machinery, such as the TATA binding protein (TBP) and TBP associated factors (39, 43). Although Sp1 is considered to be

associated with constitutive expression, it has recently been shown to participate in activities such as cell differentiation (44), the cell cycle (45), development (46), and the enhancement of drug resistance (47).

Unlike Sp1, Sp3 contains an inhibitory region between the second glutamine-rich activation domain and zinc finger region (48). Sp3 can activate or repress transcription but the molecular event that controls this dual function remains unclear. It has been reported that cellular context plays a role in the activation of transcription by Sp3. For example, Sp3 stimulated transcription from the HERV-H terminal repeat in the teratocarcinoma cell line, NTera2-D1, but acted as a repressor in HeLa and insect cells (49). Whether Sp3 acts as an activator or repressor might also depend on the promoter. Promoters containing a single binding site are activated by Sp3 whereas promoters containing multiple binding sites often are not activated or respond weakly to Sp3 (48, 50). The extent of post-translational modification also influences the role Sp3 plays in transcription. For example, Sp3 is post-translationally modified by a small ubiquiten-like modifier (SUMO) within its inhibitory domain and that SUMO modification regulates the transcriptional activity of all Sp3 isoforms (51). It has also been reported that acetylation of Sp3 controls the activator or repressor function of Sp3. Inducing acetylation of Sp3 stimulated promoter activity and enhanced transcriptional activity in MCF-7E breast cancer cells (52). Therefore, the role of Sp3 in transcription is dependent on a variety of molecular factors.

In addition to the Sp factors binding sites, the MOR proximal promoter also contains a 26-bp polypyrimidine/purine (PPy/u) nucleotides region that is able to adopt a ss DNA conformation (53). Functional analyses suggested the PPy/u region is essential for MOR promoter activity (54). A ss DNA binding protein termed mPy protein (mor polypyridimine-

binding protein) can bind to the sense strand of the PPy/u (ssPPy) region (54). A ss DNA binding protein, named poly C binding protein 1 (PCBP1) was obtained from a mouse cDNA library using yeast one-hybrid screening system, and it has been shown to bind the ssPPy element of the MOR gene in a sequence specific manner and participate in the regulation of MOR gene expression (54).

The poly C binding proteins (PCBPs) belong to the K-homology (KH) domain superfamily. The KH domain was initially identified in heterogeneous nuclear ribonucleoprotein K (hnRNP K) and has been found in a wide range of RNA binding proteins in organisms extending throughout the evolutionary spectrum (55). PCBPs are a structurally diverse family that includes hnRNP K and αCP1-4 (α complex protein) referred to as PCBP1-4 (56). They bind mRNA sequences that contain either a single C (hnRNP K) or a stretch of C's (αCPs) via their KH domains (57). Members of the αCP protein family have been implicated in a wide spectrum of posttranscriptional controls, for example, PCBP1 can bind to human α-globulin mRNA 3'UTR and form an α complex (58). This type of mRNA stabilization does not appear to be limited to α-globulin; data has suggested that binding of PCBP 1 to 3'UTR pyrimidine rich determinants may represent a general mechanism for stabilization of long lived cellular mRNA (59). In addition, PCBP1 has been shown to function as a ss DNA binding protein and a transcriptional regulator in MOR gene expression (54).

In summary, previous studies demonstrate that MOR promoter activity can be regulated by both ss and ds DNA binding proteins, implying that regulation of MOR gene expression involves an interplay between Sp proteins and PCBP. The functional interaction between Sp factors and PCBP on the MOR gene were still unknown, we therefore investigated the functional role of PCBP, Sp1, and Sp3 on the transcriptional regulation of MOR. Using functional analysis

we determined the individual and combinatory effect of each transcription factor on MOR promoter activity. We found that PCBP1 affects the transactivation of MOR promoter activity by Sp1 and Sp3 differently dependant on Sps concentration. Although the molecular mechanism by which PCBP1 exerts its affects on the Sp factors is unknown, the results implicate that Sp1 and Sp3 at a specific concentration interact differently with PCBP1 to achieve cell-specific MOR gene regulation. Since Sp1 and Sp3 are ubiquitously expressed in various cell types at indeterminate concentrations, our results further suggest that the amount of Sp proteins and PCBP1 in different cell/tissue may contribute to different level of expression of MOR gene.

Materials and Methods

Plasmid Construction

The full length cDNA of PCBP 1 was inserted into the Xho I cloning site of a drosophila expression vector pPac, to create the pPac-PCBP fusion protein. The pPac-PCBP plasmid was confirmed using restriction enzyme digestion at 37° and subjected to gel electrophoresis. pPacSp1 and pPacSp3 were generous gifts from Dr. Tijan (University of California, Berkeley) and Dr. Gantrum Suske (University of Marburg, Germany).

Transformation

Plasmid DNA was incubated with *E. coli* competent cells on ice for 30 minutes. The mixture was then heat shocked and followed by chilled on ice for a minute. The Luria Broth (LB) media was added to the mixture and incubated at 37°C for 60 minutes. The mixture was then plated onto LB agar plates with ampicillin for selection.

Plasmid Preparation

The plasmid DNA was extracted utilizing QIAGEN (Valencia, CA) Plasmid Maxi Kit. Briefly, a transformed *E. coli* bacteria culture was grown overnight and then collected by centrifugation at 4°C. The bacteria pellet was resuspended in P1 solution containing 50mM glucose, 10mM EDTA (pH 8), 25mM Tris HCl (pH 8) with RNase A. The cells were then lysed by the addition of P2 solution containing 0.2M NaOH and 1% SDS, which was then neutralized by adding P3 solution (5 M potassium acetate, glacial acetic acid). The mixture was then centrifuged at 4°C for 35 minutes, the supernatant was then applied onto a column, which was washed twice with

QC (100% Ethanol) wash buffer. The plasmid DNA was eluted using QF Elution buffer. The eluant is then mixed with isopropanol, and the DNA pellet was collected by centrifugation at 4°C for 35 minutes, and further washed with 70% ethanol.

Cell Culture

Drosophila Schneider line 2 cells (SL-2 cells) were purchased from ATCC and grown at 22-24°C in Schneider's Drosophila medium (Invitrogen) supplemented with 10% heat inactivated fetal bovine serum (FBS).

Transient Transfection

The SL-2 cells were first seeded onto 6 well plates. Cells were then transfected with Superfect lipofection method (Qiagen, Valencia, CA) according to manufacturer's instructions. Various amounts (0.1 μg, 0.5 μg, 1.0 μg) of each test plasmid were transfected into the cells. The amount of DNA used was within the linear range of the relationship between luciferase activity and the amount of DNA transfected. Forty-eight hours after transfection, cells were harvested.

Luciferase Assay

Transfected cells were lysed with Reporter Lysis Buffer (Promega, Madison, WI). The resulting lysate was centrifuged and the supernatant was used for reporter activity assay.

Each cell lysate was mixed with luficerase substrate mixtures, and the light emission from the reaction was measured and recorded in relative light units (RLUs) by a Lumat LB 9507.

Lowry Assay

To determine the protein amount of each cell lysate, the Lowry assay was carried out. A standard curve from 0 to100 μg/ml was generated using bovine serum albumin (BSA). In duplicate sets cell lysate were dispensed into each tube, followed by the addition of freshly prepared solution containing 49 ml of 0.188 M Na_2CO_3 in 0.1 M NaOH, 0.5 ml of 0.07 M sodium potassium tartrate, and 0.5 ml of 0.06 M $CuSO_4$. Each sample was then incubated with 1N Folin–Ciocalteau reagent for 30 minutes at R.T. The protein concentration was determined by colorimetric method by reading O.D. at 595 nm.

Results

Individual contribution of Sp1 on MOR gene expression

To assess whether Sp1 can modulate MOR gene expression, an Sp1 expression plasmid, pPac-Sp1, was cotransfected with the reporter gene driven by the MOR proximal core promoter into *D. melanogaster* SL2 cells. These cells are devoid of ubiquitous Sp transcription factors and are commonly used to study the activation or repression properties of the Sp family. Thus transfections of SL2 cells provide a null background to study the relationship between Sp and other factors in regulating the MOR promoter activity.

The pPac-Sp1 expression plasmid was transiently transfected into SL2 cells at 100 ng, 500 ng, and 1 μg, together with the luciferase reporter driven by the MOR proximal core promoter. Results are expressed relative to the promoter activity obtained upon transfection with pPac empty vector alone, which was defined as 100%.

Fig.1 showed that as the quantity of Sp1 plasmid (closed circle) increased form 100 ng to 1 μg, transactivation of the MOR promoter by Sp1 also increases. This result suggested that Sp1 had a positive transactivation effect on MOR proximal core promoter.

Individual contribution of Sp3 on MOR gene expression

In addition to Sp1, Sp3 also contributes to transcriptional regulation of several genes. It has been shown to regulate transcription and act as a transcriptional repressor or activator. The effect of Sp3 on MOR gene expression was examined using cotransfection assays in SL2 cells. The pPac-Sp3 expression plasmid was cotransfected at quantities of 100 ng, 500 ng, and 1 μg, together with the luciferase reporter driven by the MOR proximal core promoter. As shown in Fig 1 (open circle), at 100 ng of Sp3, MOR promoter activity is at 100%, as the concentration of

Sp3 is increased to 500 ng MOR gene expression increased by 50%. Finally at 1 μg of Sp3, MOR promoter activity nearly doubled compared to100 ng of Sp3. These findings clearly showed that Sp3 alone can transactivate the MOR proximal core promoter in a dose dependent manner.

Individual contribution of PCBP on MOR gene expression

The poly C binding protein 1 (PCBP) has been identified as a ss DNA binding protein that functions as a transcriptional regulator in MOR gene expression. To further compare the role of PCBP in MOR gene expression to those of Sps, a cotransfection assay was performed using various amounts of pPac-PCBP expression plasmid (100 ng, 500 ng, and 1 μg) with the MOR proximal core promoter in SL2 cells. MOR promoter activity increased in the presence of various amounts of PCBP expression plasmid (closed square) compared to that of the pPac expression vector alone, as shown in Fig. 1. These results suggest that PCBP can also transactivate the MOR promoter in a dose dependent manner. However, the PCBP alone showed the least transactivation activity on MOR proximal promoter compared to those of Sp1 and Sp3.

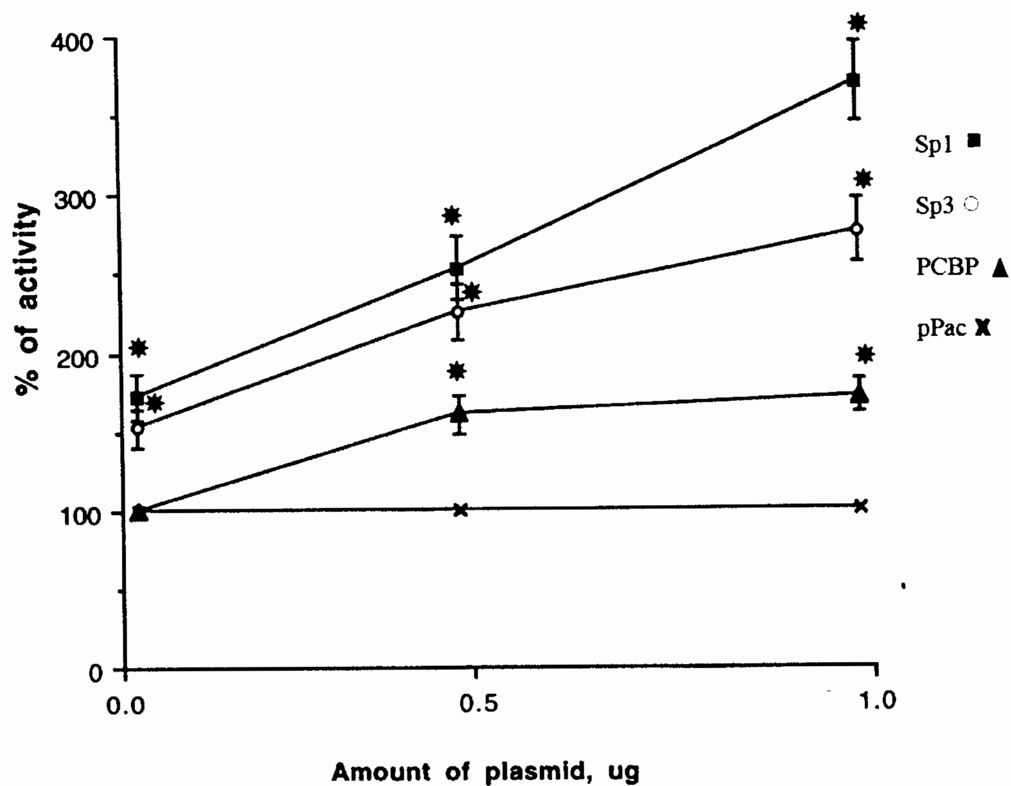

Fig. 1

SL2 cells were transiently cotransfected with indicated amounts of Sp1 (closed circle), Sp3 (open circle), PCBP expression vector (closed square), or pPac vector containing no insert (x) along with the luciferase reporter gene driven by the MOR proximal core promoter. Forty eight hours after transfection, cells were harvested and lysed, and luciferase activity was measured. The relative luciferase activity represents the fold increase in luciferase activity relative to that obtained following cotransfection of the MOR with the control empty Ppac vector, defined as 100%. These results are means of at least three independent experiments. Statistical significance in activities was determined by paired student t test. "✱" indicated $p < 0.01$.

The combinatory effect of Sp proteins at a moderate concentration and PCBP on MOR gene expression

The individual contribution of Sp proteins and PCBP on MOR promoter activity was determined; we therefore examined their combinatory effect on MOR gene expression. The combined role of the strong activator, Sp1, with PCBP was examined on the MOR promoter using cotransfection assays in SL2 cells. Transactivation of MOR was calculated as the percentage of promoter activity in the presence of 500 ng of pPac-Sp1 or pPac-Sp3 and indicated amounts of PCBP divided by the activity of the promoter in the presence of 500ng of pPac-Sp1 or pPac-Sp3 and indicated amounts of pPac empty vector. In Fig. 1, the individual contribution of pPac-Sp1 and pPac-Sp3 at 500ng on MOR promoter activity was approximately 250% and 200%, respectively, as compared to that (100%) of pPac vector alone. Here, in Fig. 2, the MOR promoter activity in the presence of pPac (vector) along with pPac-Sp1 or pPac-Sp3 at 500ng level was defined as 100%.

As shown in Fig. 2, upon cotransfection of a constant amount of Sp1 (500 ng) and increasing quantities of PCBP, an increase in MOR promoter activity is observed. This result suggested that the combination of PCBP and Sp1 displayed an additive effect on the MOR proximal core promoter.

We next examined the combinatory effect of the weak activator, Sp3, together with PCBP (Fig 3). The increase in activity of the MOR proximal promoter correlated with the increase of PCBP in the presence of Sp3 (500 ng). The results suggested the combination of PCBP and Sp3 at 500 ng concentration can also transactivate the MOR proximal core promoter in an additive manner.

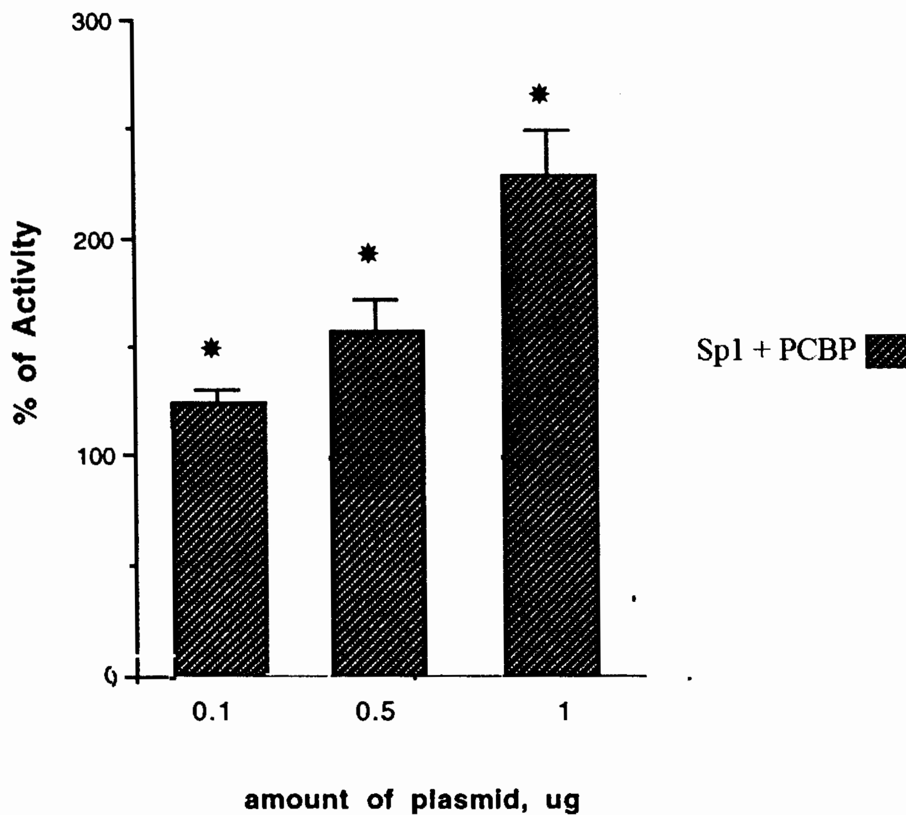

Fig. 2

SL2 cells were transiently cotransfected with constant amount of Sp1 expression plasmid (500ng), an indicated amount of PCBP expression plasmid, and the luficerase reporter gene driven by the MOR proximal core promoter. Forty eight hours after transfection, cells were harvested and lysed. The luciferase activity was then measured. The relative luciferase activity represents the fold increase in luciferase activity relative to that obtained following cotransfection of the pPac-Sp1 at 500 ng with indicated amounts of Ppac empty vector defined as 100%.. These results are means of at least three independent experiments. Statistical significance in activities was determined by student paired t-test. " * " indicated $p < 0.01$.

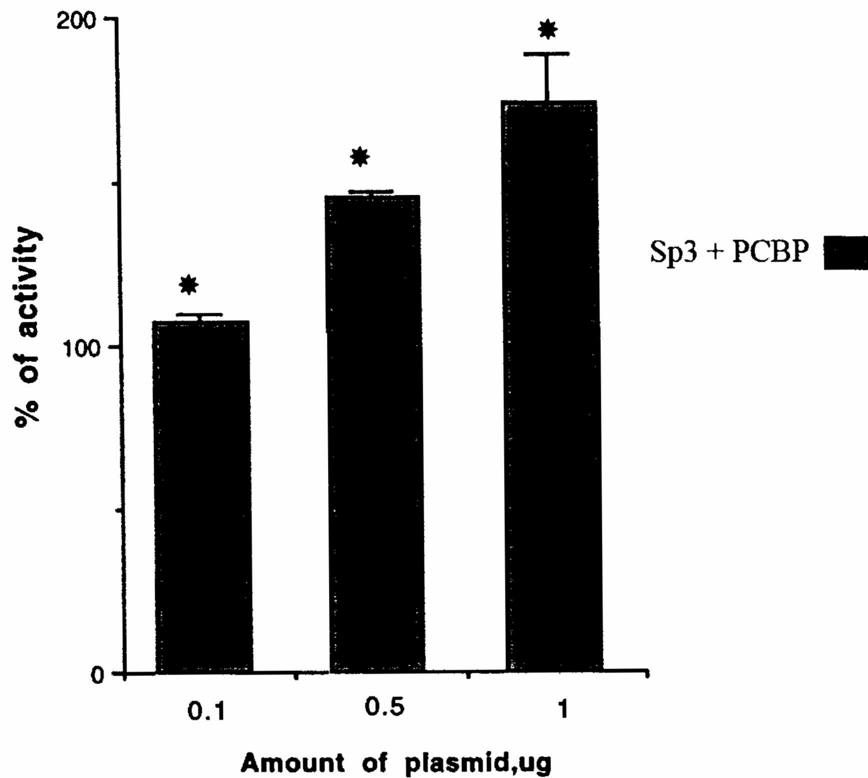

Fig. 3

SL2 cells were transiently cotransfected with constant amount of Sp3 expression plasmid (500ng), an indicated amount of PCBP expression plasmid, and the luficerase reporter gene driven by the MOR proximal core promoter. Forty eight hours later, cells were lysed and luciferase activity was measured. Data are expressed as the relative activity. The relative luciferase activity represents the fold increase in luciferase activity relative to that obtained following cotransfection of the pPac-Sp3 at 500 ng with indicated amounts of Ppac vector, defined as 100%. .These results are means of at least three independent experiments. Statistical significance in activities was determined by student paired t-test. "✱ " indicated $p < 0.01$.

The combinatory effect of Sp proteins at a low concentration with PCBP on MOR promoter activity

Since Sp1 and Sp3 are expressed at varying levels in different cell types, we therefore examined the combinatory effect of PCBP with Sp proteins at a low concentration (100 ng level) on the MOR gene expression. The combined role of Sp1 and Sp3 at 100 ng with pPac-PCBP (100 ng, 200 ng, and 500 ng) on the MOR promoter was examined using cotransfection assays in SL2 cells. The MOR proximal core promoter activity observed with Sps at 100 ng and different amounts of pPac empty vector was defined as 100%.

In Fig. 4, the results suggested that the combination of PCBP with Sp1 at a low concentration exerts an additive effect on MOR proximal core promoter activity. Interestingly, in Fig 5 we observed that Sp3 at a low concentration combined with PCBP at 500 ng had a synergistic effect on the transcriptional activation of the MOR proximal core promoter. Overall, these results demonstrated that both Sp1 and Sp3 at low concentration with PCBP activate MOR promoter, but Sp3 at 100 ng with PCBP synergistically activated the MOR proximal core promoter.

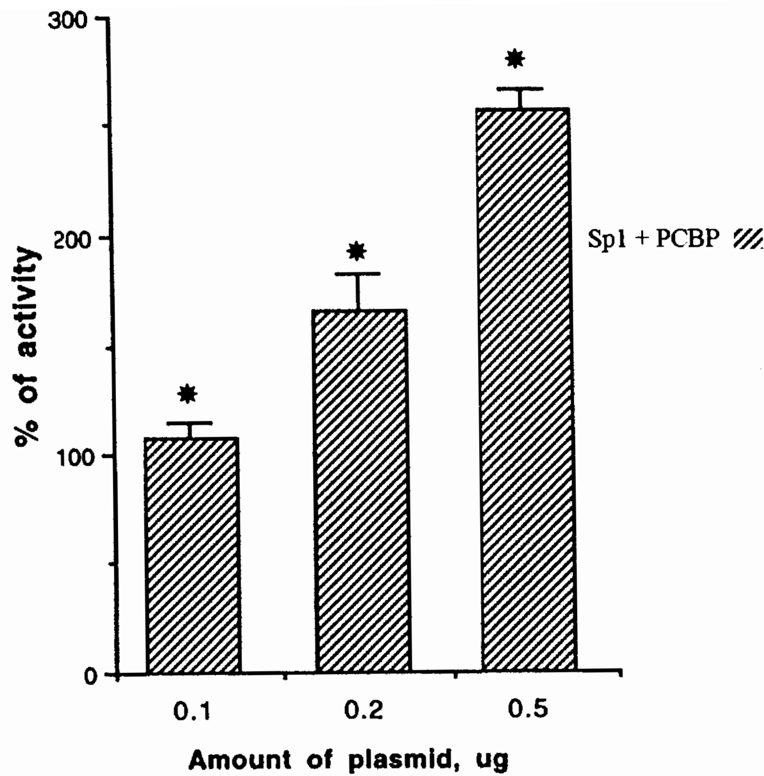

Fig. 4

SL2 cells were transiently cotransfected with a constant amount (100 ng) of Sp1 expression plasmid (hatched bar), an indicated amount of PCBP expression plasmid, and the luficerase reporter gene driven by the MOR proximal core promoter. Forty eight hours later, cells were lysed and luciferase activity was measured. Data are expressed as the luciferase activity relative to that obtained following cotransfection pPac-Sp1 at 100 ng and indicated amounts of Ppac vector as 100%. These results are means of at least three independent experiments. Statistical significance in activities was determined by student paired t-test. "✱" indicated $p < 0.01$.

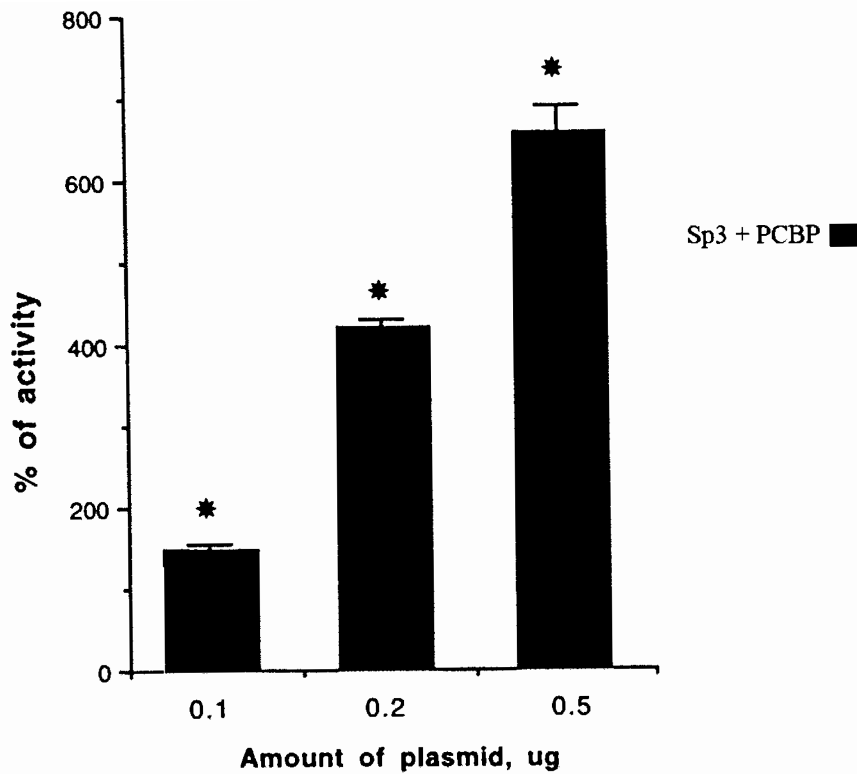

Fig. 5

SL2 cells were transiently cotransfected with a constant amount (100 ng) of Sp3 expression plasmid, an indicated amount of PCBP expression plasmid, and the luficerase reporter gene driven by the MOR proximal core promoter. Forty eight hours later, cells were lysed and luciferase activity was measured. Data are expressed as the fold increase in luciferase activity relative to that obtained following cotransfection of the pPac-Sp3 at 100 ng with indicated amounts of Ppac empty vector as 100%. These results are means of at least three independent experiments. Statistical significance in activities was determined by student paired t-test. " ✱ " indicated $p < 0.01$.

Discussion

The mu opioid receptor plays a fundamental role in analgesia and the addictive effects of opioid drugs. To obtain further insight into the regulatory events of MOR gene expression, we tested the involvement of Sp1, Sp3, and poly C binding protein 1 (PCBP), whose responsive elements have been identified on the MOR proximal promoter region (36, 53). Recently PCBP has been reported to be involved in the regulation of MOR gene expression in NMB cells (60). Using *Drosophila* SL2 cells in cotransfection experiments, our results showed that transcription of MOR is positively regulated by Sp1, Sp3, and PCBP1 (Fig 1), individually. Our studies suggested that Sp1 and Sp3 transactivated the mouse MOR proximal core promoter. Of particular interest to us was the finding that PCBP also transactivated MOR proximal core promoter in SL2 cells. Despite PCBP known function as an RNA binding protein, our functional analysis supports the function of PCBP as a positive regulator on the MOR gene expression. Interestingly, PCBP displayed a lesser transactivation effect on MOR promoter than Sp1 and Sp3 at the same concentration, suggesting that PCBP alone may not play a major role in the transcription of MOR gene.

It is well established that specific combinations of transcription factors exert unique effects on individual gene promoters. For example, Sp1 has been described to collaborate with many transcription factors including Oct-1 (61), NF-κB (62), and E2F1 (63-64) in order to regulate gene expression. We therefore examined the combinatory effect of Sp proteins and PCBP on the MOR proximal core promoter. Our results suggested that a cooperative interaction of Sps and PCBP regulated MOR proximal core promoter. The MOR proximal core promoter is activated in an additive manner by moderate concentration (500 ng) of Sp1 (Fig 2) and Sp3 (Fig 3) with PCBP. These results suggest that a functional and possibly physical interaction between

Sps at a concentration of 500 ng with PCBP is involved in regulating MOR proximal promoter. The same effect was observed between a low concentration of Sp1 (100 ng) and PCBP.

Upon cotransfection with Sps, additive and synergistic effects have been observed in numerous promoters. Whereas, Sp3 can act as an activator or repressor of Sp1 mediated activation depending on the availability of specific co-activators, co-repressors, or other transcription factors (39). Although certain promoters can be activated by either Sp1 or Sp3, and occasionally by both Sp1 and Sp3 in a synergistic manner (65-67), it is reported that promoter composition is likely to play an important role in whether Sp1 or Sp3 is recruited or is able to activate expression (68). Interestingly, the functional interaction between Sp3 at 100 ng with PCBP resulted in a synergistic activation of MOR gene transcription (Fig 5), however, the mechanism for this remains unclear. The acetylation of transcription factors such as p53, E2F1, Myo D, and EKLF has been shown to enhance transcriptional potency and affect protein-protein interactions (69). Previous studies have shown that acetylation of Sp3 enhanced its transactivation activity (52); therefore it is possible that Sp3 at 100 ng with PCBP1 may undergo acetylation which then increases MOR gene transcription.

Variation in the expression of Sp1 and Sp3 may have important consequences for transcriptional activation (40). Thus the ratio of Sp1 and Sp3 factors along with PCBP may contribute to the transcriptional activity of MOR gene in different cells.

Conclusion

In this study we have identified the role of Sp1, Sp3, and PCBP in the expression of MOR gene by utilizing cotransfection assays in SL2 cells. First we measured the effect of each transcription factor on MOR promoter activity using the luciferase assay. We established the individual effect of Sp1, Sp3, and PCBP on MOR proximal promoter. Next we performed combinatory experiments using Sp1 with PCBP and another set of combinatory experiments utilizing Sp3 with PCBP.

Our results found that Sp1 alone possessed the strongest transactivation on MOR proximal promoter activity compared to Sp3 and PCBP, with PCBP exerting the least effect on MOR promoter activity (Figure 1). Further functional analysis revealed the interaction of Sp1 at 100 ng (Figure 4) and 500 ng (Figure 2) with poly C binding protein 1 (PCBP) positively regulated transcription of the MOR gene in an additive manner. In addition our results also showed that Sp3 at 500 ng (Figure 3) with PCBP transactivated the MOR proximal promoter in an additive manner. Finally Sp3 at 100 ng (Figure 5) with PCBP synergistically activated MOR gene expression.

In summary, the present study determined the functional significance of Sp1, Sp3 and PCBP individually and in combination on MOR promoter activity. The synergistic effect observed on MOR gene expression between Sp3 at 100ng and PCBP has not been previously reported. This effect may be attributed to cell specific MOR gene expression or acetylation of Sp3 at 100 ng causing an increase in MOR gene expression. Identification of the mechanism responsible for this effect may have future therapeutic implications. Therefore, further analysis will need to be performed.

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