Involvement of a single-stranded DNA binding protein, ssCRE-BP/Purα, in morphine dependence

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Abstract We have purified a nuclear protein from mouse cerebella that binds to single-stranded oligo-DNA of cAMP response element and is modulated by morphine treatment. Isolation of the cDNA clone showed that the nuclear protein (ssCRE-BP) was identical to Purα, a DNA binding protein for single-stranded purine-rich sequences that was originally isolated as a replication factor. ssCRE-BP/Purα and mRNA were abundant in the brain. The levels of ssCRE-BP/Purα and the transcript were not changed by chronic morphine treatment, however, the levels of an activator of ssCRE-BP/Purα, which is necessary for the DNA binding, may be modulated by the treatment.

Key words: Single-stranded cAMP response element; Morphine dependence; DNA binding protein; Purα; Mouse cerebellum

1. Introduction

The development of tolerance and dependence by chronic morphine administration has been well documented in rodents using different protocols such as analgesic assays, locomotor activities and withdrawal-induced jumping activities [1]. For example, naloxone-induced jumping in mice treated chronically with morphine was used as a model for drug dependence [2]. Tolerance and dependence have been shown to continue for a certain period after morphine withdrawal. Since the long-term cellular modifications are thought to be associated with changes in protein synthesis [3], it has been speculated that the regulation of gene expression may be involved in drug tolerance and dependence. Recent studies have shown that neural activities are associated with changes of transcription factors that interact with specific *cis*-elements in the promoter region [4–6]. Morphine treatment has been shown to regulate c-*fos* expression in rat caudate putamen and phosphorylation of cAMP response element binding protein (CREB) in rat locus coeruleus [7,8].

We have recently found that the binding activity of a nuclear factor (ssCRE-BP) from the cerebellum was modified by chronic morphine treatment [9,10]. In this report, we describe the purification of ssCRE-BP from mouse cerebellum and the isolation of cDNA encoding ssCRE-BP, and investigate the mechanism by which the DNA binding activity of ssCRE-BP is decreased. This protein was identified originally as a replication factor, termed Purα [11,12]. We discuss the implication of ssCRE-BP/Purα in morphine dependence in the brain.

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Abbreviations: ssCRE-BP, single-stranded cAMP response element binding protein; GST, glutathione *S*-transferase; DAPI, diamidinophenyl indole

2. Materials and methods

2.1. DNA-protein binding assays

For all experiments, male ddY strain mice weighing 25–30 g were supplied with food and water ad libitum. Chronic morphine treatment consisted of injecting the mice subcutaneously twice daily for 8 days with morphine hydrochloride (Dainipponseiyaku, Osaka, Japan). The dose of morphine hydrochloride was doubled every day from 10 mg/kg to 80 mg/kg in 4 days, and then a dose of 100 mg/kg was maintained during the last 4 days. This treatment has been shown to induce tolerance and dependence in mice [13]. At appropriate intervals, mice were decapitated, and the brains were dissected.

Gel shift assays were carried out as previously described [9].

2.2. Purification of ssCRE-BP/Purα

Biotin-21-dUTP (Clontech Laboratories, Inc., CA) was incorporated into the 3′ terminus of the synthetic single-stranded oligonucleotide (5′-CTGGGGGCGCCTCCTTGGCTGACGTCAGAGAGAGAG-3′) using terminal deoxynucleotidyltransferase (Takara Shuzo Co., LTD., Kyoto, Japan). Reactions (100 μl) contained 100 mM sodium cacodylate (pH 7.0), 1 mM $CoCl_2$, 0.1 mM DTT, 2 nmol oligonucleotides, 10 nmol biotin-21-dUTP, and 20 U terminal transferase, and were incubated at 37°C for 30 min, and stopped by heating to 75°C for 10 min. The biotinylated oligonucleotides were precipitated by adding 15 μg poly(dI-dC), 0.3 M NaCl, and 1 ml of ice-cold 100% ethanol. The precipitate was dissolved in 100 μl of H_2O, and mixed with 500 μl of streptavidin agarose (Gibco BRL, Life Technologies, Inc., MD) with agitation for 30 min. The binding of the biotinylated oligonucleotides to the streptavidin agarose was monitored by adding ^{32}P-labeled oligonucleotides to the reaction buffer [14].

Nuclear extracts were prepared from 60 g of mouse cerebella as described previously [10]. Extracts (600 ml) were subjected to high speed centrifugation (100 000 × *g* for 1 h), concentrated with Amicon YM 100 ultrafiltration membranes (Amicon, Grace Company, Danvers, MA) to 60 ml, mixed with 6 mg poly(dI-dC), and applied on 5 ml of a DNA affinity column. After the column was washed with buffer A (20 mM 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid-NaOH, pH 7.5, 20% glycerol, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM DTT), containing 0.1 M NaCl, proteins were eluted with buffer A, containing 0.3, 0.6, and 1.0 M NaCl. Fractions eluted with 1.0 M NaCl which contained ssCRE-BP/Purα were diluted to 0.1 M NaCl before application to a 1 ml Mono-Q FPLC column (Pharmacia LKB Biotechnology, Uppsala, Sweden) equilibrated with buffer A, containing 0.1 M NaCl. Following a 10 min wash with the same buffer, elution was carried out at 0.5 ml/min using a linear gradient of 0.1–1.0 M NaCl over 30 min. DNA binding activity and purity of the fractions were measured by the gel shift assay and 10% SDS-polyacrylamide gel electrophoresis.

2.3. Protein sequence analysis and cDNA cloning of ssCRE-BP/Purα

The cDNA of ssCRE-BP/Purα was obtained as follows. The protein in buffer A (phenylmethylsulfonyl fluoride was omitted), containing 0.1 M NaCl and 2 M urea, was digested with *Achromobacter lyticus* lysyl endopeptidase (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 30°C overnight. Enzyme to substrate ratio was at 1:10 (w/w). The mixture of the products was applied to a reversed-phase column (2.1 × 150 mm, YMC-Pack MB-ODS, YMC Co., Ltd.,

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Kyoto, Japan) using a Waters HPLC system (Waters Associates, MA), eluted by a linear gradient from 0.1% trifluoroacetic acid in acetonitrile/H_2O, 1:99 (v/v) to 0.085% trifluoroacetic acid in acetonitrile/H_2O, 60:40 (v/v), at a flow rate of 0.3 ml/min for 60 min at ambient temperature, and collected as individual chromatographic fractions by monitoring at 214 nm. The N-terminal sequences of peptides were determined using automated cycles of the Edman degradation reaction with peptide sequencer (ABI-477A, Applied Biosystems).

An aliquot of template DNA from a mouse brain Quick-Clone cDNA library (Clontech Laboratories, Inc., CA) was amplified by PCR with degenerate primers from peptide sequences. A 96 bp PCR product was obtained using the sense primer (5′-GT(AGCT)-GA(CT)AT(ACT)CA(AG)AA(CT)AA(AG)-3′) and the antisense primer (5′-CTTGTT(GT)CC(GT)CC(AGT)GC(AGCT)CC(AGCT)-AC(CT)TC-3′) which encoded two of the sequenced peptides. Approximately 1.0×10^6 plaques of a mouse brain λgt10 cDNA library (Clontech) were screened by plaque hybridization at high stringency using the α-^{32}P-labeled PCR product as probe. DNA sequences were analyzed by the dideoxy chain termination method with an ABI 373A DNA sequencer (Applied Biosystems).

2.4. Northern blot analysis

Northern blot analysis was carried out according to the method of Maniatis et al. [15]. The probe used for Northern blot analysis was the 95 bp fragment of ssCRE-BP/Purα cDNA (nucleotides 165–262) labeled with [α-^{32}P]dGTP, an antisense primer (5′-TCTTCAGG-AAGCGGCCCTTGGCGTTCTGCTTCACGTCCAGGTA-3′) corresponding to nucleotides 220–262 of ssCRE-BP/Purα cDNA, the 777 bp *Pst*I fragment as a template, and Klenow fragment. Total RNA was isolated from several tissues by the acid guanidinium thiocyanate-phenol-chloroform method [16]. The amount of RNA was measured at OD_{260}. 20 μg of the RNA samples were fractionated on 1% agarose gels containing formaldehyde and transferred to nitrocellulose filters (Schleicher and Schuell). The blotted membrane was hybridized with a radiolabeled DNA probe in hybridization buffer at 65°C for 17 h [17]. After hybridization, the filters were washed in 1×SSC at 71°C, dried, and autoradiographed.

2.5. Western blot analysis

Polyclonal antisera against ssCRE-BP/Purα were raised in rabbits by immunization with GST-ssCRE-BP/Purα fusion proteins. The anti-ssCRE-BP/Purα antibody was purified on immobilized GST-ssCRE-BP/Purα proteins. A bacterial expression vector encoding recombinant GST-ssCRE-BP/Purα fusion protein with GST was constructed by subcloning 1.2 kb full-length cDNA of ssCRE-BP/Purα into the vector pGEX-2T (Pharmacia). The GST fusion protein was expressed in *E. coli* (DH5α, Gibco BRL) and purified using a glutathione Sepharose column (Pharmacia).

Nuclear extracts from mouse tissues were analyzed by Western blot as described previously [17]. Equal amounts of proteins (20 μg) from each sample were fractionated on 10% SDS-PAGE and electrotransferred to nitrocellulose filters (85A, Schleicher and Schuell). The filters were blocked with 1% skim milk (Gibco) in TS buffer (150 mM NaCl and 10 mM Tris-HCl, pH 8.0) and then incubated with the affinity purified antibody against ssCRE-BP/Purα (primary antibody) diluted in TS buffer containing 1% skim milk (1:100) for 1 h at room temperature. After washing with TST buffer (TS buffer containing 0.05% Tween 20) 4 times, the filters were incubated with horseradish peroxidase-conjugated goat antibody against rabbit IgG (Cappel) in TS buffer containing 1% skim milk (1:1000) for 1 h at room temperature. Then, after washing with TST 3 times and washing with TS buffer twice, the blotting was performed in the ECL system (Amersham) according to the protocol of the manufacturer.

2.6. Immunofluorescence

Mice were decapitated, and the brains were quick-frozen in a powder of dry ice. The frozen brains, supported in OCT compound (Tissue Tek II, Miles), were sectioned in a cryostat chamber. The sections (16 μm thick) were mounted on gelatin-coated glass slides as described previously [14], and incubated with the antibody against ssCRE-BP/Purα (diluted at 1:100) in buffer A containing 100 mM NaCl, 20 mM Na_2HPO_4, 20 mM NaH_2PO_4, pH 7.4, at 4°C for 15 h. Then the sections were washed in buffer A twice and incubated with FITC-conjugated goat antibodies against rabbit IgG (Cappel, diluted at 1:500) at 4°C for 15 h. For DNA staining with diamidinophenyl indole (DAPI), the sections were incubated with DAPI (2 μg/ml) in the buffer at room temperature for 1 h, and washed twice with the buffer. The sections were then examined and photographed under a fluorescence microscope (Zeiss). Cultured cell lines, NG108-15 mouse neuroblastoma×rat glioma cells, SK-N-SH human neuroblastoma cells, C6 rat glioma cells, and NIH3T3 mouse fibroblasts, were grown on tissue culture chamber slides (Nunc), washed with buffer A and fixed with 2% paraformaldehyde/0.1% Triton X-100 in buffer A for 30 min at room temperature. The cells were washed with 1% Triton X-100 in buffer A twice and then washed again with buffer A twice. The cells were incubated with the antibodies and DAPI.

3. Results

The single-stranded DNA binding protein ssCRE-BP was purified from 800 mouse cerebella using ultrafiltration, oligo-DNA agarose chromatography and ion exchange chromatography on a Mono Q column. A 13 500-fold purification and 20% recovery of the total ssCRE binding activity was obtained (Table 1). During the purification of ssCRE-BP/Purα, we found that addition of casein is required to restore the DNA binding activity of the purified ssCRE-BP/Purα in the gel shift assay. Analysis of molecular weight on a Superose 12 column showed ssCRE-BP/Purα to elute with a molecular mass of ~140 kDa. When analyzed by SDS-PAGE and gel shift assays, the highly purified ssCRE-BP/Purα fraction yielded two silver stained bands corresponding to 40 and 38 kDa. This indicates that ssCRE-BP/Purα is composed of a dimer or oligomer in its native state. The fractions from the Mono Q column with ssCRE-BP/Purα DNA binding activity were digested with lysylendopeptidase to provide peptide for protein sequence. Eight different fragments were separated on reverse-phase HPLC, and the N-termini were sequenced by Edman degradation (data not shown).

Degenerate oligonucleotides encoding these peptides were provided as primers for polymerase chain reactions (PCRs) using a mouse brain cDNA library as template and a PCR product with 96 bp was obtained. The mouse brain cDNA library was then screened using a ^{32}P probe prepared from this PCR fragment; several overlapping partial cDNAs were obtained. An open reading frame encoding 322 amino acids predicted ssCRE-BP to be 35 kDa, and included all eight peptides deduced from the peptide sequencing. A Genbank data base search revealed that the cDNA sequence of mouse ssCRE-BP was identical to mouse Purα [18].

Northern blot analysis was used to determine the size and tissue distribution of the mRNA encoding ssCRE-BP/Purα. The ssCRE-BP/Purα probe hybridized a 5 kb message in several tissues (Fig. 1a). We used 95 bp cDNA (nucleotides 165–262) instead of the 777 bp *Pst*I fragment (nucleotides 165–941) of the ssCRE-BP/Purα cDNA [12], because the longer probe contained CAA repeats which produced non-specific hybridization. This short DNA probe hybridized mainly with the transcript of 5 kb. The 5 kb ssCRE-BP/Purα transcript was abundant in the cerebral cortex and cerebellum. The transcript was also detected in striatum, hippocampus, thalamus, hypothalamus, pons, mesencephalon and medulla oblongata at lower levels. Examination of the effect of chronic morphine treatment on ssCRE-BP/Purα mRNA levels in these brain regions showed no significant influence by chronic morphine treatment (data not shown).

To confirm the ssCRE-BP/Purα mRNA distribution and establish that the cloned gene is capable of binding to the ssCRE DNA probe, an expression vector that expresses a

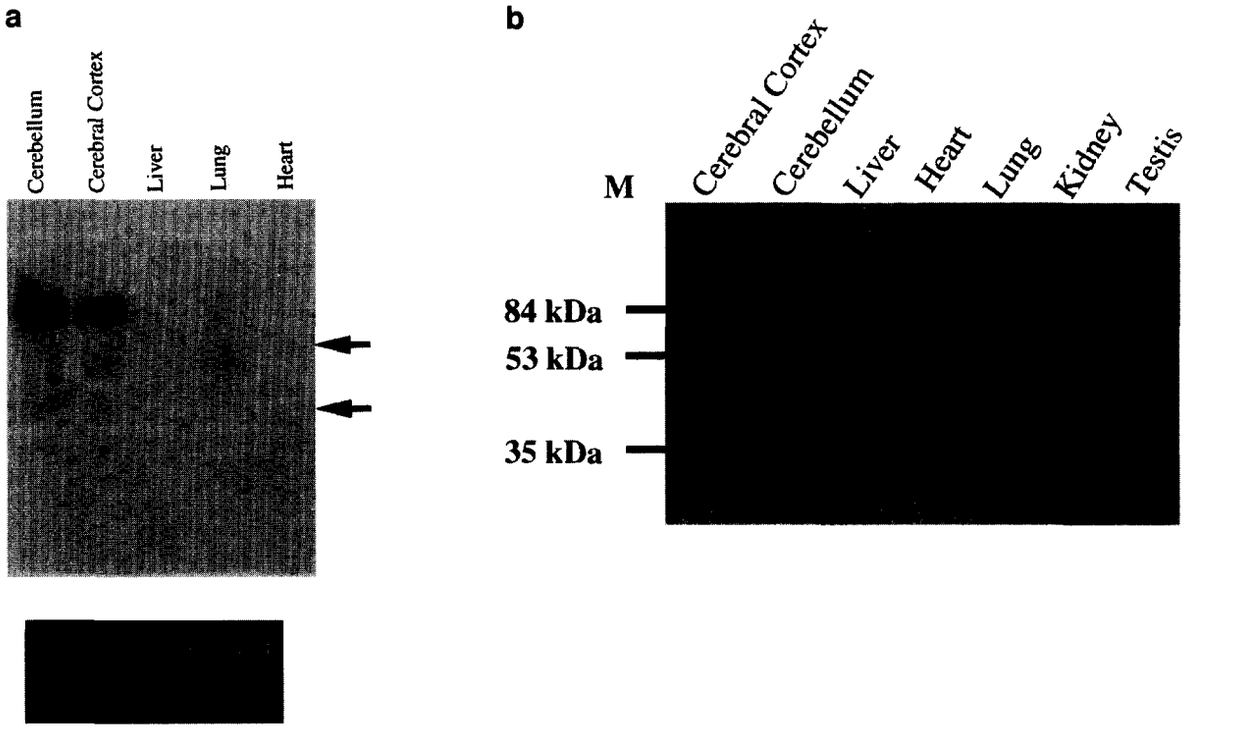

Fig. 1. Northern blot and Western blot analyses of ssCRE-BP/Purα in mouse tissues. (a) Northern blot analysis was carried out according to the method of Maniatis et al. [15]. Arrows indicate the positions of 28S and 18S rRNAs. The lower panel shows the ethidium bromide staining of the gel. (b) Nuclear extracts (20 μg protein) from mouse tissues were used for Western blot analysis with the affinity purified antibody against GST-ssCRE-BP/Purα. M indicates molecular size from markers.

cloned ssCRE-BP gene as a fusion protein to the C-terminus of glutathione *S*-transferase (GST) was constructed. The GST-ssCRE-BP/Purα fusion protein formed a sequence-specific complex with ssCRE DNA in a gel shift assay, confirming that the isolated cDNA encoded ssCRE-BP/Purα. Furthermore, the complex between ssCRE and nuclear extracts from the mouse cerebella which express ssCRE-BP/Purα was supershifted when a polyclonal antibody against GST-ssCRE-BP/Purα fusion protein was added. As control, the complex band with GST-ssCRE-BP/Purα was also supershifted by the polyclonal antibody completely (data not shown).

We examined the expression of the ssCRE-BP/Purα protein using Western blot analysis with the GST-ssCRE-BP/Purα antibody. ssCRE-BP/Purα was found to be widely expressed in the mouse brain. ssCRE-BP/Purα was abundant in the cerebral cortex and cerebellum and low levels were detected in testis (Fig. 1b). Examination of the effect of chronic morphine treatment on the amount of ssCRE-BP/Purα in the mouse brain regions, such as cerebral cortex, cerebellum, striatum, hippocampus, thalamus, hypothalamus, pons, mesencephalon and medulla oblongata, showed that ssCRE-BP/Purα was not significantly influenced by chronic morphine treatment in Western blot analysis (data not shown). These data were also confirmed by Northern blot analysis. Immunohistochemical study demonstrated ssCRE-BP/Purα protein expression in the Purkinje neurons and granular cells of the mouse cerebellum, and low expressions in the liver and heart (Fig. 2a). In the Purkinje neurons, ssCRE-BP/Purα was detected in the nucleus and cytoplasm of the cells. ssCRE-BP/Purα was mainly observed in nuclei of glial cells of the mouse cerebellum at lower levels. In some glial cells, the expression was detected in both nuclei and cytoplasm. Similar data were obtained from cultured cell lines. ssCRE-BP/Purα was localized in both the nucleus and cytoplasm of neuronal cell lines, but mostly in nuclei of C6 glioma cells (Fig. 2b); NIH3T3 cells exhibited barely above background with this antibody. The signals of the fluorescence were decreased by the preincubation of the antibody with GST-ssCRE-BP/Purα fusion proteins (0.5 μg/ml) to background, but not with GST proteins.

At each step of the protein purification, ssCRE-BP was monitored by the sequence-specific binding to the ssCRE

Table 1
Purification of ssCRE-BP

Fraction	Protein (mg)	Spec. act. (cpm × 10^{-4}/mg)	Total binding activity (cpm × 10^{-4})	Yield (%)	Fold purification
Nuclear extract	400	58	23200	100	1
Amicon YM 100	180	113	20340	88	2
Oligo DNA-agarose	0.05	148770	7439	32	2565
Mono Q	0.006	784160	4705	20	13520

Specific activity (Spec. act.) indicates the radioactivity of the complex band in the gel shift assays. After the step of oligo-DNA agarose, the binding activity was assayed in the presence of casein (1 μg).

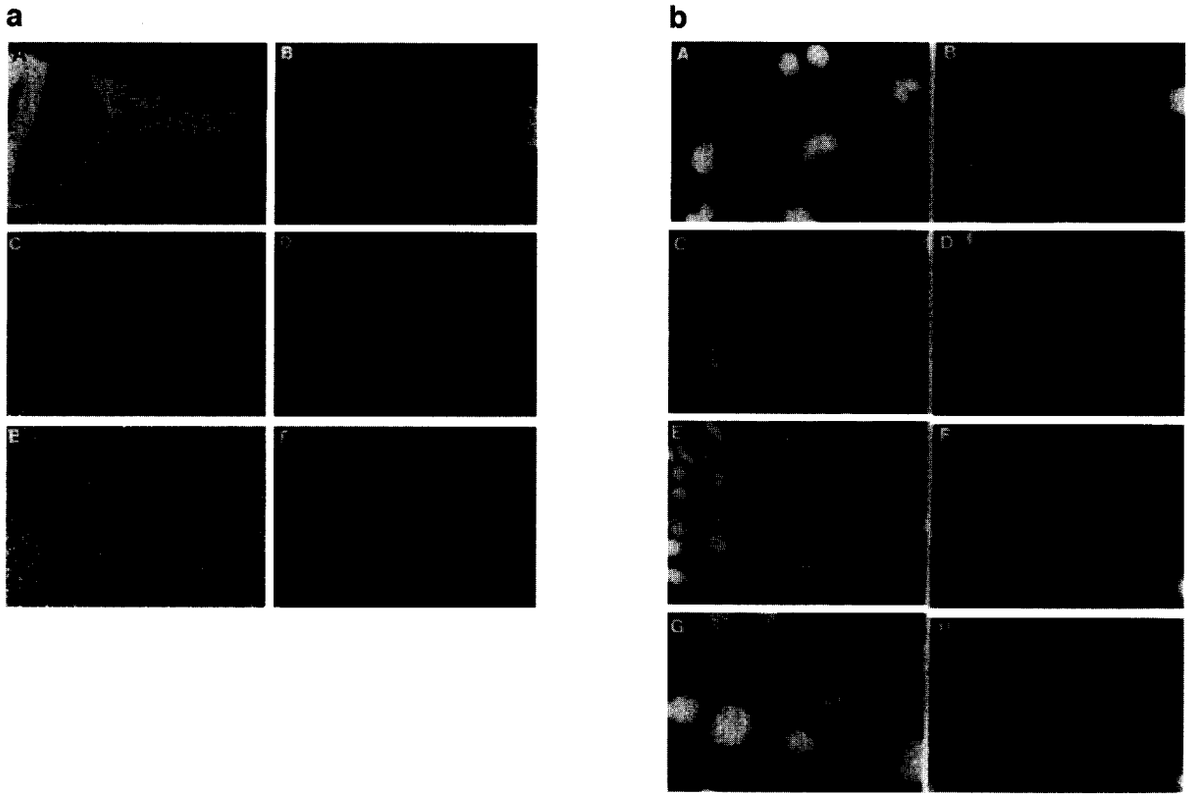

Fig. 2. Immunofluorescence micrographs of mouse tissues and cultured cell lines. (a) The sections of mouse tissues were stained with DAPI (left panels) for DNA staining or the antibody against GST-ssCRE-BP/Purα (right panels); A and B, cerebellum; C and D, heart; E and F, liver. (b) The cells were stained with DAPI (left panels) or the antibody against GST-ssCRE-BP/Purα (right panels); A and B, NG108-15 cells; C and D, SK-N-SH cells; E and F, C6 cells; G and H, NIH3T3 cells.

using gel shift assays. DNA binding activity in the 1.0 M NaCl eluate from the affinity column was no longer detected, however, when a small quantity of the 0.3 or 0.6 M NaCl flow-through fraction from the column was added to a binding mixture containing 1.0 M NaCl fraction, the DNA binding activity of affinity-purified ssCRE-BP/Purα was recovered. Since the 0.3 and 0.6 M NaCl fractions had no DNA binding activity alone, this indicates that an activator is necessary for complex formation between ssCRE-BP/Purα and DNA. To assess the putative activator, a variety of proteins were tested for the ability to increase the DNA binding activity of ssCRE-BP/Purα. As shown in Table 2, casein was capable of substituting for the activator in the DNA binding assay. Bovine serum albumin (BSA), histone and sodium pyrophosphate showed no significant increase in the DNA binding activity of ssCRE-BP/Purα.

Table 2
Effect of casein on the binding activity of ssCRE-BP

Sample	Binding activity
Nuclear extract	324 ± 11
Nuclear extract + Casein	368 ± 24
Affinity fraction	17 ± 5
Affinity fraction + flow-through fraction	2108 ± 39
Affinity fraction + casein	2254 ± 33
Affinity fraction + BSA	14 ± 6
Affinity fraction + histone	16 ± 3
Casein	0

Casein, BSA and histone were added to the DNA binding assays at 0.1 mg/ml except for the affinity chromatography flow-through fraction (0.3 and 0.6 M NaCl eluates), which was used at 0.02 mg/ml. Binding activity was tested by a gel shift assay with 2 μl of nuclear extracts (0.7 mg/ml) or affinity fractions (1.5 μg/ml).

To determine whether the activator was involved in the decrease in the DNA binding activity of ssCRE-BP/Purα observed after chronic morphine treatment of mice, GST-ssCRE-BP/Purα with or without casein was added to the nuclear extracts of cerebellum chronically treated with morphine (Fig. 3). Addition of GST-ssCRE-BP/Purα (2.5 ng) to the nuclear extracts which contained approximately 0.5 ng of endogenous ssCRE-BP/Purα resulted in two complex bands; the lower band was derived from the nuclear extract and the upper band was from GST-fusion protein (arrow in Fig. 3). The complex band formed with the morphine-treated nuclear extracts was weaker than the band with the control nuclear extracts (Fig. 3, lanes 4 and 5). The addition of excess casein to the control or morphine-treated nuclear extracts increased the DNA binding activity to a similar level (Fig. 3, lanes 8 and 9). These data strongly suggest that chronic morphine treatment decreased the DNA binding activity of ssCRE-BP/Purα by lowering the level or activity of the activator in the cerebellum.

4. Discussion

The present data demonstrate that the sequence-specific DNA binding protein, ssCRE-BP/Purα, is a neuronal and

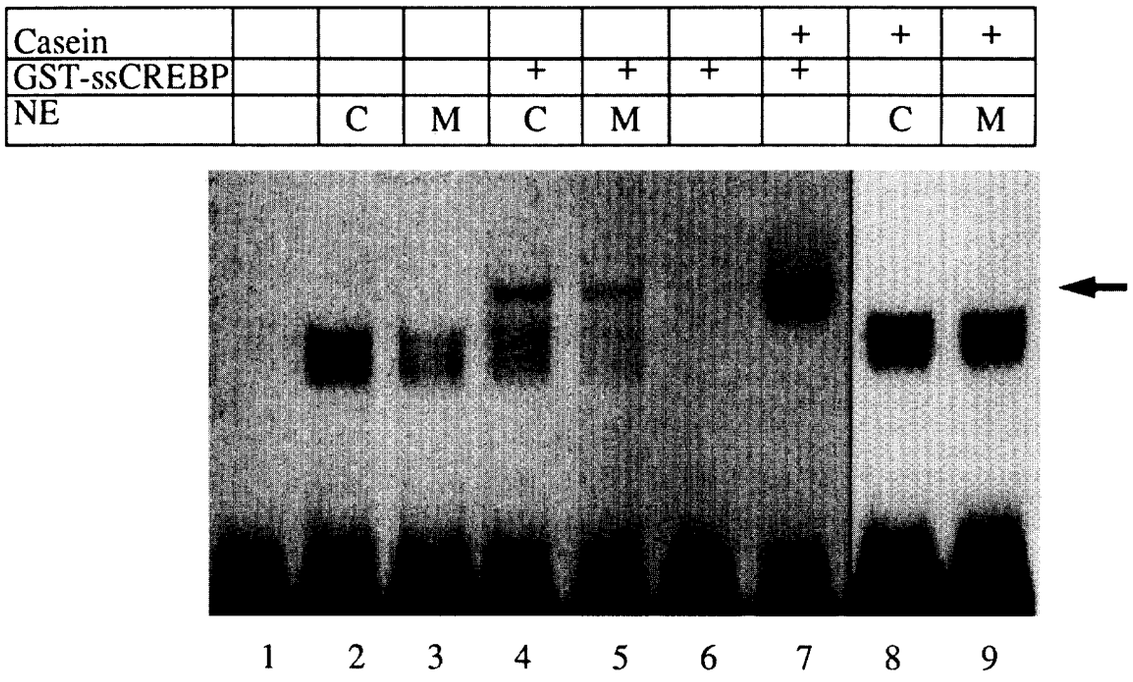

Fig. 3. Effect of chronic morphine treatment on an activator required for the DNA binding activity of ssCRE-BP/Purα. Gel shift assays were performed with 5 μl of nuclear extracts (NE) alone (lanes 2, 3), NE supplemented with 2.5 ng of GST-ssCRE-BP/Purα (lanes 4, 5) or NE plus 1 μg of casein (lanes 8, 9). NE was prepared from the cerebellum of control mice (C) or morphine treated mice (M). Band shifts were not detected with ssCRE-BP/Purα alone (lane 6); the maximal binding activity of ssCRE-BP/Purα when extracts were supplemented with casein (lane 7). The arrow indicates the complex band with GST-ssCRE-BP and oligo-DNA. Lane 1 shows free DNA. Results of the gel shift assay represent the data from four independent experiments.

glial-enriched protein. Purα protein was originally purified from HeLa cells based on its binding to a DNA replication zone of initiation [11,12]. This zone contains a GA-rich element which exists in several eukaryotic origins of DNA replication and upstream promoter regions. Recent reports show that Purα can stimulate transcription of a quail clusterin gene and is a potent activator of early gene transcription [19]. It is possible that ssCRE-BP/Purα has a dual role in DNA replication and in transcription, such as RAD25/ERCC3 protein for DNA repair and RNA polymerase II transcription [20,21]. The data that ssCRE-BP/Purα is abundant in the brain and is regulated by chronic morphine treatment indicate that ssCRE-BP/Purα acts as an important transcription factor in the brain. ssCRE-BP/Purα binds to single-stranded CRE of the somatostatin gene promoter region and preliminary results in our laboratory indicate that this protein binds to the CRE region of the tyrosine hydroxylase gene promoter which contains a GA-rich sequence. The regulation of the DNA binding activity of ssCRE-BP/Purα is likely to influence the biosynthesis of neuropeptides and catecholamine synthesis in the brain [22]. ssCRE-BP/Purα may bind to unknown promoter sequences that regulate numerous cellular processes of the brain. The different distribution of ssCRE-BP/Purα in neuronal and glial cells may imply that ssCRE-BP/Purα is translocated from cytoplasm to nucleus to bind specific sequences of DNA by some signals or factors such as casein-like activators in the neurons. ssCRE-BP/Purα may be activated for replication of DNA and cell division in the nucleus of the glial cells.

We demonstrated that the binding of ssCRE-BP/Purα to single-stranded CRE was specifically activated by casein. Casein stimulates the DNA binding activity of CBF3, a yeast centromere DNA binding protein, and casein is able to substitute for a flow-through fraction from affinity chromatography or *E. coli* cytosolic fraction [23]. Thus, the effect of casein on the DNA binding activity of ssCRE-BP/Purα seemed to be specific. Recent reports showed that ssCRE-BP/Purα interacts with viral transcription regulatory tumor antigen (TAg) of human JC polyomavirus (JCV) which induces demyelinated plaques within the brain of patients, and with the late gene transcription activator of JCV, YB-1, which inhibits the DNA binding activity of ssCRE-BP/Purα to the pur element [24]. ssCRE-BP/Purα can be associated with a variety of proteins and acquire different functions in the transcription and replication in different cells. Because the amount of ssCRE-BP/Purα does not seem to be affected by chronic morphine treatment, the DNA binding of ssCRE-BP/Purα is likely to be regulated by the activator in the brain. The distribution of the activator in the brain would explain why the decreased DNA binding activity of ssCRE-BP/Purα was observed only in the cerebellum by chronic morphine administration [10]. It is possible that the levels of the activator protein in the cerebellum may be lower than in other brain regions limiting its susceptibility to morphine treatment.

Isolation of cDNA clones for the activator and the production of antibody are required for investigating the regulation of the activator protein by morphine. The altered function of ssCRE-BP/Purα by the activator may lead to morphine dependence through the regulation of transcription of the genes that affect the production of neuropeptides and catecholamine synthesis. Further experiments will be required to elucidate the functions of ssCRE-BP/Purα and its activator in the development of morphine dependence.

References

[1] Brady, L.S. and Holtzman, S.G. (1981) Pharmacol. Biochem. Behav. 14, 361–370.
[2] Eidelberg, E and Schwartz, A.S. (1970) Nature 225, 1152–1153.
[3] Loh, H.H., Shen, F.H. and Way, E.L. (1969) Biochem. Pharmacol. 18, 2711–2721.
[4] Graybiel, A.M., Moratalla, R. and Robertson, H.A. (1990) Proc. Natl. Acad. Sci. USA 87, 6912–6916.
[5] Nguyen, T.V., Kosofsky, B.E., Birnbaum, R., Cohen, B.M. and Hyman, S.E. (1992) Proc. Natl. Acad. Sci. USA 89, 4270–4274.
[6] Vezina, P., Blanc, G., Glowinski, J. and Tassin, J.-P. (1992) J. Pharmacol. Exp. Ther. 261, 484–490.
[7] Chang, S.L., Squinto, S.P. and Harlan, R.E. (1988) Biochem. Biophys. Res. Commun. 157, 698–704.
[8] Guitart, X., Thompson, M.A., Mirante, C.K., Greenberg, M.E. and Nestler, E.J. (1992) J. Neurochem. 58, 1168–1171.
[9] Osugi, T., Taniura, H., Ikemoto, M. and Miki, N. (1991) Biochem. Biophys. Res. Commun. 174, 25–31.
[10] Osugi, T., Ikemoto, M., Tanaka, H., Wang, X.-B. and Miki, N. (1994) Mol. Brain Res. 21, 256–262.
[11] Bergemann, A.D. and Johnson, E.M. (1992) Mol. Cell. Biol. 12, 1257–1265.
[12] Bergemann, A.D., Ma, Z.-W. and Johnson, E.M. (1992) Mol. Cell. Biol. 12, 5673–5682.
[13] Babbini, M. and Davis, W.M. (1972) Br. J. Pharmacol. 46, 213–224.
[14] Wang, X.-B., Watanabe, Y., Osugi, T., Ikemoto, M., Hirata, M. and Miki, N. (1992) Neurosci. Lett. 146, 25–28.
[15] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) in: Molecular Cloning, A Laboratory Manual, pp. 7.3–7.84. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[16] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[17] Taira, E., Takaha, N., Taniura, H., Kim, C.-H. and Miki, N. (1994) Neuron 12, 861–872.
[18] Ma, Z.-W., Bergemann, A.D. and Johnson, E.M. (1994) Gene 149, 311–314.
[19] Herault, Y., Chatelain, G., Brun, G. and Michel, D. (1993) Cell. Mol. Biol. Res. 39, 717–725.
[20] Drapkin, R., Reardon, J.T., Ansarl, A., Huang, J.-C., Zawel, L., Ahn, K., Sancar, A. and Reinberg, D. (1994) Nature 368, 769–772.
[21] Guzder, S.N., Sung, P., Bailly, V., Prakash, L. and Prakash, S. (1994) Nature 369, 578–581.
[22] Nestler, E.J., Hope, B.T. and Widnell, K.L. (1993) Neuron 11, 995–1006.
[23] Johannes, L. and Carbon, J. (1991) Cell 64, 717–725.
[24] Chen, N.N., Chang, C.-F., Gallia, G.L., Kerr, D.A., Johnson, E.M., Krachmarov, C.P., Barr, S.M., Frisque, R.J., Bollag, B. and Khalili, K. (1995) Proc. Natl. Acad. Sci. USA 92, 1087–1091.