Glutamate Regulates Kainate-binding Protein Expression in Cultured Chick Bergmann Glia through an Activator Protein-1 Binding Site*

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The expression of the chick kainate-binding protein, a member of the ionotropic glutamate receptor family, is restricted to the cerebellum, specifically to Bergmann glia. Glutamate induces a membrane to nuclei signaling involved in gene expression regulation. Exposure of cultured chick Bergmann glia cells to glutamate leads to an increase in kainate binding protein and mRNA levels, suggesting a transcriptional level of regulation. The 5’ proximal region of the chick kainate binding gene was cloned and transfected into Bergmann glia cells. Three main regulatory regions could be defined, a minimal promoter region, a negative regulatory region, and interestingly, a glutamate-responsive element. Deletion of this element abolishes the agonist effect. Moreover, electrophoretic mobility shift assays, cotransfection experiments, and site-directed mutagenesis clearly suggest that the glutamate effect is mediated through an AP-1 site by a Fos/Jun heterodimer. The present results favor the notion of a functional role of kainate-binding protein in gluta

Glutamate is the major excitatory neurotransmitter in the vertebrate central nervous system. To elicit its functions, Glu activates two types of receptors: ligand-gated ion channels and metabotropic receptors coupled to G-proteins (1). Kainate-binding proteins (KBPs) are members of the ionotropic Glu receptors’ gene family, the expression of these proteins is enriched in non-mammalian vertebrates (2). In the chick, KBP is expressed as a 49-kDa glycosylated polypeptide that is restricted to the cerebellum. In situ hybridization and immunohistochemical studies have shown that KBP is expressed exclusively in Bergmann glia (3, 4).

The chick KBP is the best characterized of the non-mam-

malian vertebrate KBPs and is the major binding site for kainate within the cerebellum. Several lines of evidence suggest a functional role of chick KBP; for example, the expression of KBP follows by several days the onset of Bergmann glia development and matches the time of migration of granular cells (2). In addition, KBP expression is up-regulated by an imprinting stimulus in ducks (5). Nevertheless, with the exception of toad KBP, no functional properties have been reported for this protein. No ligand-gated ion channel activity is observed, even when chick KBP is coexpressed with other ionotropic receptor subunits. Interestingly, when the transmembranal domain of the chick KBP is linked to the Glu binding site of membrane α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate (AMPA/KA) receptors in a chimera construction, this transmembranal domain can function as an ionic pore (6).

Bergmann glia extends processes through the molecular layer, ensheathing Purkinje cell dendrites and the excitatory synapses between Purkinje cells and the parallel and climbing fibers. Because of this specific localization, it has been suggested that these cells participate in the modulation of the efficacy of excitatory synapses surrounded by them (4). Bergmann glia cells (BGC) carry in their membranes AMPA/KA, N-methyl-D-aspartate, and metabotropic receptors (7). The activation of these receptors promotes calcium influx, phosphoinositide hydrolysis, PKC translocation to the membrane, activation of the MAPK (mitogen-activated protein kinase) pathway, increase in DNA binding activity of the activator protein-1 (AP-1) and changes in Glu receptors expression (8). Parallel fiber stimulation induces depolarization of Bergmann glia, an effect mediated by AMPA/KA receptors and by the Na+ dependent Glu transporter (9). These responses suggest that long term changes within Bergmann glial cells occur as a consequence of synaptic activity. In this sense, chick KBP could be an important molecule related to long term changes in glial function, glia-neuron communication, or synaptic plasticity.

The relative expression of the various Glu receptors is not static but is remodeled during development, ischemia, seizures, repetitive activation of afferents, or chronic administration of a variety of drugs (10). Nevertheless, little is known of the molecular identity of the promoter regions involved in such regulation, mainly because in the promoter regions reported thus far, none of them predicts the interaction of inducible transcription factors. An interesting exception is the chkbp promoter, in which a putative AP-1 binding site has been reported (11, 12). In the present work, we provide evidence for a Glu-induced transcriptional regulation of KBP expression mediated through an AP-1 site.

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1 The abbreviations used are: KBP, kainate-binding protein; gluRE, glutamate-responsive element; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; KA, kainic acid; BGC, Bergmann glia cells; AP-1, activator protein 1; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-PCR; CAT, chloramphenicol acetyltransferase; bp, base pair(s).
EXPERIMENTAL PROCEDURES

Cell Culture and Stimulation Protocol—Chick cerebellar BGC were prepared as detailed elsewhere (8). Briefly, 14-day-old chick embryos were used, and the cerebellum was dissected and homogenized mechanically. Cells were plated at a density of 1 × 10^6 cells /ml in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, and 50 µg/ml gentamicin. The cells were incubated at 37°C in 5% CO₂ and used after 5–6 days in culture. Cultures at monolayers were exposed to the indicated concentrations of agonists for varying periods of time; antagonists were added 10 min before the agonists. Incubation was stopped by removing the medium, and samples were processed as detailed below.

SDS-Polyacrylamide Gel Electrophoresis and Western Blots—The cells were harvested and washed several times with 10 mM K-HPO₄/KCl, pH 7.4. Cells at 2 × 10⁵ cells/ml were lysed in 50 mM Tris-HCl pH 7.5 with protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, 1 mM mg/ml aprotinin, and 1 mg/ml leupeptin), and aliquots of this suspension were used for protein concentration determination and boiled for 5 min in Laemmli's sample buffer. Equal amounts of protein (approximately 50 µg) were resolved on 10% SDS-polyacrylamide gels and electroblotted to nitrocellulose membranes. Blots were incubated with PAb against p53 to confirm that protein loading was equal in all lanes. Filters were soaked in PBS to remove the Ponceau S and incubated in PBS containing 5% dried skimmed milk and 0.1% Tween 20 for 1 h to block the excess of nonspecific protein binding sites. Filters were then incubated for 12 h at 4°C with primary antibodies diluted in 0.25% bovine serum albumin, 0.1% Tween 20 in PBS buffer followed by secondary antibodies. The antigenic specificity of the monoclonal antibody p53, polyclonal anti-p53 (Santa Cruz Biotechnology), and anti-rabbit antibodies conjugated to horseradish peroxidase (Amersham Pharmacia Biotech). Finally, the proteins were detected using an ECL chemiluminescence kit (Amersham Pharmacia Biotech).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)—For semiquantitative RT-PCR, reverse-transcription was performed as described (7). Total RNA was isolated from monolayers as described by Chomczynski and Sacchi (13). First-strand cDNA was synthesized using 2 µg of total RNA, 200 units of Moloney murine leukemia virus-reverse transcriptase, and 40 pmol of oligo(dT) as primer. The amplification of an internal control house-keeping S17 chick ribosomal protein mRNA was performed. Cycling conditions were 95°C for 1 min, 55°C for 1 min, 72°C for 1.5 min, by 30 cycles. PCR products were analyzed by 1.2% agarose-gel electrophoresis. Data were quantified by densitometric analysis and the indicated time periods and concentrations. Protein lysates were obtained as follows. Cells were harvested in TEN buffer (40 mM Tris-HCl pH 8.0, 1 mM EDTA, 15 mM NaCl) and centrifuged at 12,000 × g for 3 min. Equal amount of protein lysates (~80 µg) were incubated with 0.25 µCi of [3H]chlorpromazine (50 Ci/mmol, Amersham Pharmacia Biotech) and 0.8 mM acetyl-CoA (Sigma) at 37°C. Acetylated forms were separated by thin-layer chromatography and quantified using an AMBIOS 4000 Scannal Instruments radioanalytic imaging analyzer. CAT activities were expressed as the acetylated fraction corrected for the activity in the pCAT-Basic vector and are expressed as relative activities to non-treated control cell lysates. Cotransfection experiments were done under the same protocol using the indicated amounts of the expression vectors.

Electrophoretic Mobility Shift Assays—Nuclear extracts were prepared as described previously (15). All of the buffers (unless otherwise stated) contained protease inhibitors mixture (Roche Molecular Biochemicals) to prevent nuclear factor proteolysis. Protein concentration was measured by the Bradford method (16). Nuclear extracts (approximately 15 µg) from control or agonist-treated BGC (1 mM, 2 h, for indicated times) were incubated on ice with 1 µg of poly(dI-dC) as non-specific competitor (Amersham Pharmacia Biotech) and 1 ng of 32P end-labeled double-stranded oligonucleotides as follows: chAP-1, 5'-AACATGTTGTTCTGTCGAC-3'; AP1SV, 5'-TCTATACGGTTCTGTCGAC-3'; SIEm67STAT3, 5'-CGGCTGGATGGGAATGACCAAGG-3'; S17 antisense, 5'-CCGCTGGATGGGAATGACCAAGG-3'; S17 sense, 5'-CCGCTGGATGGGAATGACCAAGG-3'; AP1, 5'-CGGCTGTGACCAAGGAATGACCAAGG-3'; Sp1, 5'-CCGCTGGATGGGAATGACCAAGG-3'. Incubation was done at room temperature in 20 µl of binding buffer (50 mM Tris-Cl, pH 7.5, 2 mM MgCl₂, 75 mM NaCl, 10% glycerol, 200 µM each of dATP, dCTP, dGTP, and dTTP). The reaction mixtures were subjected to electrophoresis on 6% polyacrylamide gels in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA) and visualized by autoradiography. For competition studies, the reaction mixtures were pre-incubated with the indicated amount of unlabeled competitor oligonucleotide before the addition of labeled DNA. For gel supershift experiments, reactions with the DNA-protein complexes were incubated at 4°C with anti-c-Jun (sc-45, Santa Cruz Biotechnology) or anti-p53 (sc-123, Santa Cruz Biotechnology) for 2 h prior to electrophoresis.

RESULTS

Glutamate Regulates KBP Promoter Activity—Treatment of cultured chick BGC with 1 mM Glu elicits a time-dependent accumulation of the characteristic 49-kDa KBP polypeptide as detected with an antisera raised against the purified protein (Fig. 1A). The maximal response is obtained after 12 h of Glu exposure and is still present after 24 h of agonist treatment. The dose dependence of the effect was explored, and an EC₅₀ of 200 µM was determined (Fig. 1B). A pharmacological profile of the receptors involved in chick KBP accumulation was undertaken. Exposure of the cultures for 12 h to a concentration of 500 µM kainate (KA), as well as to 200 µM N-methyl-D-aspartate plus 10 µM glycine, 100 µM 1-amino-4,5-cyclopentane-trans-1,3-dicarboxylate, or 100 µM quisqualate, led to a similar increase in KBP levels as treatment with 1 mM Glu (Fig. 2A), suggesting the involvement of

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ionotropic and metabotropic receptors in the phenomena. This puzzling result was further confirmed when specific antagonists were used to block the Glu effect. Preincubation with the specific antagonists 6-cyano-7-nitroquinoxaline-2,3-dione at a concentration of 50 μM, 5-methyl-10,11-dihydro-5H-dibenzo-cyclohepten-5,10-imine (MK-801) at 5 μM, or L-2-amino-3-phosphonopropionic acid at 500 μM resulted in a reduction in the Glu-mediated increase in KBP.

Glu Regulates KBP mRNA Levels—To gain insight into the level of regulation being modified to Glu exposure, we first measured chick KBP mRNA levels by means of semiquantitative RT-PCR from control and Glu-treated cells, using the small ribosomal protein 17 (S17r) as an internal control (17). Linear amplification was found between 24 and 30 cycles, as demonstrated by scanning of the ethidium bromide staining of the electrophoresed PCR products (not shown). Treatment of the cells with 1 mM Glu resulted in a time-dependent increase in chKBP mRNA (Fig. 3A). These results were further confirmed by Northern blot analysis (Fig. 3B). Therefore, the increased amount of KBP in Glu-treated cells may result from a transcriptional regulation of the kbp gene elicited by Glu receptor activation in BGC.

The chkbp 5′-Noncoding Region: Identification of a Glu-responsive Element—The sequence previously reported as the putative promoter of chick KBP had not been functionally characterized (11, 12). Therefore, to further explore the transcriptional regulation of KBP, we isolated chick DNA and performed PCR experiments with specific primers to amplify the putative promoter sequence (12). The predicted band of around 500 bp was cloned into a pBluescript II plasmid and sequenced. As depicted in Fig. 4, the sequence obtained showed only minor changes in comparison with the sequences previously reported (11, 12).

To perform functional assays, the putative promoter sequence was subcloned into the reporter plasmid pCATBasic, and BGC cultures were transfected in basal conditions (cells not stimulated with Glu). The p435kbpCAT construct (nt 2435 to 188) shows a lower expression of the CAT reporter gene in comparison with p250kbpCAT or p170kbpCAT constructs (Fig. 5A). Deletion of 185 or 265 bp of the 5′ region significantly increased the promoter activity after deletion, suggesting that the region upstream nt 170 could contain several negative regulatory elements. Maximal transcriptional activity in nonstimulated conditions can be defined in the −170/+88 region.

Treatment with 1 mM Glu of cultured BGC transfected with the p435kbp construct resulted in a time-dependent increase in activity (Fig. 5B). Exposure to 1 mM KA mimicked this effect (Fig. 5C). Interestingly, when the deleted constructions p250kbpCAT and p170kbpCAT were tested for Glu induction, none of them responded to the agonist treatment (Fig. 5C). These results clearly suggest that the −435/−250 region con-
fers the response to Glu treatment, constituting a Glu-responsive element (gluRE).

**AP-1 Drives Glu-induced Transcription of chkBp**—The promoter sequence of chkBp includes several putative binding sites for inducible transcription factors (Fig. 4; Refs. 11 and 12). Taking into consideration that Glu promotes an increase in the binding activity of AP-1 in Bergmann glial cells (18), it was tempting to speculate that Glu might be regulating KBP expression through the putative AP-1 binding sequences within its promoter. Interestingly, an AP-1-like sequence is present at nt 337–329 (within the gluRE). We therefore decided to explore and detect nuclear factors capable of interacting with the gluRE, using electrophoretic mobility shift assays as well as functional experiments with several new generated constructs.

![Figure 3](image3.png)

**Fig. 3.** chKBp mRNA is up-regulated by Glu. Cells were exposed to 1 mM Glu by the indicated time periods (h), and total RNA was extracted. Quantitative PCR (panel A) and Northern blot using KBP cDNA as probe (panel B) were performed. A representative of at least three independent experiments is shown.

![Figure 4](image4.png)

**Fig. 4.** chKBp 5′ noncoding region. KBP putative promoter region (−435 to +88 bp) was amplified by PCR using the oligonucleotides kbpup and kbplow and cloned in pCAT-Basic vector XbaI site. The sequence was obtained (GenBank accession number AF208519), analyzed, and compared with previous reported sequences. Changes in sequences are noted. Putative binding sites for transcription factors (C/EBP, CAAT/enhancer-binding protein; Oct-1, octamer-binding protein-1; Sp1, stimulating protein 1) and TATA box are underlined (MatsInspector 2.0 searching). The broken arrow indicates the transcription starting site. Restriction sites are shown.

![Figure 5](image5.png)

**Fig. 5.** chKBp 5′ noncoding region transcriptional activity in BGC under basal and Glu-stimulated conditions. BGC were transfected with 3 μg of total plasmid DNA in all cases and harvested 48 h post-transfection. Panel A, p435kbpCAT full-length construction containing the chkbp promoter region and two deletions created using the HindIII and BspM1 restriction sites. Activities are expressed relative to the full-length construct from at least three independent experiments. Panel B, BGC were transfected with p435kbpCAT, stimulated with 1 mM Glu for different time periods, and harvested 24 h post-stimulation. Panel C, deletion-containing constructs activity in BGC under stimulated conditions using Glu or KA (1 mM, 2 h). For all induction experiments, activities are expressed over the non-stimulated particular construct (control) used in each experiment. Values are mean of three independent experiments ± S.E. A gluRE can be defined between nt positions −435 to −250.

and detect nuclear factors capable of interacting with the gluRE, using electrophoretic mobility shift assays as well as functional experiments with several new generated constructs. A 180-bp gluRE fragment containing the putative chick AP-1 site was labeled and incubated with nuclear extracts prepared from control or stimulated BGC. As depicted in Fig. 6A, KA induces both an increase in the intensity of two retarded bands (solid arrows) as well as the appearance of a new retarded complex (open arrows). As expected, a significant increase in binding to the SV40 AP-1 site (AP1SV oligonucleotide) is elicited by KA (8, 18). Note that treatment with KA does not result in an increase on STAT3 binding to the SIS-inducible element (SIE) (19). The characterization of the chick AP-1 site was performed using an end-labeled 25-bp double-stranded nucleo-
tide (chAP-1). Several specific complexes were retarded with nuclear extracts of Glu-exposed BGC. Binding activity was increased in BGC treated with or without agonist. Gel shift assays were performed using as \(^{32}\)P end-labeled probes, the oligonucleotides: AP1SV (AP-1 site from SV40 virus), chAP1 (chkbp promoter AP-1 site, nt 337–329), and STAT3 (m67SIESTAT3 site from the c-fos promoter) (1 ng each) or the 180-bp fragment containing the Glu responsive element ch180. Panel A, comparison of DNA binding activity between nuclear extracts from control (N/S, non-stimulated) or KA-stimulated (S) (1 mM 1 h) BGC using different probes. Open arrows indicate changing complexes and black arrows increasing complexes after stimulation. Panel B, binding and competition experiments using BGC nuclear extracts from 1 mM Glu-treated cells. Panel C, supershift assay using anti-c-Jun, anti-c-Fos, and anti-p53 antibodies. 100 and 150 indicate folds of unlabeled competitor; −, no competitor added; *, free probe. a, AP-1-related complex. SS, c-Jun supershifted complex.

Fig. 6. chKBP glutamate responsive element contains binding sites recognized by AP-1 factor. Nuclear extracts (Nucl. Extract) were obtained from BGC treated with or without agonist. Gel shift assays were performed using as \(^{32}\)P end-labeled probes, the oligonucleotides: AP1SV (AP-1 site from SV40 virus), chAP1 (chkbp promoter AP-1 site, nt 337–329), and STAT3 (m67SIESTAT3 site from the c-fos promoter) (1 ng each) or the 180-bp fragment containing the Glu responsive element ch180. Panel A, comparison of DNA binding activity between nuclear extracts from control (N/S, non-stimulated) or KA-stimulated (S) (1 mM 1 h) BGC using different probes. Open arrows indicate changing complexes and black arrows increasing complexes after stimulation. Panel B, binding and competition experiments using BGC nuclear extracts from 1 mM Glu-treated cells. Panel C, supershift assay using anti-c-Jun, anti-c-Fos, and anti-p53 antibodies. 100 and 150 indicate folds of unlabeled competitor; −, no competitor added; *, free probe. a, AP-1-related complex. SS, c-Jun supershifted complex.

A mutational approach was used to define the role of the chAP-1 site in the gluRE. Supershift experiments with anti-c-Jun polyclonal antibodies result in a reduction of the mobility of the upper chAP-1 complex and a new super-retarded one. These results suggest that c-Jun is part of the transcription complex bound to the chkbp promoter upon Glu exposure. With anti-Fos antibodies, the reduction was as shown in Fig. 6C.
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Fig. 7. Identification of AP-1 as the glutamate responsive element. Panel A, analysis of Glu response (indicated as fold induction) of wild type (p435kbpCAT) and the chAP-1 mutant (p435kbpAPM). Panel B, wild type and mutant versions of the chAP-1 DNA motif found in the gluRE were analyzed for Glu responsiveness by cloning them upstream of the SV40 heterologous promoter (panel B) or the −250/+88 chkbp region (panel C). Cells were transfected as indicated before and stimulated with Glu 1 mM for 2 h.

response, demonstrating that chAP-1 mediates the Glu effect. Furthermore, the chAP-1 element is also capable of conferring Glu-induced transcriptional activity to a heterologous promoter, the SV40, that is present in the pCAT-Promoter vector (Fig. 7B). This activity is dependent on both the integrity and orientation of the chAP-1 sequence; mutated or antisense versions do not confer Glu-responsiveness. Interestingly, when the chAP-1 site is fused to the −250/+88 chkbp region, a dramatic transcriptional increase (−30-fold) is obtained upon exposure to Glu (Fig. 7C).

In line with the mentioned results, a Western blot analysis of BGC nuclear extracts from Glu-stimulated cells shows a time-dependent increase in c-Jun and c-Fos levels (Fig. 8A). Accordingly, when the amount of c-Jun or c-Fos is artificially augmented by co-transfection of the pSGS/c-Jun, or pSGS/c-Fos expression vectors along with the p435kbpCAT construct, a concentration-dependent increase in CAT activity is observed (Fig. 8). Thus, c-Jun and c-Fos are implicated in the increased levels of AP-1 binding to the chkbp gluRE upon stimulation of Glu receptors.

DISCUSSION

It has become clear that Glu does not confine its excitatory actions to neurons but also interacts with glia cells. Because neurons and glia share the same subtypes of Glu receptors, an active role for glia cells in neurotransmission is now evident. Applied on cultured glial cells, Glu regulates the opening of receptor channels, activates second messenger cascades, causes the release of neuroactive substances, and triggers the binding to DNA of inducible transcription factors (20, 21). In astrocytic cultures, Glu produces changes these in gene expression: induces the expression of neuronal growth factor, basic fibroblast growth factor, fibroblast growth factor receptor-1, and the transcriptional factors c-Fos, FosB, c-Jun, Jun B, zif/268, and NF-κB (22). In situ, Glu-mediated gene expression has also been reported in astrocytes, for example following transient ischemia, the expression of the Glu transporter, GLAST, is up-regulated (23). Requirement for protein and mRNA synthesis has been documented for long term memory (24). Therefore, the analysis of regulatory mechanisms of Glu-induced gene expression in glial cells is a prerequisite to postulate long-lasting modulation of glial cells in neuronal communication or function.

A stimulus-transcription coupling elicited by Glu has been documented in cultured BGC (18). Moreover, an imprinting stimulus leads to an increase in Bergmann glu-specific KBP mRNA levels in ducks (5). The fact that Glu receptor activation is involved in the acquisition of a wide variety of learning paradigms suggests that Glu receptors might play a role in KBP gene expression regulation. This is indeed the case; Glu receptor activation results in increased levels of KBP. Apparently, the effect is mediated through ionotropic as well as metabotropic receptors, suggesting that the signaling cascades activated by these receptors either converge or that the level of regulation modified is different each receptor subtype. This issue remains to be determined.

As mentioned, downstream targets for ionotropic receptor-activated signaling pathways in cultured BGC include an increase in AP-1 DNA binding (18). Taking into consideration that an AP-1 site is present in the putative promoter region of chkbp (12), we speculated that the increase in KBP levels detected upon Glu exposure was the result of a transcriptional regulation through this AP-1 site. First, it was important to demonstrate that the reported putative promoter region could drive the transcription of a reporter gene. Second, the reporter activity should be inducible by Glu. Third, deletion or mutation of this AP-1 site would necessarily abolish the Glu effect. Not only these conditions were fulfilled, but also three main regions could be defined: 1) the promoter region, nt −170/+88, containing the characteristic TATA and GC boxes, with a higher transcriptional activity but not inducible by Glu; 2) a region containing negative regulatory elements (nt −250/+170), strongly acting over the promoter but unaffected by Glu; 3) a Glu-responsive region, in which the AP-1 site is present, functional, and essential for response.

A major role for the Fos/Jun heterodimer in Glu-mediated transcription is possible for a number of reasons. Nuclear extracts prepared from Glu-treated cells are able to reduce the electrophoretic mobility of a bona fide AP-1 site, the SV40

Fig. 7. Identification of AP-1 as the glutamate responsive element. Panel A, analysis of Glu response (indicated as fold induction) of wild type (p435kbpCAT) and the chAP-1 mutant (p435kbpAPM). Panel B, wild type and mutant versions of the chAP-1 DNA motif found in the gluRE were analyzed for Glu responsiveness by cloning them upstream of the SV40 heterologous promoter (panel B) or the −250/+88 chkbp region (panel C). Cells were transfected as indicated before and stimulated with Glu 1 mM for 2 h.
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Fig. 8. c-Jun and c-Fos stimulate chkbp promoter activity. Panel A, Western blot analysis of nuclear extracts from Glu-stimulated cells for the indicated time periods, revealed with anti-c-Jun or anti-c-Fos antibodies. Panel B, Glu-induced activity is compared with the over-expression of c-Jun/pSG5 and c-Fos/pSG5 constructs (1 to 5 µg) that were co-transfected with p435kbPCAT. Cells were harvested 48 h post-transfection. The results are from at least three independent experiments and are expressed as activity relative to the p435kbPCAT co-transfected with pSG5 vector alone.

Fig. 9. Functional elements in the chkbp regulatory region.

AP-1 site. Moreover, this oligonucleotide sequence competes with the AP-1 containing a 25-bp fragment of the putative Glu-responsive element of chkbp (chAP-1) for the binding of nuclear proteins of Glu exposed cells. Anti-c-Jun antibodies as well as anti-c-Fos antibodies supershifted the chAP-1 complex. One could argue that, in addition to an increase in AP-1 binding, Glu favors the binding of other putative elements predicted to interact within this region (OCT1, CAAT, CEBP/B) that might mediate the activating effect. Because over-expression of c-Jun or c-Fos results in a concentration-dependent increase in promoter activity that reproduces the Glu effect, additional cooperativity with other activating factors may be less important. Site-directed mutagenesis of chAP-1 rules out this possibility (Fig. 8). Furthermore, when chAP-1 was fused to the SV40 promoter, it was able to confer Glu inducibility. It is important to note that when chAP-1 was fused to the SV40 promoter, it was able to confer Glu-induced activity. Careful analysis of putative DNA-protein interactions in the negative regulatory region did not reveal a well known repressor binding site. Nevertheless, octamer binding sites are recurrent in this region, and interestingly, Oct-2 has been reported to act as an ambivalent transcription factor, acting either as activator or repressor in epithelial and neural cells (21). A detailed study is needed to establish the interaction and functional role of each factor in the context of Glu-induced transcriptional activity.

In summary, the present study results identify, for the first time, a gluRE and provide a major input to our present understanding of Glu-mediated gene expression regulation. Concomitantly, they suggest that whatever the function of KBP may be, it may play a significant role in long term changes in glia physiology.

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