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Organization and expression of the gene encoding chick kainate binding protein, a member of the glutamate receptor family

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The gene encoding chick cerebellar Bergmann glia-specific kainate binding protein (chKBP), has been isolated, characterized and expressed in heterologous systems. The structural gene spans 11.2 kb and contains 11 exons and 10 introns. Several of the exons encode specific receptor domains, including each of the predicted transmembrane regions. Exon/intron boundaries flanking the second, putative channel-forming transmembrane domain are conserved between chKBP and other glutamate/kainate receptor subunits. The putative promoter region 5′ to the first exon displays high GC content and TATA, CAAT and AP1 consensus sequences. Transcription of the chKBP gene is evident prior to full cerebellar cortical maturation. Transcripts are abundant in cells consistent with Bergmann glia, as revealed by in situ hybridization. Transfection of 293 kidney cell cultures with chKBP cDNA or chKBP gene expression constructs confers CNQX-sensitive kainate binding with the pharmacological specificity displayed by both chKBP and kainate receptors. However, expression of the same constructs in *Xenopus* oocytes fails to yield detectable agonist-activated currents.

INTRODUCTION

Glutamate, the principal brain excitatory neurotransmitter, acts on a variety of membranous receptors with distinct structural, pharmacological and physiological properties[27,36,46]. One approach to understanding the complexity of glutamate receptors involves the characterization of genes encoding the subunits of these receptors.

The chick cerebellum harbours dense binding sites for the glutamate agonist kainic acid, which are carried by a 49 kDa polypeptide called the chicken kainate binding protein (chKBP)[19]. Immunoelectron microscopy shows that chKBP is exclusively present on Bergmann glia[40], whereas a closely related frog KBP is present mostly in neurons[13]. The pharmacological specificity of ligand binding to chKBP is similar to that of mammalian brain glutamate/kainate receptors, although binding affinities are lower[1,19]. Cloning of the cDNAs encoding KBPs[20,45] revealed transmembrane topologies homologous to other ligand-gated channels, and marked sequence homologies with the C-terminal half of the ~ 100 kDa subunits of the ionotropic glutamate receptors[1,6,7,14,21–23,28,34]. In contrast with the established signal-transducing functions of ionotropic and metabotropic glutamate receptors, the role of the KBPs as glutamatergic signalling devices remains elusive. The frog KBP is inactive as a kainate receptor channel[45] and only indirect evidence describes the channel activities of chKBP[30].

As a step in the investigation of chKBP function, we now report studies of its gene organization and expression. We describe the structural chKBP gene and compare its intron/exon arrangement with that of other glutamate receptor genes. Analysis of sequences 5′ to the first exon reveals putative promoter and regulatory control elements. Expression of this gene in chick cerebellum is elucidated by in situ hybridization and Northern blot analyses. The functional properties of expressed chKBP are studied by measurements of

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xainate binding, Western blot analysis and electrophysological recordings.

MATERIALS AND METHODS

General methods

Southern blotting, restriction enzyme digests, subcloning of DNA ragments and bacteriophage manipulations were carried out by tandard methods[25]. Polymerase chain reactions[33] were carried out n 50 μl volumes using λ DNAs as template and 1 μM primers with he Perkin Elmer Cetus (Norwalk, CT) kit. Amplification was with hermal cycler cycling 94°C, 1.5 min, 45–55°C, 2 min, and 72°C 2–4 in. DNA sequencing with denatured double-stranded plasmid templates were carried out by the chain-termination method[35] with the equenase kit (US Biochemicals; Cleveland, OH). Sequences were nalysed using University of Wisconsin Genetics Computer Group JWGCG) software. Poly(A)$^+$ RNA was isolated with a commercial it (Invitrogen; San Diego, CA), analysed on 1.2% formaldehyde-garose gels and blotted on Nylon membrane[25]. Northern blots were ashed to a final stringency of 0.1× SSPE, 56°C.

olation and characterization of genomic clones

The entire 3.3 kb cDNA encoding chKBP (KBP-3.3 cDNA)[20] was diolabeled[16] and used to screen a library of chicken genomic DNA bacteriophage λ EMBL-3 (Clontech; Palo Alto, CA) using high ringency hybridization conditions[25]. Screening of about 500,000 aques revealed 3 positive clones which were plaque-purified. Reriction mapping and Southern analysis indicated that the three ıge DNAs contain overlapping inserts. DNA of phage clone λ #7 ıs restricted with *Sal*I and *Bam*HI. The resulting 8.5 kb restriction ıgment was subcloned into pKS Bluescript phagemid (Stratagene; ı Jolla, CA) and used for sequencing. Introns were located by PCR th phage DNAs as templates and cDNA-derived oligonucleotides primers. Amplification products were characterized by elecphoresis on a 1% agarose gel and detected with ethidium mide[25]. The antisense oligonucleotide primers corresponding to e 3′ ends of cDNAs KBP-2.2 and KBP-3.3[20] were 5′-CCAGCTC-\TCCCTGTCAC-3′ (CHP-12) and 5′-GTGTTTATTTTAAT-\TGC-3′ (CHP-13), respectively.

situ RNA hybridization histochemistry

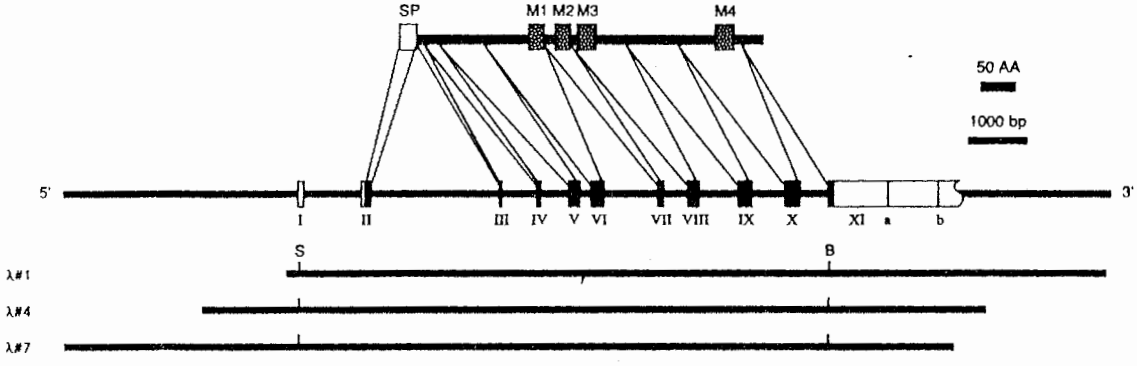
A 45 base oligonucleotide complementary to bases 700–744 of : KBP-3.3 cDNA[20] was used as a hybridization probe since its quence is not found in other members of the glutamate receptor nily. In situ hybridization techniques were performed as deibed[42,43]. Eighteen-day-old chick embryos (Truslow Farms, MD) re sacrificed by intracardiac perfusion with periodate-lysine-paramaldehyde-glutaraldehyde solution under pentobarbital anesthesia. The brains were post-fixed in the same fixative, soaked in buffered sucrose solution, frozen on dry ice, and sectioned as described. Ten μm sagittal sections were cut using cryostat and thaw-mounted onto slides pretreated with Denhardt's solution, treated with HCl and proteinase K, dehydrated, dried, and stored until used as described[43]. Radiolabeling of the 45 base oligonucleotide was carried out by a primer extension method in the presence of α-[^{35}S]dATP, with Klenow fragment of DNA polymerase I and an antisense 15-mer as a primer[43]. In some experiments, a ^{35}S-labeled cDNA corresponding to the large cytoplasmic loop of chKBP (nucleotides 930–1231[20]) was used as a probe. Sections were hybridized with ^{35}S-labeled oligonucleotide (10,000 cpm/μl) at 42°C in 50% formamide, 3× SSC, 1× Denhardt's solution, 10% dextran sulfate, 0.1 mg/ml sheared salmon sperm DNA, for 24 h and washed with 2× SSC for 1 h at 50°C. Then dehydrated, dried, dipped in NTB2 emulsion (Kodak; diluted 1:1 with 0.6 M ammonium acetate) exposed for 2 weeks at 4°C, developed in Kodak D19 developer and counterstained with Toluidine blue.

Expression in mammalian cells

The plasmid pcKBP was constructed by subcloning the KBP-3.3 cDNA[20] into the eukaryotic expression vector pcDNA I (Invitrogen), which contains a cytomegalovirus promoter. To construct plasmid pGKBP, the DNA of clone λ #7 was first restricted with *Sal*I yielding an 11 kb fragment spanning sequences from within exon I through exon XI. This 11 kb genomic fragment was subcloned into the Xho I site of the plasmid pcDNA I and the orientation of the insert was confirmed by sequencing. Human embryonic kidney 293 cells (ATCC CRL 1573) were transfected by the calcium phosphate precipitation method in low levels of CO_2[9,32]. 15 μg of plasmid DNAs were used per 100 mm dish. Transfection efficiencies were about 50%, as measured by parallel transfections with pCMVβ (Clontech). Cells were harvested 48–72 h after transfection. Membrane preparation and binding experiments were performed as described[19].

Expression in oocytes

Oocytes from adult *Xenopus laevis* were harvested, incubated with collagenase (2 mg/ml; Sigma, type Ia) in calcium-free ND96 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM Hepes, pH = 7.5) for 2 h, and manually defoliculated[2,10]. For nuclear injections, oocytes were injected into the center of the dark pole with 1–20 ng of plasmid DNAs in 10 nl of water[3,10]. Injected oocytes were incubated in ND96 solution containing 1.8 mM $CaCl_2$, 2.5 mM pyruvate, penicillin (50 U/ml) and streptomycin (50 μg/ml) for 3–5 days at 19°C. Electrodes were filled with 3 M KCl and whole cell currents were recorded under voltage-clamp conditions at −70 or −100 mV[2,3]. During the recording, oocytes were bathed in frog Ringer's solution and their resting membrane potentials were −35 to −45 mV. As a positive control for nuclear injections, we used the



1. Organization of the chKBP gene. The relationships (drawn to scale) of the three lambda phage DNA inserts (λ #1, #4 and #7) and their riction map with *Sal*I (S) and *Bam*HI (B) are shown (each clone also has a flanking λ-borne *Sal*I site). Upper part shows exon assignment to coding region of the chKBP cDNA. The signal peptide (SP) is marked by a box and the transmembrane regions (M1-M4) are indicated by oled boxes. Exons are numbered in Roman numerals and shown with untranslated regions depicted by open bars and translated regions by solid bars. Alternate 3′ end processing of the last exon is indicated.

plasmid pGABArho, which contains a GABA receptor cDNA[12] in the pcDNA I expression vector. In oocytes injected with this plasmid, GABA (10 μM) elicited large currents, with amplitudes up to 500 nA. In several experiments, pGABArho was co-injected with pcKBP or with pGKBP, and oocytes responding to GABA were selected for sensitivity to kainate and glutamate. Some oocytes were also treated with Concanavalin A prior to recording[14].

RESULTS

Structure of the chKBP gene

The size of the chKBP gene was estimated by probing Southern blots of genomic DNA with the 3.3 kb cDNA encoding chKBP (KBP-3.3 cDNA)[20]. The sum of all hybridizing Pst I restriction fragments was 11 kb; Hind III fragments summed to 13 kb[21]. These results were most consistent with a relatively compact, single copy gene. Screening a chicken genomic bacteriophage library with KBP-3.3 cDNA yielded three λ clones (Fig. 1) whose inserts displayed several identical restriction fragments, suggesting their origin from the same gene. Further analysis by PCR and DNA sequencing showed that these clones span approximately 19 kb of genomic sequence including the chKBP structural gene with 4.5 kb 5′ upstream and 2.5 kb 3′ downstream flanking sequences (Fig. 1). Subsequent sequencing of all exons encoding chKBP-3.3 cDNA, except for the 3′-untranslated portions of the exon XI, have identified the intervening sequences. The structural gene contains ten introns (Figs. 1 and 2), with splice junction donors and acceptors conforming to consensus sequences[24].

The first exon contains the 5′ untranslated sequence of KBP-3.3 cDNA. Two different approaches suggest that the immediate 5′ flanking 4.5 kb genomic region is not transcribed. Northern analysis failed to demonstrate hybridization with the 4.5 kb *Sal*I fragment located just 5′ to exon I. A RNA/PCR assay using a primer corresponding to sequences 35 bp upstream from the 5′ end of KBP-3.3 cDNA (Fig. 3) also yielded negative results. Sequence analysis of the 5′ flanking region reveal several sequence elements consistent with roles as promoter and enhancer elements. The first 200 5′ base pairs display a sudden rise in GC content to between 58 and 64%, and a higher than expected frequency of CG dinucleotide. Both factors are good indicators of eukaryotic promoters[24]. A TATA box consensus sequence surrounded by GC rich sequences is found 41 bp upstream of the 5′ end of KBP-3.3 cDNA, while a CAAT box is found 200 bp upstream[8,24] (Fig. 3). These observations, combined with the above mentioned data suggesting that these regions are not transcribed, are consistent with a promoter role for regions immediately upstream of exon I.

Exon Boundary		5′ Donor	Intron Size	3′ Acceptor	
Exon I/II		TGCT gtaagt	...~1.1 kb...	tttaacctcaacag	GAAG
Exon II/III		ACAG gtgaga	...~2.4 kb...	gttattttttcag	GAGCA
	1	ThrG			lyAla
Exon III/IV		ATTAAG gtatgt	...0.56 kb...	tttttatttgtag	CCCAAT
	11	IleLys			ProAsn
Exon IV/V		ATCTTG gtaaaa	...~0.5 kb...	ccctgtttatgcag	GAAGAT
	21	IleLeu			GluAsp
Exon V/VI		AGACAG gtaatt	...0.18 kb...	acttctctttacag	GAAGCA
	91	ArgGln			GluAla
Exon VI/VII		GCCAG gtataa	...~1.0 kb...	ttatttcctcatag	GCTG
	167	AlaAr			gLeu
Exon VII/VIII		CAAG gtgagc	...~0.4 kb...	ctctggtgtcgcag	GTGTG
	200	GlnG			lyVal
Exon VIII/IX		TTCAAG gtgcag	...~0.7 kb...	ttttggggttgcag	AACTCC
	272	PheLys			AsnSer
Exon IX/X		CCCAGG gttagt	...~0.6 kb...	tccactgctgccag	CATCGC
	347	laGlnA			laSerP
Exon X/XI		ATCAAG gtgggt	...~0.5 kb...	ctcttcttttccag	AAATCC
	430	IleLys			LysSer

Fig. 2. Sequences across the intron/exon boundaries of the chKBP gene. The nucleotide sequences of the splice junctions are shown and the encoded amino acid sequences are indicated. The amino acid sequence is numbered from the first residue of the mature protein[20]. Nucleotides in uppercase letters represent exonic sequences, while those in lower-case letters represent intronic sequences. The approximate sizes of introns were estimated by electrophoresis of PCR-derived DNAs of clone λ #7.

The protein-coding sequence is borne on ten exons (Fig. 1). Exon II carries a translational initiation consensus sequence, the entire signal peptide and its initiator methionine codon identified by cDNA sequenc-

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-572   TTGTGGTTTACCTTGAAGCTCAGGCCTTTCTTTCAGCATTCTGG
-528   GATGTGAGGGGGATACTCGTGTATGTAACTGGTTTGTGAGGGTT
-484   CTGATGTTGTACCTGAATCCCCAACTGATC[redacted]ATCACGG
-440   AGAAAGCCTTTAGCTTGCAGCAGCAGTAGACCGGGGAGATCGGC
-396   ACAGCTGAATAGGGAACGGTACTGCAGAAAGAAAGCTTTTACAG
-352   GACCACACAGCAGGCCACGCAGCAGTATCTGTATTTTCCGAGTC
-308   [redacted]GTAGCATTGAGCCGCTGGACTGACCTGCTTCCAAACTTC
-264   TTTTTGCTGGGGGTGCTCCCCTTTCTCTGTTCCCAAGGCAAGGT
-220   CCTGGAGCCTGCACTGCCAGCTGGCACGTCCTTCCCGCAGGCCA
                                     31
-176   GCCTGGCTCCTCCCGCTGTCCT[redacted]CAGCGTGGATGCAGCGA
                             *            SalI
-132   GCATTCCCGTTCCAGCAGTTCCATTTGCCTGCTCGTCGACGTGC
- 88   TGCTGCTGGGACCCAGTCAGACAGACCAAGCTGGAGAGGCTGAA
- 44   GGAATTGCTGAAGTTTCCTCTAGACAGTAGCTATTTCTGCTGCA
                     33
+  1   ATGGACAAAGGACTTCACTTTATATTCTGT...
       MetAspLysGlyLeuHisPheIlePheCys...
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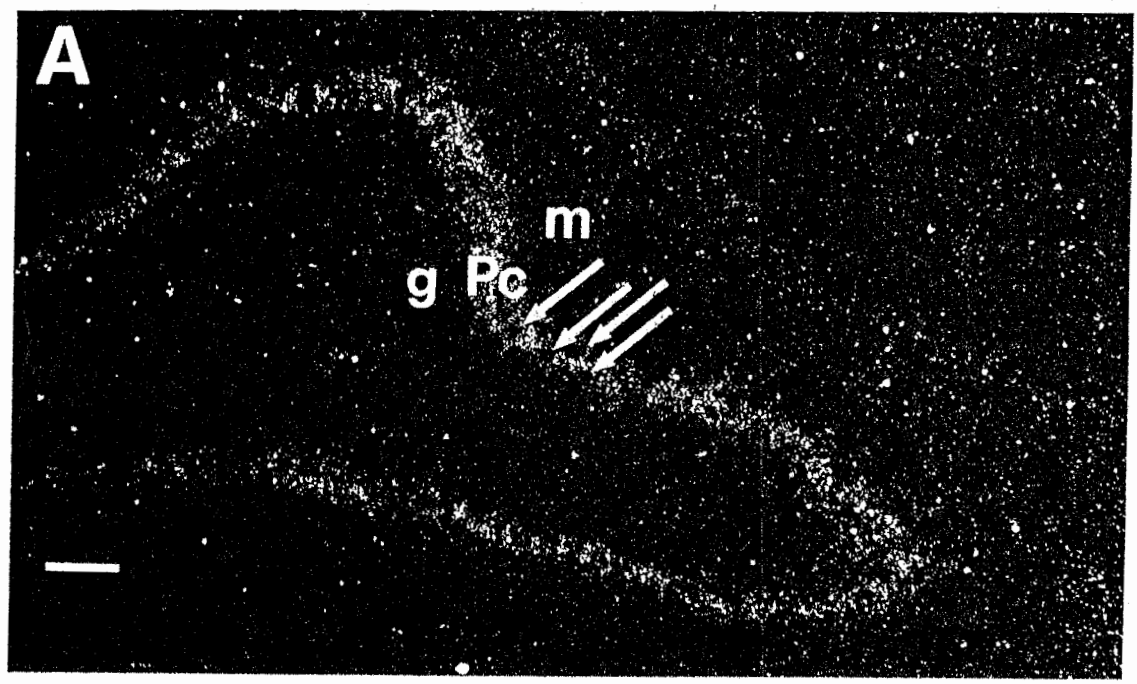
Fig. 3. Nucleotide sequences at the 5′ limit of the chKBP gene. The 5′ end of KBP-3.3 cDNA is indicated by an asterisk, and the unique Sal I site is shown. Oligonucleotide positions used in RNA/PCR are indicated as solid lines with arrows above the sequence; oligonucleotide #33 was antisense. Consensus sequences of promoter elements – TATA box, CAAT box and AP1 site are indicated by shaded boxes.

Fig. 4. Comparison of intron positions within different members of glutamate-gated ion channel/receptor gene family[38]. Positions of three introns with respect to the central transmembrane segments M1 and M2 are marked by arrows.

ing[20]. The first amino acid residue of the mature protein, a threonine, is followed immediately by the largest intron of about 2.4 kb. Exon III is the smallest, encoding only 11 amino acids. Exons III–V and a portion of exon VI encode the proposed N-terminal extracellular domain which might harbor the agonist binding sites[26,28,34].

Exons VI–VIII and X encode sequences containing the putative transmembrane domains. Interestingly, each of the four transmembrane domains is encoded by a separate exon. Furthermore, the intron/exon boundaries of the chKBP gene align with homologous functional regions of the genes encoding glutamate receptor subunits GluR5 and GluR2/GluR-B[38] (Fig. 4). Although the sizes of respective introns are different, the positions of three introns are precisely conserved between GluR5 and chKBP genes. The arginine codon



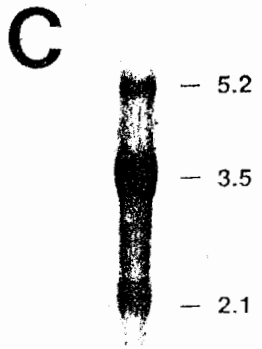


Fig. 5. Localization of chKBP mRNA in cerebellar Bergmann glia by in situ hybridization. A: dark-field photomicrograph of an emulsion-dipped coronal section showing accumulation of silver grains over Purkynje-Bergmann layer. Arrows indicate negatively stained Purkinje cell bodies. m, molecular layer; Pc, Purkinje cell layer; g, granular layer. B: bright-field photomicrograph of a section at higher magnification, showing accumulation of silver grains over small-cell soma. Bars represent 10 μm and 100 μm respectively. C: Northern hybridization of RNA from 11-day-old embryonic chick cerebellum with a restriction fragment of KBP-3.3 cDNA (nucleotides 930–1231) corresponding to the large cytoplasmic loop. The lane contained 5 μg of poly(A)$^+$ RNA. The blot was over-exposed to visualize minor transcripts.

following the first transmembrane domain and the glycine codon following the second transmembrane domain are split by introns in similar fashions.

The last exon encodes the carboxy-terminus and also the two 3′ untranslated sequences found previously at the 3′ ends of KBP-3.3 and KBP-2.2 cDNAs[20] and which contain polyadenylation signals AATAAA just preceding their cognate poly(A) tails[20,21]. Analysis of genomic clones by PCR with primers CHP-12 and CHP-13, corresponding to the 3′ ends of cDNAs KBP-2.2 and KBP-3.3, reveal that the two alternate 3′ ends are separated by about 0.6 kb (data not shown). These two alternate 3′ ends are indicated as XIa and XIb in Fig. 1.

Transcription of the chKBP gene in chick cerebellum

To localize the RNA transcripts encoding chKBP, in situ hybridization was performed with a radiolabeled oligonucleotide complementary to mRNA. Strong hybridization was observed along the Purkinje-Bergmann cell layer and in a neighboring zone in the molecular layer. Darkfield microscopy reveals that this pattern is repeated throughout the entire cerebellum. The Purkinje cell bodies are unlabeled and yield apparent "holes" in the dense band of grains (Fig. 5a). At higher magnification, most of the silver grains are seen between and below the Purkinje cell bodies (Fig. 5b), and associated with small somas consistent with Bergmann glia. Furthermore, labeling of lower density is observed in the molecular layer. Labeling in the granular layer is close to background level.

Northern blot analysis of 11-day-old embryonal RNA shows cerebellar-specific 3.5 kb major transcript and minor transcripts of 5.2 and 2.1 kb (Fig. 5c). The same transcripts can be observed in 14- and 18-day-old embryos, as well as in 2-week-old chicks. Oligonucleotide CHP-13 corresponding to the 3′ end of the KBP-3.3 cDNA hybridized with the 3.5 and 5.2 kb mRNA species but not with the 2.1 kb mRNA species (data not shown), consistent with the possibility of alternative 3′ end processing.

Properties of the chKBP in heterologous expression systems

Western blots of membrane proteins extracted from human 293 kidney cells transfected with the pcKBP expression construct reveal a strong immunoreactive band of 49 kDa detected by a polyclonal anti-chKBP antiserum. Faint bands at molecular weights > 90 kDa are also detectable (data not shown). These transfected cells display avid binding of [^{3}H]kainate. Transfection with a plasmid construct containing the entire chKBP structural gene (pGKBP) yields identical kainate binding (Fig. 6). Scatchard analysis of the binding data are consistent with the existence of a homogenous population of sites with a K_d = 560 nM (Fig. 6a). The binding

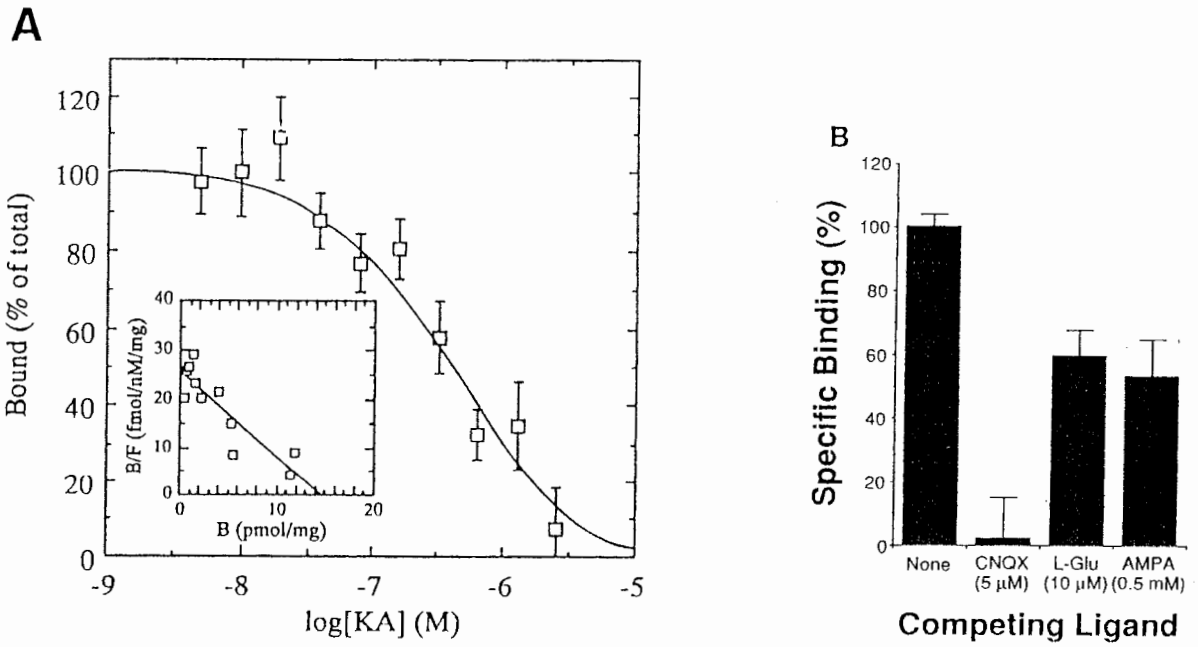


Fig. 6. Analysis of [^{3}H]kainate binding to membranes of human 293 kidney cells transfected with pGKBP. A: displacement curve of 25 nM [^{3}H]kainate binding with non-radioactive kainate. Inset shows Scatchard analysis of the data. B: displacement of 25 nM [^{3}H]kainate binding with glutamatergic ligands.

of [^{3}H]kainate can be totally abolished by the antagonist 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Fig. 6b) and partially prevented by 10 μM L-glutamate but only high concentrations of α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA; 0.5 mM). In control experiments, cells transfected with pcDNA vector displayed no specific [^{3}H]kainate binding.

Electrophysiological responses of expressed chKBP were examined in three fashions in two expression systems. *Xenopus* oocytes injected with capped mRNA transcribed from the KBP-3.3 cDNA, and oocytes injected into the nucleus with the expressible chKBP constructs pcKBP and pGKBP were examined under two-electrode voltage clamp conditions. In neither case, using more than 15 oocytes, did kainate (up to 10 mM), glutamate (100 mM), L-aspartate (100 mM) or domoate (1 mM) elicit any currents. COS cells transfected by electroporation with pGKBP yielded specific kainate binding, but no kainate-induced currents were noted under whole-cell voltage clamp conditions in recordings from 10 cells (data not shown).

DISCUSSION

Organization of the chKBP gene

The present data constitute one of the first reports of the gene organization of a member of the glutamate receptor family, and establish that chKBP is encoded by a compact but mosaic single copy gene. The entire structural chKBP gene is 11.2 kb long; 10 introns total 7.9 kb. The 11 exons that we have defined account for both the length of the nearly full-length KBP-3.3 cDNA[20] and the 3.5 kb mRNA detected in Northern analyses. However, the possibility of additional facultative exons such as the "flip/flop" exons[37] cannot be excluded.

As in many genes[11,18,24], several exons correspond to structural domains. Each of the four proposed transmembrane regions of chKBP as well as the signal peptide are encoded by separate exons. The strength of this concordance even suggests that the initially-predicted boundaries of the second transmembrane domain may require revision so that this domain's C-terminus is no longer split by an intron[39].

Comparison of these exon/structural domain features with available data concerning other glutamate receptor genes reveals interesting conservation in exon boundaries corresponding to the first two transmembrane segments (Fig. 4). Three exon boundaries are precisely conserved between chKBP, GluR5 and GluR6 genes[38]. One exon boundary, that preceding transmembrane region M1, is also conserved in genes encoding GluR-B (GluR2), GluR-C (GluR3) and GluR-D (GluR4)[38]. Two other exon boundaries, as well as the alternate exons[37], are not present in these three genes. Thus, the structural features of the chKBP gene are more closely related to the kainate-preferring GluR5 and GluR6 genes than to the AMPA-prefering glutamate receptor genes[10,15]. As revealed by analyses of alternately spliced cDNAs encoding GluR4c[17] and a novel isoform of GluR6 (Gregor and Yang, unpublished data), the position of the last intron in the C-terminal part of chKBP appears to be conserved in both GluR4 and GluR6 genes.

These comparisons imply that the respective portions of genes derive from common ancestral genes. They further suggest that the genomic organization of these exons was established prior to the divergence of birds and mammals. The maintenance of these structures over more than 270 million years of independent existence of these species is consistent with the key biological functions of the various members of the glutamate receptor family.

Comparing the intron/exon arrangement of the chKBP gene with other cloned ligand-gated ion channels reveals different numbers of exons and positions of introns. However, the genomic organization of the chKBP is reminiscent of the chicken γ and δ AChR subunits[29]. These genes each have four transmembrane regions encoded by separate exons and also contain similar numbers of exons (12 in γ and δ subunit genes).

The genomic region immediately preceding exon 1 is probably the chKBP promoter. This region is not transcribed, it has high GC content and a relatively high frequency of the CG dinucleotide. Further, it contains several putative regulatory sequence elements. It is likely that the 5′ limit of the structural gene is within a 20 bp genomic region just preceding the 5′ end of KBP-3.3 cDNA (indicated as asterisk in Fig. 4), since this region contains a TATA box, a structure with a defined location 20-36 bp upstream from transcriptional startpoint[8,24]. The CAAT box, at position −196, also fits into the appropriate relationship with the same transcriptional startpoint[8]. Even the AP1 consensus sequences[24] (Fig. 4) are often found within several hundred bases of the transcriptional startpoint. Conceivably, this often-active promoter element could contribute to the high-level of expression of this gene in its clearly-defined cell type(s) in the nervous system.

Just before the submission of this manuscript, another study describing a very similar intron/exon structure of the chKBP gene appeared[15]. Primer extension and RNase protection experiments suggested that the RNA cap site is 6 bp upstream from the 5′ end of

KBP-3.3 cDNA[15], in agreement with the above predictions.

Expression of the chKBP gene in Bergmann glia, and in vitro

The in situ hybridization data accord well with the results of a previous immunohistochemical study of chKBP distribution at the electron microscopic level[40]. This work used a monoclonal antibody raised against purified chKBP[19] to demonstrate chKBP-like immunoreactivity in cell bodies and processes of Bergmann glia in the chick and fish cerebellar cortex[40]. Our in situ RNA hybridization results, in spite of the low level of anatomic resolution, are entirely consistent with this localization, revealing that transcripts are abundant in the Purkinje-Bergmann layer of the entire cerebellum (Fig. 5; see also ref. 15), and absent from at least most Purkinje cells. Northern blot analyses of chick cerebellar RNA show that the chKBP transcripts are evident in the immature cerebellum, at least 2 days prior to the appearance of kainate binding sites[44] and of anti chKBP immunoreactivity[19]. These data may indicate translational regulation of the chKBP transcripts. The minor transcript of 2.1 kb has not been previously described[20], possibly due to its low abundance or preferential expression in immature cerebellum.

In transfected cells, specific [^{3}H]kainate binding with pharmacological specificity similar to mammalian kainate receptors was readily detected. However, when chKBP cDNA or gene were expressed in oocytes or COS cells, no ion channels were detected. Other investigators have also failed to find channel formation from the expressed amphibian KBP homolog[45]. Several other glutamate receptor subunit cDNAs do not produce functional homo-oligomeric channels in heterologous expression systems[5,47]. Conceivably, failure to function could relate to channel properties, such as small unitary conductance, low open probability or rapid desensitization[5,47], or to a requirement for either post-translational modification or combination with additional subunit(s). There is little evidence for the presence of other subunits in purified preparations of chKBP[19–21]. However, Western blot analysis of fish cerebellar membrane proteins reveals two equally abundant polypeptides of ~49 and 52 kDa[19]. cAMP-dependent phosphorylation of chKBP can be demonstrated; conceivably it could be required for the activity of the ion channel[31,41].

The function of chKBP remains obscure and we cannot exclude the possibility, that it may not mediate ion currents. However, the presence of the core ~400 amino acid region, that is conserved in other glutamate receptor subunits and includes the M2 putative channel-forming domain, predicts that chKBP could participate in an ion channel forming structure.

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