Activity-Dependent Expression of Acyl-Coenzyme A-Binding Protein in Retinal Muller Glial Cells Evoked by Optokinetic Stimulation

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Long-term horizontal optokinetic stimulation (HOKS) decreases the gain of the horizontal optokinetic reflex and evokes the second phase of optokinetic afternystagmus (OKAN-II). We investigated the possible molecular constituents of this adaptation. We used a differential display reverse transcriptase-PCR screen for mRNAs isolated from retinas of rabbits that received HOKS. In each rabbit, we compared mRNAs from the retina stimulated in the posterior→anterior (preferred) direction with mRNAs from the retina stimulated in the anterior→posterior (null) direction. Acyl-CoA-binding protein (ACBP) mRNA was one of four mRNAs selected by this screen, the proteins of which interact with GABA receptors. HOKS in the preferred direction increased ACBP mRNA transcription and ACBP protein expression. ACBP was localized to Muller glial cells by hybridization histochemistry and by immunohistochemistry. ACBP interacts with the α_1 -subunit of the GABA_A receptor, as determined by a yeast two-hybrid technique. This interaction was confirmed by coimmunoprecipitation of ACBP and the α_1 -subunit of the GABA_A receptor using an antibody to GABA_A α_1 . The interaction was also confirmed by a “pull-down” assay in which histidine-tagged ACBP was used to pull down the GABA_A α_1 . ACBP does not cross the blood–brain barrier. However, smaller truncated proteolytic fragments of ACBP do, increasing the excitability of central cortical neurons.

Muller cells may secrete ACBP in the inner plexiform layer, thereby decreasing the sensitivity of GABA_A receptors expressed on the surface of ganglion cell dendrites. Because retinal directional sensitivity is linked to GABAergic transmission, HOKS-induced expression of ACBP could provide a molecular basis for adaptation to HOKS and for the genesis of OKAN-II.

Key words: adaptation; astrocyte (astroglia); diazepam; glia; nystagmus; retina

Introduction

The horizontal optokinetic reflex (HOKR) is adaptive. It is evoked by rotation of a cylindrical optokinetic drum about a vertical axis and consists of conjugate, horizontal, compensatory eye movements interspersed with anticomensatory fast phases. In rabbits, if horizontal optokinetic stimulation (HOKS) is stopped after 1 hr, the first phase of optokinetic afternystagmus (OKAN-I) persists, in which conjugate eye movements continue in the same direction of the former HOKS for ~0.5–5 min.

Prolonged HOKS (12–48 hr) progressively reduces the gain of the HOKR (Fig. 1*E*). When HOKS stops, an afternystagmus, OKAN-II, develops in which the eyes move oppositely to the direction of the former HOKS (Fig. 1*D*). OKAN-II lasts for tens of hours (Barmack and Nelson, 1987; Pettorossi et al., 1999; Bar-

mack et al., 2002). The reduction in gain of the HOKR and the genesis of OKAN-II are the hallmarks of optokinetic adaptation.

During binocular HOKS, one eye is stimulated in the preferred posterior→anterior direction, increasing the discharge of ON- and ON-OFF direction selective ganglion cells (DSGCs), whereas the opposite eye is stimulated in the null, anterior→posterior direction. DSGCs have large receptive fields and are concentrated at highest densities in the visual streak. The visual streak lies just below and parallel to retinal vessels and myelinated nerve fibers that extend horizontally from the optic nerve head (Hughes, 1971; Choudhury, 1981). DSGCs are sensitive to stimulus velocities of 0.1–10°/sec and respond to light–dark edges (Collewijn, 1975; Oyster et al., 1980; Simpson et al., 1988; Soodak and Simpson, 1988).

GABAergic pathways are most clearly linked to direction selectivity. Asymmetric release of GABA by amacrine cell axon terminals on ganglion cell dendrites during stimulation in the preferred and null directions could account for directional selectivity (Barlow and Levick, 1965; Euler et al., 2002; Fried et al., 2002). A role for GABA_A receptors in direction selectivity is suggested by the observation that null responses of DSGCs in retinal eye cups are abolished by perfusion with GABA_A-specific antagonists (Kittila and Massey, 1995, 1997).

Horizontal DSGCs project centrally to the contralateral nucleus of the optic tract (NOT), one of the nuclei of the accessory optic system (Gioli et al., 1985, 1988). NOT cells project to ipsi-

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Figure 1. Long-term HOKS decreases gain of the HOKR and evokes OKAN-II. *A*, Rotation of the optokinetic drum about a vertical axis evokes HOKR. *B*, Monocular HOKS in preferred direction (5°/sec) evokes a conjugate HOKR at high gain. *C*, Monocular HOKS in the null direction evokes lower-gain HOKR. *D*, Gain of the HOKR decreases with increasing duration of HOKS. *E*, After 18–24 hr of binocular HOKS, OKAN-II is evoked when HOKS is stopped and the rabbit is placed in total darkness (indicated by the arrow). Note that the slow phase of the evoked OKAN-II is reversed with respect to the slow phase of the HOKR. R, Right; P→A, posterior→anterior; A→P, anterior→posterior.

lateral cells in the nucleus tegmenti pontis, dorsal cap of the inferior olive, pontine nuclei, dorsal lateral geniculate nucleus, medial vestibular nucleus, and nucleus prepositus hypoglossi (Terasawa et al., 1979; Mustari et al., 1994; Schmidt et al., 1995). The axons of dorsal cap neurons, climbing fibers, project to Purkinje cells in the contralateral flocculus and nodulus of the cerebellum (Alley et al., 1975). Floccular and nodular Purkinje cell axon terminals synapse on cells in the subjacent vestibular nuclei, which in turn have direct and indirect projections onto horizontal oculomotor neurons. Adaptation to HOKS could occur at one or several sites in this circuitry.

Although optokinetic circuitry is known, little is understood about how this circuitry adapts to prolonged HOKS. We used differential display reverse transcriptase (DDRT)-PCR to screen different parts of the circuitry for mRNAs, the transcription of which is differentially affected by long-term HOKS. In the retina, four transcripts related to GABAergic synaptic function were identified by DDRT-PCR. One of these was a transcript for acyl-CoA-binding protein (ACBP), a protein with increased expression in Muller glial cells during HOKS in the preferred direction.

Materials and Methods

Anesthesia and surgery. In preparatory operations, pigmented rabbits were anesthetized with intramuscular injections of ketamine hydrochloride (50 mg/kg), xylazine (6 mg/kg), and acepromazine maleate (1.2 mg/kg). The head of each rabbit was aligned in a stereotaxic apparatus. Under aseptic surgical conditions, a 2 cm midline incision on the dorsal surface of the cranium was made. The periosteum was removed. Two stainless-steel screws (8–32) were anchored to the calvarium with four smaller peripherally placed stainless-steel screws (2–56) and dental cement. The two larger screws mated with devices to restrain head movement during optokinetic stimulation. Rabbits were housed and handled according to the guidelines of the National Institutes of Health on the use of experimental animals. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Oregon Health and Science University.

Long-term binocular HOKS. Rabbits were placed in a restrainer at the center of a cylindrical optokinetic drum with a contour-rich pattern on the interior wall (diameter, 110 cm; height, 115 cm). The head of the rabbit was fixed to the restrainer by a spring-loaded coupling that mated with implanted head screws. The coupling maintained a head pitch angle of 12° with respect to earth horizontal. This angle corresponds to that maintained by unrestrained rabbits and aligns the visual streak and the horizontal semicircular canals in the horizontal plane (Hughes, 1971; Barmack and Nelson, 1987; Soodak and Simpson, 1988). The head coupling permitted small movements in the sagittal plane but prevented head movements in the horizontal plane. This method of restraint caused no pressure on any part of the body. The rabbit maintained its normal posture. All four paws remained in contact with the support surface.

The optokinetic drum was rotated at 5°/sec, stimulating the left eye of the restrained rabbit in the posterior→anterior direction and the right eye in the anterior→posterior direction. Optokinetic stimulation lasted 48 hr. Every 8 hr, the rabbit was removed from the drum and allowed access to food and water under ambient illumination reduced by the opaque roof of the feeding cage. When the optokinetic stimulation was stopped, the rabbit was immediately anesthetized (see above), and the left and right retinas were removed.

The efficacy of long-term HOKS in evoking differential changes in retinal gene transcription was inferred from previous experiments in which identical long-term HOKS evoked changes in the expression of corticotropin-releasing factor mRNA in neurons in the dorsal cap of the inferior olive (Barmack and Young, 1990). Dorsal cap neurons receive direction-selective inputs from the nucleus of the optic tract. NOT neurons receive projections from ON-DSGCs in the contralateral eye. Changes in relative transcription levels of dorsal cap neurons are detected after only 6 hr of HOKS and reach a sevenfold difference after 48 hr of HOKS.

DDRT-PCR. We used a modification of the DDRT-PCR technique (Liang and Pardee, 1992) in which 12 3'-oligo-dT anchored primers and four 5'-arbitrary primers were used to reverse transcribe mRNA to cDNA and to further amplify cDNA for gel analysis. Each of the anchored 3'-primers had two non-dT bases attached to a poly-T tail. This facilitated priming and first-strand cDNA synthesis from different subpopulations of the mRNA pool. Anchored 3'-primers were 31 bases long, including a 17 mer T7 phage promoter sequence upstream of a 12 mer oligo-dT region. Each of the 5'-arbitrary primers included an M13 promoter. First-strand fragments were converted into one, or at most a few, different double-stranded cDNA fragments during subsequent DDRT-PCR amplification using different upstream arbitrary 5'-primers.

Rectangular retinal tissue samples (2×15 mm) were removed and included the visual streak of each retina. The samples were frozen in isopentane cooled by dry ice. The tissue was homogenized in a Trizol (Invitrogen, Gaithersburg, MD) solution. RNA samples had an $A_{260/280}$ ratio ≥ 1.8 .

First-strand synthesis of cDNA was done with 0.2 μ g of DNase-treated total RNA using avian myeloblastosis virus reverse transcriptase (Invitrogen) in the presence of 12 different oligo-dT anchored primers and 20 μ M deoxy-NTP for 1 hr at 42°C. After heat inactivation of reverse transcriptase at 95°C for 5 min, PCR amplification was performed in the presence of [32 P- α]deoxy-ATP (dATP) (PerkinElmer Life Sciences, Emeryville, CA) using *Taq*DNA polymerase (Invitrogen).

Amplified cDNA was separated on a 4.5% polyacrylamide denaturing gel. The dried gel was exposed to BioMax MR film (Eastman Kodak, Rochester, NY) for 1–2 d. Identified cDNA fragments were recovered and reamplified by PCR using the appropriate corresponding set of primers. The reamplified cDNA was separated by agarose gel electrophoresis. It was purified using a GeneClean II kit (BIO 101, Vista, CA) and cloned into the pT7Blue vector (Novagen, Madison, WI). Plasmid DNA was prepared, and the insert was used as a probe for Northern blots and for cDNA library screening.

DNA sequencing: library screening and extension of DDRT-PCR gene fragments. A rabbit brain 5'-stretch plus cDNA library (Clontech, Palo Alto, CA) was used to obtain longer sequences from DDRT-PCR gene fragments cloned into a pT7Blue vector. Positive plaques were rescued into the plasmid vector Bluescript SK[−]. The inserts were sequenced using a thermosequase radiolabeled terminator cycle sequencing kit (United States Biochemicals, Cleveland, OH). A GeneRacer Kit (Invitrogen) was also used to obtain complete gene sequences.

Northern blots. Total RNA was isolated with Trizol (Invitrogen) from retinal tissue samples weighing 15–20 mg. Equal amounts of mRNA were separated on agarose gels (1.2%), transferred onto nylon membranes (MSI, Westboro, MA) with $20 \times$ SSPE (in M: 3 NaCl, 0.02 EDTA, and 0.25 NaH_2PO_4 , pH 7.4) for 12 hr, and then fixed by exposure to ultraviolet light. The membrane was hybridized with a probe containing the gene of interest generated from a plasmid and labeled with a 32 P deoxy-CTP kit (Amersham Biosciences, Piscataway, NJ). The nylon membrane was prehybridized for 6 hr at 42°C using prehybridization solution (Orbach et al., 1990). Hybridization was conducted by adding labeled oligonucleotide probes to the prehybridization solution ($\sim 1.8 \times 10^6$ cpm/ml). Hybridization was performed overnight at 42°C. The nitrocellulose membrane was washed twice for 15 min with $0.02 \times$ SSPE in 0.5% SDS at room temperature and once for 15 min at 65°C. The membrane was exposed to x-ray film (X-Omat AR; Eastman Kodak) at -80°C with an intensifying screen for 2 d.

Hybridization histochemistry. Fresh frozen tissue sections were cut 14 μ m thick and thaw mounted onto slides (Barmack and Young, 1990). Oligonucleotide probes were end labeled with [32 P- α]dATP (PerkinElmer Life Sciences) and purified with a Princeton Spin20 separation column. Each probe had an activity of 40–60,000 cpm/ μ l. After hybridization, the sections were exposed to an x-ray film (Amersham Biosciences) for 24 hr. On the basis of the results of this initial x-ray film exposure, sections were coated with NTB2 nuclear emulsion (Eastman Kodak) and exposed for 6–9 d. After development of the nuclear emulsion, sections were counterstained through the emulsion with neutral red.

Preparation of ACBP antibody. A bacterial expression system (TOPO TA expression kit; Invitrogen) generated sufficient amounts of ACBP to

immunize chickens and goats. The correct expression sequence of ACBP was confirmed by N-terminal protein sequence analysis performed on an N-terminal protein sequencer (model 492; Applied Biosystems, Foster City, CA) using N-terminal Edman degradation chemistry. These antibodies were affinity purified and used for immunolabeling Western blots and for immunohistochemistry.

Preparation of retinal subfractions. After dissection, the central retina was immediately homogenized with a $10 \times$ volume of Tris-buffered saline (TBS) (in mM: 20 Tris-HCl and 150 NaCl, pH 7.5). To TBS was added 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 μ g/ml aprotinin, and 10 μ g/ml leupeptin. All subsequent procedures were performed at 4°C. The homogenate was centrifuged at 14,000 rpm for 10 min. The supernatant contained the cytosolic fraction. The remaining pellet was sonicated for 30 sec in TBS containing 2% SDS. After centrifugation at 14,000 rpm for 10 min, the supernatant contained the membrane-associated fraction.

Gel electrophoresis and immunoblotting. Proteins from different cell fractions were separated either on a 15% SDS-PAGE gel or on a 12.5% urea gel (Laemmli, 1970). After electrophoresis, proteins were transferred onto Immobilon-P-membranes, blocked with PBS Tween 20 (PBST) containing 5% milk, incubated with primary antibody in PBST, and visualized with horseradish peroxidase-conjugated anti-goat IgG using enhanced chemiluminescence (Amersham Biosciences).

Immunocytochemistry. Retinas were fixed by a 1 hr perfusion in freshly depolymerized 4% paraformaldehyde and 0.15% saturated picric acid in 0.1 M PBS, pH 7.2, at room temperature. The neural retina was removed and postfixed for 20 min. The retina was then rinsed in PBS and dehydrated in graded sucrose (10–30%) in 0.1 M PBS, pH 7.4, before being frozen, sectioned on a cryostat at 10–14 μ m, and thaw mounted. Tissue sections were incubated on the slide with the primary antibody and developed with a double peroxidase antiperoxidase protocol.

Yeast two-hybrid system for identification of protein interaction. A yeast two-hybrid method detected protein–protein interactions, specifically interactions between ACBP and the α_1 -subunit of the GABA_A receptor. The yeast two-hybrid method uses the relative separation of the activation domain (AD) and binding domain (BD) of a reporter gene to indicate protein interaction. Attached to separate proteins that interact, the two domains are brought into close proximity, and transcription of the reporter gene occurs. Attached to separate proteins that do not interact, the domains will remain distant and transcription does not occur. Transcription in the reporter gene leads to a visually detectable signal. In the present experiment, activation and binding domains were coupled to the transcription of a reporter gene that expressed β -galactosidase (Chien et al., 1991).

Immunoprecipitation of ACBP from rabbit brain lysates with antibody to GABA_A α_1 . Lysates of rabbit brain were prepared by sonication three times for 15 sec in a lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EGTA, 0.25% Igepal, plus a Complete protease inhibitor mixture tablet for each 15 ml of buffer) (Roche, Branchburg, NJ). The lysates were centrifuged at $12,000 \times g$ for 10 min at 4°C to remove insoluble debris. The supernatant was precleared with protein A/G plus agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA) to remove proteins that bind nonspecifically to the beads. The beads were removed by centrifugation at $2000 \times g$. The cleared lysates were incubated with 4 μ g of polyclonal antibody to GABA_A α_1 for 2 hr at 4°C. The A/G plus agarose beads were then added to the mixture for 1 hr. The beads were removed by centrifugation at $200 \times g$ and washed three times with lysis buffer. The immunoprecipitate was boiled for 10 min to dissociate the proteins immunoprecipitated with the beads. The dissociated solution was run subsequently on a 10% SDS-PAGE gel.

Protein “pull-down” assay using histidine-tagged ACBP. QNR/K2 cells were transfected with the pCDNA 3.1 expression vector containing the histidine-tagged ACBP gene. Expressed ACBP was purified using nickel-impregnated agarose beads (Ni-NTA; Qiagen, Hilden, Germany). The beads were poured into a column and washed with three column volumes of low imidazole-containing buffer (20 mM imidazole in 50 mM Na_2PO_4 , pH 8.0, 300 mM NaCl). The washed beads were incubated with rabbit brain lysate. Proteins pulled down by ACBP were removed from

Table 1. Gene products, identified by DDRT-PCR after long-term optokinetic stimulation

Gene fragments	Primers
Acyl-CoA-binding protein	4
Tumor necrosis factor (TNF) receptor-associated factor-TNF receptor-associated protein	1
Cytochrome c oxidase subunit Vic	1
Light intermediate chain-2 dynein	1
Recoverin	1
Mitochondrial protein	4
GABA receptor-associated protein	2
TATA box binding protein	2
Bicarbonate cotransporter	2
14-3-3- ϵ protein	2
14-3-3- θ protein	2
P55-related membrane-associated guanylate kinase-like protein	2

Each of these gene products was elevated in the retina of the eye stimulated in the preferred direction, relative to those in the retina of the eye stimulated in the null direction. The number of different primer pairs out of the 48 combinations of "anchored" (12) and "arbitrary" (4) primers that identified a differentially transcribed gene product is listed in the right column.

the beads and separated from ACBP with a wash of 250 mM imidazole buffer.

Fixation of the head and implantation of EEG electrodes. Ten pigmented rabbits were anesthetized with a ketamine mixture as described above and fitted with head-restraint screws. Two EEG electrodes made from 30 gauge nichrome wire were inserted into the posterior cortex. One of the implanted anchor screws was used as a recording reference electrode.

Intravenous and intracisternal injection of ACBP. Previously implanted stainless screws were mated with a bar used to support the head in a three-axis vestibular rate table. During vestibular stimulation, the body of the rabbit was encased in foam rubber and fixed with elastic straps to a plastic tube aligned with the longitudinal axis of the rate table. ACBP was administered intravenously through a 22 gauge intravenous cannula placed in the marginal ear vein. Intravenous injections were 0.5 ml (1–2 mg/ml) administered over an interval of 30–60 sec. Control injections of vehicle alone were made before ACBP injections.

Intracisternal injections of ACBP were made in lightly anesthetized rabbits (30 mg/kg). The muscles overlying the cisterna magna were locally anesthetized before reflecting them. A microdrive was used to advance a 30 gauge needle through the cisterna magna. Typically, injections of 100–400 μ l (0.5–0.7 mg/ml) were made over an interval of 1–3 min.

Eye movement recording. Eye movements were measured by an infrared light projection technique. The right eye was topically anesthetized with proparacaine hydrochloride. A small suction cup (diameter, 3 mm) bearing a light-emitting diode (LED) was affixed to the right eye. The entire suction cup–LED assembly weighed 135 mg. The narrow projection beam angle of the LED was aligned with the visual axis and detected with a photosensitive X–Y position detector (United Detector Technology, Hawthorne, CA) fixed relative to the head. The detector had a diameter of 3.8 cm and provided continuous X–Y voltages proportional to the horizontal and vertical position of the incident centroid of infrared light. Eye movements were calibrated by moving the LED on a model of the rabbit eye through a known angular displacement. The system had a sensitivity of 0.2 min of arc and was linear to within 5% for eye deviations of 15° and to 8% for deviations of 30° (Barmack, 1981).

Results

DDRT-PCR identification of changes in retinal transcription evoked by HOKS

Optokinetic stimulation was used to generate differences in activity of directionally selective ganglion cells in the left and right retinas. DDRT-PCR was used to detect whether long-term differences in activity caused systematic changes in transcription of any retinal genes. After 48 hr of unidirectional HOKS, total RNA was isolated from the central region of each retina and reverse transcribed to cDNA. Differentially transcribed gene products were

reamplified and sequenced. Several differentially transcribed gene products were identified (Table 1). Four of these gene products were implicated potentially in the regulation of GABA receptors: (1) ACBP, (2) GABA receptor-associated protein (GABA-RAP), (3) 14-3-3- ϵ protein, and (4) 14-3-3- θ protein. Herein we focus on ACBP.

ACBP, named for its ability to bind thiol esters of coenzyme A, provides a pool of long chain acyl-coenzyme As and transports them from the cytosol to the membrane (Kragelund et al., 1999). ACBP is also known as the diazepam binding inhibitor, because it displaces diazepam at GABA_A receptors and consequently reduces the efficacy of GABA in evoking inhibition mediated by the GABA_A receptor (Costa and Guidotti, 1991; Knudsen et al., 1993). Perfusion of cultured motoneurons with 10 μ M ACBP reduces IPSCs evoked by iontophoretic application of GABA (Bormann et al., 1987, 2000).

ACBP full-length sequence

Transcripts of ACBP mRNA and of the other mRNAs identified by DDRT-PCR were found in greater abundance in the retina stimulated in the posterior→anterior (preferred) direction (Fig. 2). The full-length sequence was obtained by cloning DDRT-PCR gene fragments into a pT7Blue vector and using the cloned sequence to screen a rabbit brain 5′-stretch plus cDNA library (Clontech). The 5′ and 3′ cDNA end sequences were obtained using a Gene Racer Sequencing kit (Invitrogen). Protein sequences were deduced from full-length cDNAs.

In the rabbit, ACBP consists of 87 aa. ACBP has two potential phosphorylation sites that fit the pattern for protein kinase C (PKC) phosphorylation and three that fit the pattern for casein kinase phosphorylation (Table 2A). ACBP is highly conserved in mammals (Table 2B).

Northern blots for ACBP confirm results from DDRT-PCR

The optokinetically evoked increases in transcription of ACBP mRNA in a retina stimulated in the preferred direction compared with a retina stimulated in the null direction were confirmed by Northern blot (Fig. 2C). Forty-eight hours of binocular HOKS in the posterior→anterior (preferred) direction with respect to the left eye caused a relative increase in transcription of ACBP mRNA in the central retina of the left eye. The transcription of the "housekeeping gene" glyceraldehyde-3-phosphate dehydrogenase was not affected by the HOKS.

Hybridization histochemistry localizes ACBP to Muller glial cells

Hybridization histochemistry was used to determine specific cells in the retina that express ACBP. Three oligonucleotide probes for rabbit ACBP mRNA were hybridized with histological sections of the retinas in six rabbits: (1) 5′-ACGTCACCCACGGTTGCC-TGTTTGTAATGGCTGTA-3′, (2) 5′-CCCATCATCTGCAG-TATTAGAAAAACAAGGCCTATTGGTTAG-3′, and (3) 5′-GAT-TCCGTACTTCTGCTTGAGCTCCTCCACTTTGTCCACGTAC-GCTC-3′. These corresponded to 132–166, 345–387, and 262–308, respectively, of the full-length rabbit ACBP cDNA sequence (Table 2).

In a retinal whole mount, the ACBP1 probe labeled regions of the retina that flanked the myelinated nerve fibers that extended from the optic nerve head. The region just inferior to these myelinated nerve fibers was most densely labeled (Fig. 3E). Probes ACBP1 and ACBP3 were from the coding region and hybridized in the retina to a region extending from the ganglion cell layer to the inner nuclear layer (Fig. 3A–D). None of the neuronal cell

Figure 2. DDRT-PCR. *A*, A rectangular strip was cut from the neural retina just below the horizontally aligned myelinated fiber band and blood vessels extending from the optic nerve head. This strip included the visual streak, the retinal region of peak ganglion cell density. Ganglion cell isodensity contour lines are indicated (Hughes, 1971). Total RNA was extracted, and mRNAs were reverse transcribed to cDNAs. cDNAs were preferentially amplified using 48 combinations of primer pairs. *B*, Samples from the left and right retinas, amplified by the same set of primers, were run on adjacent lanes of a denaturing gel and separated by electrophoresis. Brackets indicate left (L)–right (R) samples that were differentially transcribed during 48 hr of HOKS. *C*, A Northern blot compared the ACBP mRNA extracted from a pair of retinas, one stimulated in the preferred direction and the other in the null direction. ACBP mRNA increased in the retina stimulated in the preferred direction. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

types in this region (amacrine cells, ganglion cells, bipolar cells) hybridized with the ACBP probes. Rather, the probes colocalized to Muller glial cells. The probes also hybridized with another glial astrocyte, the Bergman glial cell, in the cerebellum (Fig. 3*F*).

Optokinetic stimulation changes retinal ACBP expression: Western blots

Western blots were used to determine whether changes in retinal transcription of ACBP mRNA led to changes in protein expression. Retinal lysates were prepared and divided into cytosolic and membrane-associated fractions. These fractions were then run on a 15% SDS gel, using a bacterially expressed ACBP as a control (Fig. 4*A*). These blots showed that ACBP is an ~14 kDa protein found as a single band in the cytosol. Membrane-associated ACBP appeared as a double band. One band corresponded to the 14 kDa found in the cytoplasm. A second band, 20 kDa, was also observed. When the lysates were run on a 12.5% urea denaturing

gel, the heavier band in the membrane-associated fraction was reduced (Fig. 4*B*).

Cytosolic and membrane-associated fractions were compared from the left and right retinas of rabbits receiving 48 hr of HOKS. Cytosolic ACBP expression was greater in the retina (left) that received HOKS in the posterior→anterior (preferred) direction, as opposed to the retina (right) that received HOKS in the anterior→posterior (null) direction (Fig. 4*C*). This difference was not evident in the membrane-associated fractions run on the SDS gel but was evident in the bands run on the urea gels (Fig. 4*C,D*).

The optical densities of the cytosolic fractions from the left and right retinas were measured in five rabbits exposed to 48 hr of HOKS. The optical density of the cytosolic fraction isolated from retinas stimulated in the preferred direction was greater than the optical density of the cytosolic fraction isolated from the retinas stimulated in the null direction ($n = 4$; mean, posterior→anterior = 64.5 ± 6.9 ; mean, anterior→posterior = 35.6 ± 7.0 ; one-tailed t test; $p < 0.001$).

ACBP localized to Muller cells by immunohistochemistry

ACBP antisera were used to immunolabel the retina and to further localize cells that expressed ACBP. Muller cells, particularly in the inner plexiform and ganglion cell layers, were immunolabeled by the ACBP antisera. Immunolabeled Muller cell processes extended transversely in the lower part of the outer nuclear layer. Muller cells were clearly identified in retina stained with 1% toluidine blue (Fig. 5*A*). Muller cells were immunolabeled by ACBP antibodies (Fig. 5*B*). The Muller cell foot in the ganglion cell layer was particularly well immunolabeled, frequently obscuring ganglion cell bodies, which were not.

ACBP interacts with GABA_Aα₁

In spinal motoneurons, ACBP reduces the sensitivity of GABA_A receptors (Bormann, 1991). In the retina, although several transmitter systems may contribute, GABAergic pathways are most clearly linked to direction selectivity. When GABA_A receptors are blocked, direction selectivity is reduced (Caldwell et al., 1978; Kittila and Massey, 1995, 1997). If ACBP expression and secretion by Muller cells alter the sensitivity of GABA_A receptors of retinal neurons, then ACBP should interact with at least one of the GABA_A receptor subunits.

We used a yeast two-hybrid protocol to test whether ACBP interacted with GABA_Aα₁. ACBP was linked to the BD, and the α₁-subunit of GABA_Aα₁ was linked to the AD of a reporter gene that expressed β-galactosidase (Fig. 6*A*). The affinity of ACBP for GABA_Aα₁ brought the AD and BD into close proximity and activated transcription of the reporter gene (*lexAop*)₈-*lacZ* (Fig. 6*B*). No transcription was detected when either GABA_Aα₁ or ACBP was omitted (Fig. 6*C,D*). In a separate negative control, transcription–expression of β-galactosidase was not detected when two proteins known not to interact, human lamin C and simian virus 40 (SV40), were coinubated (Fig. 6*F*). Conversely, transcription was detected when two proteins known to interact, murine p53 and SV40, were coinubated (Fig. 6*E*). This indicates that the yeast two-hybrid system accurately reported the interaction of ACBP and GABA_Aα₁.

The interaction of GABA_Aα₁ with ACBP was demonstrated further using a coimmunoprecipitation assay. An antibody to GABA_Aα₁ was used to immunoprecipitate it from a retinal lysate. Subsequent immunostaining of the immunoprecipitate showed that ACBP was coimmunoprecipitated with GABA_Aα₁ (Fig. 7*A*).

Additional evidence of the association of ACBP with GABA_Aα₁ was obtained using a pull-down assay, in which histidine-tagged

Table 2. Full-length cDNA and protein sequence of rabbit ACBP AF407578 and comparison with protein sequences from other mammals

A																			
		M S Q A E F																6	
		tctctgcttctccgtggtcccgaggatttttgcacgcgcggccagc																65	
		E K A A E E V K N L K T K P A D A E																24	
		GAG AAA GCT GCA GAG GAA GTT AAG AAC CTC AAG ACC AAG CCA GCA GAC GCA GAG																119	
		M L F I Y S H Y K Q A T V G D V N T																42	
		ATG CTG TTT ATC TAC AGC CAT TAC AAA CAG GCA ACC GTG GGT GAC GTC AAC ACA																173	
		E R P G M L D L K G K A K W D A W N																60	
		GAG CGG CCT GGG ATG TTG GAC CTC AAA GGC AAG GCC AAG TGG GAT GCC TGG AAC																227	
		E L K G T S K E S A M R A Y V D K V																78	
		GAG CTG AAA GGG ACT TCC AAG GAG AGC GCC ATG AGA GCG TAC GTG GAC AAA GTG																281	
		E E L K Q K Y G I																87	
		GAG GAG CTC AAG CAG AAG TAC GGA ATC taagggcccgatgaggctcccgccgcacactta																343	
		actaaaccaataggccttgtttttctaatactgcagatgatgggtgtgccagcatggaatagccagcgaac																415	
		cccacgtccagggtcctcagatggggctctagcagagtggggctgaacggctcattgacctgctgagt																487	
		agccccccatccgagatccattaaagttcatttgccagcgcaaaaaaaaa																536	
B																			
ACBP-human	1	MSQAEEFKAAEEVRHLKTKPSDEEMLFYGHYKQATVGDINTERPGMLDFTGKAKWDANNEK																63	
ACBP-cow	1	MSQAEEFDKAAEEVKHLKTKPADEEMLFYSHYKQATVGDINTERPGMLDFKKGAKWDANNEK																63	
ACBP-pig	1	MSQAEEFKAAEEVKHLKTKPADEEMLFYSHYKQATVGDINTERPGILDGKKGAKWDANNEK																63	
ACBP-mouse	1	MSQAEEFDKAAEEVKRLKTKPTDEEMLFYSHYKQATVGDVNTDRPGLLDLKGAKWDANNEK																63	
ACBP-rabbit	1	MSQAEEFKAAEEVKHLKTKPADEEMLFYSHYKQATVGDVNTDRPGLLDLKGAKWDANNEK																63	
ACBP-rat	1	MSQVEFEEMACSLKQLKGLSLDQEKLLVYSFYKQATQGDCTIPVPPATDVKAKAKWEAMVNK																63	
ACBP-human	64	GTSKEDAMKAYINKVEELKKKYGI																87	
ACBP-cow	64	GTSKEDAMKAYIDKVEELKKKYGI																87	
ACBP-pig	64	GTSKEDAMKAYINKVEELKKKYGI																87	
ACBP-mouse	64	GTSKESAMKTYVEKVDLKKKYGI																87	
ACBP-rabbit	64	GTSKESAMRAYVDKVEELKKKYGI																87	
ACBP-rat	64	GMSKMDAMRIYIAKVEELKKNETC																87	

A, A large gene fragment was obtained from the retina by DDRT-PCR (bolded letters). This sequence was expanded using a cDNA stretch library and a GeneRacer kit (Invitrogen). The gray shading indicates the region showing the truncated form of ACBP, which comprises the ODN. B, The rabbit sequence is compared with ACBP sequences from several other mammals. The dark gray shading indicates complete homology for all species. The light gray shading indicates homologies based on equally charged amino acids.

ACBP was expressed by QNR/K2 cells transfected with the PCDNA 3.1 expression vector containing the histidine-tagged ACBP gene. Western blots of pulled-down proteins were probed with antibodies to ACBP and GABA_Aα₁ (Fig. 7B). These Western blots had immunoreactive bands to both antibodies, demonstrating an association between ACBP and GABA_Aα₁.

Central actions of ACBP

If ACBP interacts with GABA_Aα₁, then this interaction should be reflected centrally as well as peripherally. In three rabbits, intravenous injections of 0.5 ml of ACBP (0.5–1 mg/ml) failed to cause any change in the EEG or in the gain of the vertical vestibulo-ocular reflex (VVOR). The VVOR was used as a behavioral index of arousal. The gain of the VVOR increases with increased arousal. In two rabbits, intravenous injections of 0.5 ml of ACBP (0.5–0.8 mg/ml) were combined with coinjections of mannitol (1.4 M) to open the blood–brain barrier. Integrated EEG activity and the gain of the VVOR increased within 2 min and slowly decreased over the next 30 min.

In one rabbit, an intravenous injection of 1 ml of octadecanoneuropeptide (ODN) (0.8 mg/ml), QATVGDVNTDRPGMLDLK, a truncated derivative of ACBP, failed to increase cortical activity unless it was also coinjected with mannitol.

In three rabbits, intravenous injections of 0.5 ml of a truncated 6 aa residue GMLDLK, which included the C-terminal lysine of ODN (0.5–1 mg/ml), increased cortical activity and the gain of VVOR. This effect did not require coadministration of mannitol. These data suggest that although ACBP does not cross the blood–brain barrier, one or more of its proteolytic products does and excites cortical neurons.

In three rabbits, intracisternal injections of 100–400 μl of ACBP (0.5–0.7 mg/ml) increased cortical activity and the gain of the VVOR (Fig. 8A). Increased cortical activity was quantified by determining the relative duration during which the integrated EEG exceeded the preinjection mean EEG activity by >2 SDs. This was normalized for each rabbit during intervals of 2 min. Intracisternal injections of ACBP increased the normalized mean EEG activity by 50% (Fig. 8B). This ACBP-evoked increase lasted 20 min.

Discussion

ACBP and other GABA-related mRNAs increased by optokinetic stimulation

We isolated four gene fragments related to GABAergic synaptic transmission. Although we focused on ACBP, the other mRNAs detected by the DDRT-PCR screen might also participate in the regulation of sensitivity of GABAergic signaling pathways.

Figure 3. Hybridization histochemistry localizes ACBP mRNA to the inner nuclear, inner plexiform, and ganglion cell layers of the retina. Two oligonucleotide probes for rabbit ACBP mRNA (ACBP1 and ACBP3) were used for hybridization histochemistry. *A, B*, Dark-field view of hybridization in the ganglion cell layer and inner nuclear layer of the retina. The probes did not colocalize to either ganglion or amacrine cells. *C, D*, Bright-field view of the hybridization to the inner nuclear, inner plexiform, and ganglion cell layers. The region of greatest hybridization corresponds to the region of Muller glial cell processes. *E*, Hybridization of probe to the area surrounding the myelinated nerve fibers in a rabbit retinal whole mount. Arrowheads indicate the ventral border of fibers radiating from the optic nerve head. *F*, A probe (ACBP3) was also applied to a sagittal section of cerebellar nodulus. The probe hybridized with Bergman glial astrocytes. *G*, The inset shows the absence of hybridization with Purkinje cell bodies at higher magnification. GL, Ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; PL, photoreceptor layer; S, superior; T, temporal; N, nasal; I, inferior.

GABA-RAP

GABA-RAP is a small 117 aa polypeptide that recognizes and binds to the intracellular loop of the γ_2 -subunit of the GABA_A receptor. GABA-RAP does not interact with the $\alpha_{1,2,4-6}$ or β_{1-3} subunits of the GABA_A receptor (Nymann-Andersen et al., 2002). Possibly, it links the γ_2 -subunit to microtubules for transport within the cell (Wang et al., 1999; Wang and Olsen, 2000). Although it binds to gephyrin, a protein implicated in the clustering of GABA_A receptors, GABA-RAP does not colocalize with gephyrin in brain synapses (Kneussel et al., 2000). GABA-RAP promotes clustering of GABA_A receptors in recombinant cell expression systems, suggesting a role in directing GABA_A receptors to synapses (Chen et al., 2000). However, the exact role of GABA-RAP in receptor trafficking and synaptic localization remains unknown.

14-3-3 proteins

Originally named for their migration positions in two-dimensional gel electrophoresis of brain extracts, the family of 14-3-3 proteins is highly conserved and acts as adapter or scaffold proteins that bind to and interconnect several proteins involved in signal transduction, cell-cycle regulation, and apoptosis (Aitken, 1996). Isoforms of 14-3-3 interact with GABA_B receptors

(Couve et al., 2001) as well as PKC- γ , PKC- ϵ (Yaffe et al., 1997), PKC- θ (Meller et al., 1996), PKC- ζ (Van Der Hoeven et al., 2000), and PKC- μ (Hausser et al., 1999).

Action of ACBP in retina

ACBP is one of several retinal gene products, the expression of which is affected by HOKS. It is increased in the retina receiving HOKS in the preferred direction relative to the retina stimulated in the null direction. If ACBP interacts with GABA_A receptors localized to dendrites of ON-DSGCs and amacrine cells, then it must be secreted by Muller cells. A secretory role for glia has been demonstrated in cultured cortical astrocytes for atrial natriuretic peptide (Krzan et al., 2003). Cultured Muller glial cells secrete apolipoprotein E (apoE) (Amaratunga et al., 1996) and NGF (Dicou et al., 1994) as well as a factor that induces M₂ muscarinic receptor expression in the retina (Belmonte et al., 2000). In intact eyes, apoE is internalized by retinal ganglion cells and transported centrally in the optic nerve. Although the secretion of ACBP from Muller cells has not been measured *in vivo*, model Muller cells (QNR/K2 cells) maintained in tissue culture release ACBP when depolarized by increases in extracellular KCl (T. Bilderback, Z. Qian, and N. H. Barmack, unpublished observations).

Once released, ACBP could interact with GABA_A receptors on ganglion cell dendrites that receive a GABAergic direction-selective signal from Starburst amacrine cell axon terminals (Euler et al., 2002). Amacrine cell axon terminals and ganglion cell dendrites are intertwined

with horizontal Muller cell processes in the inner plexiform layer (Dreher et al., 1992), the region of maximal hybridization signal for ACBP mRNA. This suggests the possibility that Muller cells, depolarized locally in the inner plexiform layer by the discharge of GABAergic amacrine cells, secrete ACBP and thereby reduce the sensitivity of GABA_A receptors on ganglion cell dendrites, providing a local negative feedback loop.

The hypothesized, long-latency feedback loop provided by OKS-evoked transcription, expression, and secretion of ACBP is not the only mechanism by which Muller cells influence GABAergic synaptic transmission at ganglion cells. Muller cells also express high-affinity GABA transporter (GAT) proteins, GAT-1 and GAT-3 (Biedermann et al., 2002). Extracellularly applied GABA is cleared by these GABA transporters within 100 msec. Consequently, GABAergic transmission between amacrine and ganglion cells is regulated by at least two separate mechanisms having different dynamics. Both are dependent on the Muller cell.

A third possible mechanism for the postsynaptic regulation of GABAergic transmission in the retina is represented by altered transcription of GABA-RAP. This protein, expressed by ganglion

Figure 4. ACBP expression in the retina after 48 hr of HOKS. Retinal lysates were separated into cytosolic and membrane fractions. These fractions were immunoprecipitated. *A*, Western blots were run on a 15% SDS-PAGE gel and immunolabeled with an antibody to ACBP (chicken host). The membrane fraction appears as a dimer, having two bands at 14.4 and 20 kDa. *B*, Immunoprecipitates were also run on a 12.5% urea gel, reducing the larger of the two membrane bands. *C*, Samples from the left (L) and right (R) retinas of a rabbit that received optokinetic stimulation for 48 hr were run on a 15% SDS-PAGE gel. The expression of ACBP in the cytosolic fraction was greater in the left retina, stimulated in the preferred direction, than in the right retina, stimulated in the null direction. *D*, Samples from the same left and right retinas were also run on a 12.5% urea gel. This gel showed greater expression of ACBP in the left cytosolic fraction and in the lower band of the left membrane fraction. The bands at 40 kDa indicate controls for sample loading using an antibody to actin.

Figure 5. Immunohistochemical localization of ACBP to Muller glial cells. *A*, Normal retina stained with 1% toluidine blue. *B*, Immunohistochemical staining of retina with ACBP anti-serum and the PAP method. G, Ganglion cell; GL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; M, Muller cell; ONL, outer nuclear layer; PL, photoreceptor layer.

cells, could alter the sensitivity of these cells to GABA by regulating the number of GABA_A receptors inserted into the postsynaptic membrane.

Molecular interactions of ACBP

In other neural systems, the expression and release of a signaling molecule is associated with its phosphorylation or dephosphorylation. One mechanism by which the signaling molecule PKC is activated is through phosphorylation of specific threonine, serine, and tyrosine residues by other protein kinases (Parekh et al., 2000; Fukunaga et al., 2001; Zheng et al., 2002). ACBP has five potential phosphorylation sites: two threonine sites fit a PKC phosphorylation pattern (42–44 TeR and 65–67 TsK). Two fit a casein kinase-2 (CK2) pattern (36–39 TvGD and 65–68 TskE). One serine site fits a CK2 pattern (2–5 SqAE) (Table 2).

The crystal structure of bovine liver ACBP provides insight into how phosphorylation sites might be distributed in space

Figure 6. Interaction between ACBP and GABA_Aα₁ shown by yeast two-hybrid assay. *A*, Schematic of yeast two-hybrid technique. ACBP is linked to the AD. Bacterially expressed GABA_Aα₁ is linked to the BD on the upstream activation sequence (UAS). The affinity of ACBP for GABA_Aα₁ brings the AD and BD into close proximity and activates transcription of the reporter gene (*lexAop*)₈-*lacZ*. *B*, Two separate tests of interaction yielded positive results (transcription of the reporter gene). *C*, Negative control; no transcription was detected when GABA_Aα₁ was omitted. *D*, Negative control; no transcription was detected when ACBP was omitted. *E*, Positive control; transcription was detected when two proteins known to interact, murine p53 and SV40, were co-incubated. *F*, Negative control; transcription was not detected when two proteins known not to interact, human lamin C and SV40, were co-incubated.

(Andersen and Poulsen, 1992). Only 6 of 87 aa of rabbit retinal ACBP differ from those of the bovine liver ACBP. In five of six of these, the discrepant amino acids share homologies based on charge. In crystalline ACBP, the five threonine and serine residues are located close to the protein exterior, making these sites available for phosphorylation.

ACBP may interact with other transduction molecules such as PKC and CK2, major serine–threonine kinase families, capable of phosphorylating specific sites on ACBP. Several PKC isoforms are expressed in the retina. PKC-α is found in bipolar cells and photoreceptor outer segments (Cuenca et al., 1990; Suzuki and Kaneko, 1990; Osborne and Barnett, 1992; Udovichenko et al., 1993). PKC-β and PKC-γ are expressed by bipolar cells, ganglion cells, and amacrine cells (Negishi et al., 1988; Osborne and Barnett, 1992; Kolb et al., 1993). PKC-δ is expressed by Muller cells (Osborne et al., 1994, 1995). A variety of responses have been ascribed to PKC, including modulation of GABA receptor sensitivity (Wood et al., 1997). Two of the subunits of the GABA_A receptor, β and γ₂, also contain putative PKC phosphorylation sites (Krishek et al., 1994). GABA_A receptor channel function may be downregulated by PKC (Kellenberger et al., 1992; Leidenheimer et al., 1992, 1993; Lin et al., 1994). PKC activity can itself be modulated by the action of 14-3-3 isoforms, and these interactions may be isoform specific (Robinson et al., 1994; Acs et al., 1995; Aitken et al., 1995).

CK2 is a ubiquitous serine threonine kinase that is highly conserved, suggesting that it plays an important role. CK2 is more abundant in the brain than in any other tissue. It has >200 substrates (Sarno et al., 2002). Many substrates are found in synaptic and nuclear compartments and have roles in development, neurogenesis, synaptic transmission, synaptic plasticity, information storage, and survival (Blanquet, 2000). In the retina, CK2 is

Figure 7. Coimmunoprecipitation of ACBP from rabbit brain lysate with $GABA_A\alpha_1$. *A*, ACBP was coimmunoprecipitated from a rabbit brain lysate along with $GABA_A\alpha_1$ using antibody to $GABA_A\alpha_1$. Lane 1, Rabbit brain lysate was run as a control without immunoprecipitation by antibody to $GABA_A\alpha_1$. Lane 2, $GABA_A\alpha_1$ and ACBP were coimmunoprecipitated from rabbit brain lysate by an antibody to $GABA_A\alpha_1$. Lane 3, Bacterially expressed ACBP was run as a control. The portion of the blot identified by the antibody to $GABA_A\alpha_1$ is not presented, because it was complicated by the migration of the heavy chain of $GABA_A\alpha_1$ antibody to the same position as $GABA_A\alpha_1$. *B*, Histidine (His)-tagged ACBP, expressed by QNR/K2 cells, was purified with a column of His-binding beads. The purified His-tagged ACBP was incubated with rabbit brain lysates mixed with His-binding beads. The beads were separated by centrifugation and washed. His-tagged ACBP and the proteins with which it interacted were eluted and run on a Western blot. $GABA_A\alpha_1$ associated with the His-tagged ACBP. The top half of the blot was probed with an antibody to $GABA_A\alpha_1$. The bottom half was probed with an antibody to ACBP. Lane 1, Bacterially expressed His-tagged ACBP was run as a control. Lane 2, Lysate from untransfected QNR/K2 cells was coincubated with His-binding beads without rabbit brain lysate. Lane 3, Lysate from QNR/K2 cells transfected with His-tagged ACBP gene was incubated with His binding beads. Lane 4, Rabbit brain lysate was coincubated with His-binding beads without His-tagged ACBP. Lane 5, Rabbit brain lysate was coincubated with His-tagged ACBP and His-binding beads. Lane 6, A positive control for $GABA_A\alpha_1$ was prepared from a microsomal fraction of rat brain. This fraction was not incubated with QNR/K2 cells transfected with the His-tagged ACBP gene. Nonspecific binding of $GABA_A\alpha_1$ to the His beads is evident in lane 4. However, the optical density of the specific band in lane 5 is $10\times$ greater than that of the nonspecific band in lane 4.

found in rod photoreceptors, in which it phosphorylates a cGMP-gated channel (Warren and Molday, 2002).

Action of ACBP and its truncated forms on the CNS

Digestion of ACBP with trypsin yields an ODN that includes amino acid residues 34–51 (Table 2). The ODN segment of ACBP is located on the exterior of the protein. ODN is pharmacologically active and has the sequence QATVGDVNTERPGM-LDLK. ODN displaces benzodiazepines from binding sites on neurons and glial cells with a binding affinity similar to that of ACBP (Bender and Hertz, 1986). ODN decreases pentobarbital-induced sleep in mice, suggesting that ODN modulates $GABA_A$ responses (Dong et al., 1999).

ODN has two phosphorylation sites, one for PKC and one for CK2. The C-terminal region of ODN is critical. Amidification of the C-terminal lysine eliminates its pharmacological activity. This raises the question of whether phosphorylation of one or more of the sites on ACBP is either necessary or sufficient for its interaction with $GABA_A\alpha_1$. An alternative possibility is that the interaction of ACBP with $GABA_A\alpha_1$ depends on the lysine at position 51 rather than the phosphorylation of either the PKC or CK2 sites. A third possibility is that the first two possibilities are both correct. The pharmacological activity of intact ACBP may depend on the phosphorylation sites to orient the protein correctly so that lysine-51 is exposed. In the truncated ODN, Lys-51 may be exposed without phosphorylation.

Figure 8. Intracisternal injection of ACBP increases EEG activity and increases gain of vertical vestibulo-ocular reflex. An intracisternal cannula was inserted in a lightly anesthetized rabbit (ketamine hydrochloride, 30 mg/kg). Background EEG was maintained constant during sinusoidal roll-tilt vestibular stimulation about the longitudinal axis (20° , 0.2 Hz). ACBP was intracisternally injected ($400\ \mu\text{l}$, 0.7 mg/ml, 2 min). The intracisternal injection of ACBP caused an increase in EEG activity and an increase in gain of the VVOR. *A1*, An EEG trace was obtained from chronically implanted cortical electrode. The duration of the intracisternal injection is indicated by the bar. *A2*, The EEG was full-wave rectified and integrated. *A3*, Vertical eye movements were recorded with a photoelectric technique. *A4*, Gain of the VVOR was calculated for each cycle of stimulation. *B*, Three rabbits received intracisternal injections of ACBP ($100\text{--}400\ \mu\text{l}$, 0.7 mg/ml, 2 min). Normalized mean change in EEG activity was plotted for the group. The injection onset is indicated by the arrow.

The C-terminal region of ODN is critical. Amidification of the C-terminal lysine eliminates all pharmacological activity (Berkovich et al., 1990). Three smaller peptides, consisting of 6–8 aa, all of which include the C-terminal lysine, have binding characteristics similar to that of ODN. The 8 aa peptide has an efficacy equal to the full-length ACBP in modulating glucose-stimulated secretion of insulin from rat pancreatic islets (Borboni et al., 1991).

In the present experiment, a 6 aa peptide containing the C-terminal lysine crossed the blood–brain barrier and increased EEG activity. Because plasma levels of ACBP and its truncated forms are elevated in epileptic patients (Ferrarese et al., 1998), it is possible that some forms of epilepsy may be triggered either by defects in the blood–brain barrier or by increased plasma concentration of truncated forms of ACBP.

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