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Cloning and *in situ* localization of a brain-derived porin that constitutes a large-conductance anion channel in astrocytic plasma membranes

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ABSTRACT **We have cloned a protein from bovine brain, brain-derived voltage-dependent anion channel 1 (BR1-VDAC), that is identical to a recently sequenced plasmalemmal-bound porin from human lymphocytes. mRNA hybridization indicates that BR1-VDAC is widely distributed throughout nervous and nonnervous tissues. *In situ* localization substantiated that the BR1-VDAC is associated with the plasmalemma of astrocytes. A monoclonal antibody that recognizes the N terminus of the BR1-VDAC protein completely blocks an astrocytic high-conductance anion channel that has electrophysiological similarities with the mitochondrial VDAC. Since the high-conductance anion channel in astrocytes has been shown to respond to hypoosmotic solutions, its molecular identification provides the basis for a better understanding of volume regulation in brain tissue.**

Astrocytes are involved in the local homeostatic balancing of the interstitial cerebral fluid in brain. The correct condition of the interstitial cerebral fluid is crucial for normal functioning of the neuronal network. Astrocytes have a complement of neurotransmitter receptors and ion channels that form the backbone of this regulative capacity. Although physiological data on the diverse astrocytic receptor and channel types have been collected (1, 2), knowledge of their molecular composition is rare. Determining the molecular mechanisms involved in the homeostatic balancing of the interstitial cerebral fluid is, therefore, indispensible for an understanding of the properties of astrocytes and their concerted action in maintaining normal brain function. Among the various channel types electrophysiologically characterized in astrocytes (2, 3) is a large-conductance anion channel, described in cultured astrocytes (4–6) and cultured rat Schwann cells (7) and reported (8) to be present in astrocytes of intact optic nerves. Jalonen *et al.* (9) have further characterized this channel. Its basic channel properties are (*i*) a large unitary conductance (>400 pS; refs. 6–8), (*ii*) the existence of multiple subconductance states (9), (*iii*) voltage dependence and symmetrical closing in the voltage range of ±20 mV (8), and (*iv*) preferred anion permeability in the high-conductance state, which occurs around 0 mV and includes Cl^- but also larger anions such as methyl sulfate and isothionate (10). It shows some remarkable biophysical properties reminiscent of the mitochondrial voltage-dependent anion channel (mi-VDAC).

mi-VDACs (also known as mitochondrial porins) are channel-forming proteins in the outer mitochondrial membrane of eukaryotic cells. They consist of a small but abundant protein (30–34 kDa) and are considered to provide the pathway for the movement of nucleotides and various other substances into a variety of metabolic pathways (11–13). Compared with the large-conductance anion channel of astrocytes, they exhibit almost identical basic channel properties. (*i*) Single-channel conductance is roughly 450 pS. (*ii*) They show ion selectivity for Cl^- in the high-conductance state, which occurs at 0 mV transmembrane voltage. (*iii*) They respond symmetrically with respect to gating properties; i.e., they are in the high-conductance state at 0 mV and close to low-conductance states in response to application of both positive and negative potentials (14). The characteristic voltage-dependent gating and the anion preference at low potentials have led to the term VDAC (11, 12).

VDACs in eukaryotes have been considered to be localized exclusively in mitochondria. Recently, however, an apparently plasmalemmal VDAC (plm-VDAC) protein highly homologous to mitochondrial porins has been sequenced from a human lymphocyte cell line by Edman degradation (15). Subsequently, another VDAC-like protein was found to copurify with the plasmalemmal-bound central type A γ-aminobutyric acid ($GABA_A$) receptor complex (16). This protein was 70% identical with the 31HL porin (HL-VDAC) by its predicted amino acid sequence. This suggested that VDACs are localized not only in the mitochondrial compartment but also in the plasma membrane. The striking similarity of the biophysical properties of mi-VDAC and the large-conductance anion channel of astrocytes, respectively, prompted us to search for molecular identities. Here we report the isolation, cloning, and *in situ* localization of brain-derived plm-VDAC1 (BR1-VDAC) that is identical to the HL-VDAC and is predominantly expressed in astrocytes.¶ A site-specific monoclonal antibody (mAb) directed to the N-terminal sequence of the HL-VDAC completely blocked the large-conductance anion channel in excised patches of cultured astrocytes, suggesting that this channel is in fact a member of the VDAC family.

MATERIALS AND METHODS

Separation of BR1-VDAC-Enriched Membranes from Bovine Brain. Isolation of BR1-VDAC membrane fractions

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Abbreviations: VDAC, voltage-dependent anion channel; BR1-, BR2-, mi-, plm-, and HL-VDAC, brain-derived VDAC1, brain-derived VDAC2, mitochondrial VDAC, plasmalemmal VDAC, and 31HL porin, respectively; mAb, monoclonal antibody; pAb, polyclonal antibody; $GABA_A$ receptor, type A γ-aminobutyric acid receptor.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. X75068).

entails the separation of myelin from a bovine brain homogenate (17) and the subsequent detergent treatment of pellets containing the plasma membrane fraction.

Fresh bovine brains were suspended in 2 mM sodium bicarbonate (BB, pH 8.5) in the presence of a protease inhibitor (phenylmethylsulfonyl fluoride) and homogenized with the aid of an Ultra-Turrax. Myelin at the interphase between 0.32 M and 0.85 M sucrose was discarded. The pellet was used for preparation of the detergent-resistant membrane fraction. For the preparation of this fraction the homogenized pellets were poured into 650 ml of freshly prepared 5 mM Tris 10/1.1% (wt/vol) sodium lauroyl sarcosine. The mixture (1.2 liters) was continuously stirred at room temperature for 10 min, distributed equally into six 250-ml tubes, and centrifuged at 11,000 rpm in a AH29 (Sorvall) rotor. Pellets were collected and resuspended in 60 ml of 5 mM Tris 10/0.3% deoxycholic acid (DOC). The samples were then loaded onto a two-step sucrose gradient of 10 ml of 35% sucrose/5 mM Tris 10/0.3% DOC and 10 ml of 49% sucrose/5 mM Tris 10/0.3% DOC. Sample tubes were centrifuged at 25,000 rpm in a AH29 (Sorvall) rotor for at least 2 hr, and the layer constituting the detergent-resistant membrane fraction at the 35–49% interface was collected and washed three times in 10 times the volume of ice-cold BB.

Antibodies and Western Blot Analysis. Immunoblots were performed as described (18). mAb1 was propagated by injection of the electroeluted 34-kDa protein (30 μg/ml) into BALB/c mice. Samples of spleen lymphocytes were taken after the third booster injection and processed for hybridoma production (31). A site-specific polyclonal antibody (pAb) directed to positions 156–174 of the BR1-VDAC was raised by injecting HPLC-purified synthetic peptides (100 μg/kg) into New Zealand rabbits. Characterization and specificity of mAb2 has been described (19). The anti-mitochondrial F_1-ATPase subunit antibody was kindly provided by A. W. Wolkoff (Albert Einstein College of Medicine, New York).

Microsequencing. Microsequencing was performed from fragments of tryptic-digested 34-kDa protein. For this purpose, the 34-kDa band was excised from one-dimensional SDS/15% polyacrylamide gels (20), destained, washed, and freeze-dried. Tryptic digestion was performed within the gel by using 0.2 M hydrogen carbonate containing 1 μg of trypsin per 100 μl. The supernatants were collected, and the tryptic fragments were purified by reverse-phase HPLC. Microsequencing was done with an Applied Biosystem (model 477A) sequencer with online phenylthiohydantoin analyzer (model 120).

Tissue Culture of Purified Astrocytes. Brain cortex was dissected from postnatal rats (postpartum day 2), minced, and incubated for 30 min in 0.1% trypsin (GIBCO) at 37°C and for 5 min with DNase I (1 mg/ml; bovine pancreas; Sigma). After removal of the enzyme solution, tissue was dissociated by trituration with a small-bore Pasteur pipette and subsequent passage through a 50-μm nylon mesh. Cells were mounted on glass coverslips or plastic tissue culture dishes (Nunc). Cultures were grown in 45% (vol/vol) minimal essential medium/45% (vol/vol) Ham's F-12/10% (vol/vol) fetal calf serum/penicillin (50 μg/ml)/streptomycin (50 μg/ml)/glutamine (2 mM), buffered with 21 mM bicarbonate. Cultures were maintained in a 5% CO_2/95% air atmosphere at 37°C at ≈100% relative humidity.

Immunofluorescence and Immunogold Labeling. Immunofluorescence was carried out on subconfluent cultures between the second and third week of culture. Processing for immunofluorescence was performed as follows: In brief, cells were fixed in absolute ethanol at −20°C for 20 min, washed with Dulbecco's phosphate-buffered saline (PBS), and incubated in PBS, supplemented with 0.1% albumin. Incubation with primary antibody (mAb2) was performed for 60 min at room temperature, followed by three washes in PBS and then incubation with the secondary antibody, mouse anti-IgG coupled to fluorescein isothiocyanate or tetramethylrhodamine isothiocyanate (Sigma). Double immunofluorescence was performed in the same manner. The primary antibodies [mAb2 and an anti-glial fibrillary acidic protein pAb (Dianova, Hamburg, F.R.G.)] were added simultaneously, followed by the secondary antibodies. Immunogold labeling was performed by incubation of the cultured astrocytes with mAb2 for 60 min, washing in warm PBS (37°C), and fixation in 3% (wt/vol) paraformaldehyde with 0.2% glutaraldehyde in PBS for 60 min. After fixation and additional washing in PBS, rabbit anti-mouse IgG coupled to immunogold (15 nm) was applied to the cultures as the secondary antibody. Labeled cells were then washed three times in PBS and processed for transmission electron microscopy.

PCR and Cloning of the BR1-VDAC. An antisense primer, ACCGTCGACTTT(A/G)TAICCIACIGC(A/G)AA(A/G)TC, and a sense primer, TACGAATTCGGIGCI(T/C)TIGTI(T/C)TIGGITA(T/C)GA(A/G)GG, were constructed to match the left and right sides of peptide P1 (indicated by arrows above the respective residues in Fig. 2). Underlined nucleic acids represent modifications of *Sal* I and *Eco*RI restriction sites, respectively, to facilitate subcloning. First-strand cDNA was synthesized by incubating 1 μg of poly(A)$^+$ RNA from total bovine brain, 1 μg of antisense primer, 50 mM Tris (pH 8.3), 250 mM KCl, 7.5 mM $MgCl_2$, all four dNTPs (each at 1 mM), 5 mM dithiothreitol, 25 units of RNase inhibitor, and 20 units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) for 1 hr at 42°C. Then, 1 μg of sense primer and 5 units of *Taq* polymerase (Boehringer Mannheim) were added and the mixture was diluted 1:5 with water. Twenty-five PCR cycles were performed for 1 min at 92°C, 1 min at 50°C, and 2 min at 72°C. An amplification product of 120 bp was obtained, subcloned, and verified by sequencing to be the coding cDNA of peptide 1. This cDNA fragment was used as a probe to screen 250,000 recombinant plaques of a commercial cDNA library from bovine brain (Stratagene). A single positive plaque was identified and isolated by *in vivo* excision, and the entire insert was sequenced on both strands.

Northern Blot Analysis. Total RNA was extracted from tissues by lysis in guanidine thiocyanate and centrifugation through cesium chloride gradients. Total cellular RNA (10 μg per lane) was loaded on 1.2% agarose/formaldehyde gels and blotted to nitrocellulose (21). Blots were hybridized to a *Hin*dIII–*Eco*RI cDNA fragment, nt 833–1733, of the BR1-VDAC cDNA in buffers containing 50% (vol/vol) formamide at 42°C. Final washes were performed in 0.2× standard saline citrate/0.01% SDS at 68°C for 2 hr.

Electrophysiology and Blocking Experiments. Patch clamp experiments were performed using the inside-out or outside-out configuration. Pipettes were pulled from borosilicate glass capillaries showing a resistance of 12–16 MΩ. After digitizing data were stored on video tape. For analysis and records shown in Fig. 5, data were filtered at 0.5 kHz. The antibody was applied through a capillary tube by using a peristaltic pump (after diluting 1:10 to 2.5 μg/ml). Excised patches were moved into the mouth of the tube. Pipette and bath solutions are given in Fig. 5.

N-terminal peptide was diluted in dimethyl sulfoxide to a stock concentration of 250 μg/ml. For preabsorption, the antibody was added to this stock solution to give a final concentration for mAb2 of 1.5 μg/ml and for N-terminal peptide of 100 μg/ml.

RESULTS AND DISCUSSION

Isolation and Cloning of the BR1-VDAC. Detergent-resistant membrane fractions from brain tissues were isolated by a two-step procedure including myelin separation (17) and

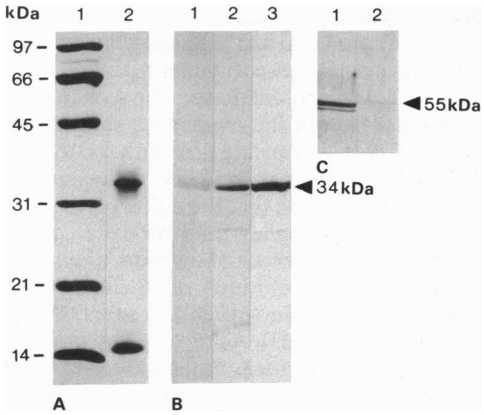

FIG. 1. Isolation and immunochemical identification of BR1-VDAC in detergent-resistant membrane fractions from bovine brain. (*A*) Lanes: 1, marker lane of molecular mass standards; 2, SDS/PAGE of the detergent-resistant membrane preparation. (*B*) Western blots using mAb1 (lane 1), pAb1 (lane 2), and mAb2 (lane 3). The faint band at ≈25 kDa (lanes 2 and 3) is probably a degradation product of the BR-VDAC protein, since freshly prepared cultured astrocytes (see Fig. 4) did not have a low molecular mass band under identical conditions. (*C*) Western blots with an anti-mitochondrial ATPase β subunit antibody of a bovine brain mitochondrial preparation (lane 1) and of a detergent-resistant membrane fraction (lane 2).

subsequent treatment with a nonionic detergent, *N*-lauroylsarcosine, followed by a two-step discontinuous sucrose gradient. Separation of the proteins collected at the 35–49% interface on one-dimensional SDS/polyacrylamide gels showed two dominant bands, at 16 kDa and 34 kDa (Fig. 1*A*). The 34-kDa band, which is in the range of the mi-VDAC, was excised from the gel and microsequenced (11). Four peptides were obtained after tryptic digestion (Fig. 2) that proved to be identical to segments of the recently published sequence of the HL-VDAC. Two degenerate oligonucleotides were synthesized matching the C-terminal and the N-terminal amino acids of peptide 1. By using these primers and poly(A)$^+$-selected RNA from bovine brain, a cDNA fragment coding for the entire peptide 1 was amplified by reverse PCR. This fragment served as a probe to screen a λZAPII cDNA library from bovine brain. A single clone with a 1.7-kbp cDNA insert was obtained. Fig. 2 shows the nucleotide and predicted amino acid sequence. This bovine clone appears to encode the same protein as the human HL-VDAC with 277 of 282 aa being identical.

Northern blot analyses were performed under high-stringency conditions using a cDNA probe containing almost the entire 3′ noncoding sequence to avoid cross-hybridization to other porin mRNAs. This probe hybridizes to a single gene under high-stringency conditions, as verified by Southern

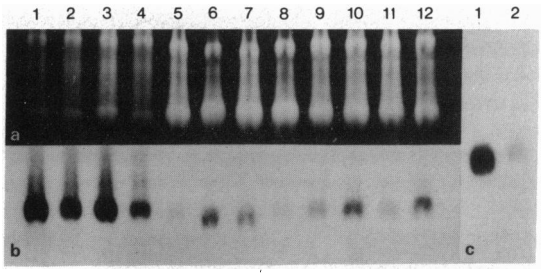

FIG. 3. Analysis of tissue-specific expression of the BR1-VDAC in bovine brain (lanes 1–4) and in diverse nervous and nonnervous tissues of rat (lanes 5–12). (*A*) Ethidium bromide staining of total RNA (10 μg per lane) from the following sources. Lanes: 1, frontal cortex; 2, hippocampus; 3, cerebellum; 4, brain stem; 5, frontal cortex; 6, cerebellum; 7, brain stem; 8, ovary; 9, kidney; 10, heart; 11, brain (total); 12, skeletal muscle. (*B*) Corresponding Northern blots (exposure time, 21 hr at 20°C). (*C*) Northern blots of total rat brain RNA (lane 1) compared to rat liver RNA (lane 2). Exposure times for both blots were identical (22 hr at −80°C).

blot analysis of bovine genomic DNA (data not shown). Thus we can rule out that the hybridization signal results from cross-hybridization to a number of different VDAC mRNAs. Results from Northern blots (Fig. 3) demonstrate that BR1-VDAC mRNA is widely distributed in bovine and rodent central nervous systems. Regional differences in mRNA levels between both species especially in the frontal cortex (compare Fig. 3*b*, lanes 1 and 5) are less likely to be due to a lack of homology, especially when the high degree of conservation between the human HL-VDAC and the bovine BR1-VDAC is considered, but rather are due to species-specific variations of local expression.

The same 3′ cDNA probe was also used to examine the expression of BR1-VDAC in various nonnervous rodent tissues. As indicated in Fig. 3*b*, there is a variable expression of BR1-VDAC in peripheral organs with high-level expression in skeletal and cardiac muscles (Fig. 3*b*, lanes 10 and 12). Interestingly, liver, a rich source of mitochondrial porins, showed a low level of BR1-VDAC mRNA expression compared to total brain (Fig. 3*c*).

***In Situ* Localization of the BR1-VDAC.** To obtain information on the cellular distribution of the BR1-VDAC, we used the electroeluted 34-kDa protein to produce a mAb (mAb1). In addition, a site-specific pAb (pAb1), directed to a peptide fragment (aa 156–174) of the cloned protein, was also applied. Both antibodies recognized a band of ≈34 kDa on immunoblots of bovine brain plasma membrane preparations (Fig. 1*B*) with mAb1 being less efficient on Western blots than pAb1. A further mAb (mAb2) against the N terminus of HL-VDAC (aa 1–19) gave a strong signal at the appropriate position (Fig. 1*B*). To exclude the possibility that our 34-kDa

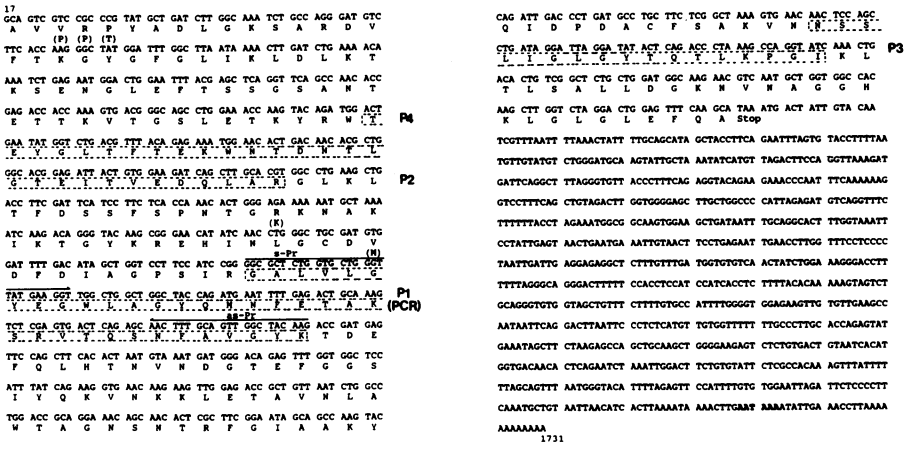

FIG. 2. Nucleic acid and amino acid sequences of the BR1-VDAC and its predicted open reading frame. Position 17 represents the first coding residue after the linker. Boxed residues represent peptide sequences P1–P4 obtained by tryptic digestion and subsequent Edman degradation. Arrows above P1 indicate the location of sense and antisense primers used for the PCR amplification. Amino acids differing in the human HL-VDAC are shown in brackets below the respective residues. A polyadenylylation signal at nt 1701–1706 is in boldface type.

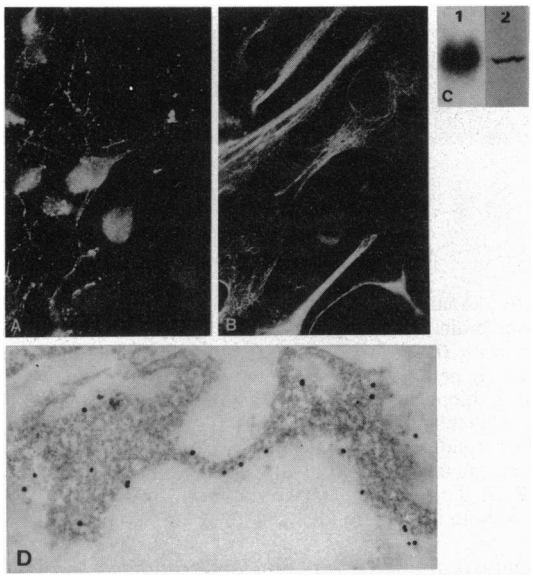

FIG. 4. Detection of BR1-VDAC expression in cultured astrocytes. (*A* and *B*) Double immunofluorescence demonstrating the presence of the BR1-VDAC in cultured subconfluent astrocytes using mAb2 and an anti-glial fibrillary acidic protein (GFAP) pAb (Sigma) in identical sets of cells. (*A*) Detection of BR1-VDAC immunoreactivity using antibody mAb2 (secondary antibody, fluorescein isothiocyanate-conjugated anti-mouse IgG). (*B*) Same set of cells counterstained with anti-GFAP as an astrocytic marker (secondary antibody, tetramethylrhodamine isothiocyanate-conjugated anti-rabbit IgG). (*C*) Northern (lane 1) and Western (lane 2) blot analyses of BR1-VDAC expression in cultured astrocytes. (*D*) *In vitro* immunogold labeling of cultured astrocytes (exposure time to mAb2, 15 min). The gold particles are primarily confined to the outer face of the plasma membrane, indicating plasma membrane association of the BR1-VDAC.

protein was derived from contaminating mitochondrial outer membranes, we analyzed the detergent-resistant plasma membrane fraction for the presence of a mitochondrial marker. As indicated in Fig. 1*C*, lanes 1 and 2, there is only minute reactivity for mitochondrial F_1-ATPase (β subunit) in comparison to brain mitochondrial preparations, rendering a mitochondrial source of the 34-kDa protein unlikely.

In situ immunolocalization of BR1-VDAC with both mAbs (mAb1 and mAb2) and the pAbs showed significant immunolabeling in astrocytes. In addition, mAb1 extensively stained hippocampal neurons of the dentate gyrus and of Purkinje cells in the cerebellar cortex (data not shown). Immunofluorescence labeling of cultured astrocytes showed well-defined plasmalemmal-bound immunoreactivity for mAb1 and mAb2 and also some intracytoplasmatic immunoreactivity (Fig. 4 *A* and *B*). Northern and Western blots of cultured astrocytes substantiated a high level of BR1-VDAC expression (Fig. 4*C*). For a better definition of the plasmalemmal association of the BR1-VDAC, we used mAb2 for *in vivo* staining. This antibody has been shown to recognize the N terminus of the HL-VDAC (19), which is highly homologous with our BR1-VDAC at the predicted amino acid level. Perhaps the N terminus of the HL-VDAC forms an extracytoplasmatic α-helix accessible for antibody detection (22). Fig. 4*D* shows *in vivo* immunogold labeling of the outer surface of cultured astrocytes exposed for 15 min to mAb2. The gold particles were found to be primarily confined to the cell surface, indicative for an extracytoplasmatic localization of the N-terminal portion of the BR1-VDAC.

VDAC-Like Activity in Astrocytes Can Be Blocked by the N-Terminal Specific Antibody. Single-channel recordings from patch-clamped cultured astrocytes revealed, in 52 of 81 cases, activity of a large-conductance channel (Fig. 5*A*). The channels opened at low transmembrane voltage from approximately −30 mV to +20 mV. Single-channel current vs. voltage curves from 15 experiments yielded a mean slope conductance of 434 pS. Increased ion concentration (500 mM) at the inner side of the membrane shifted the reversal potential by +3.5 mV, causing a negative current at 0 mV (Fig. 5*B*). This current can only be a net anion outward current. We thus conclude that the anion permeability of the BR1-VDAC is clearly higher than its cation permeability. Anion permeability is lower, however, than that of the Cl^--selective channel of cultured rat skeletal muscle where 400 mM KCl at the inside shifted the reversal potential to values of about +17 mV (23). As reported by others (8, 9), the large-conductance activity was found only in excised patches after exposure to the bath solution for 5–10 min. This procedure was necessary regardless of the patch configuration. The delay in the appearance of channel activity suggests that regulative factors are involved in preventing the channel from entering the large conductance state under cell-attached patch-clamp conditions.

To further test whether the large-conductance activity is due to the presence of BR1-VDAC, we performed blocking experiments using both antibodies against BR1-VDAC (mAb1 and pAb1) and the N-terminal-specific anti-HL-VDAC antibody (mAb2). mAb2, whose antigenic epitope resides on the extracytoplasmic side, blocked the large-conductance activity completely when the patch was super-

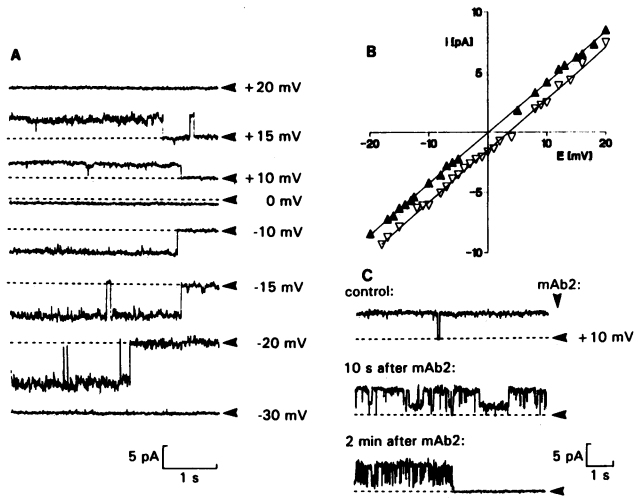

FIG. 5. VDAC-like channel activity in patch clamp recordings is blocked by the antibody mAb2. (*A*) Demonstration of voltage dependence. Current traces from excised inside-out patches showing activity with lower (−28 mV) and upper (+18 mV) threshold. Dashed lines indicate the closed level. Buffer on outside of membrane: 135 mM NaCl/3 mM KCl/5 mM Hepes·NaOH/1.9 mM $CaCl_2$. Buffer on inside of membrane: 28 mM NaCl/110 mM KCl/5 mM Hepes·NaOH/1.9 mM CaCl. Both buffers are at pH 7.4 and 22°C. (*B*) Demonstration of Cl^- preference. Current–voltage relationship in a symmetrical (143 mM) KCl solution (▲) and after exchanging the internal solution with a 500 mM KCl solution (▽). The reversal potential is shifted by +3.5 mV, indicating Cl^- preference of the channel. The symbols represent mean values of four experiments from different cells. Straight lines illustrate single-channel conductances of 425 pS (▲) and 431 pS (▽). Both solutions contained 10 mM Hepes·KOH, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 11 mM glucose at pH 7.4. (*C*) Block by the antibody. Control, before application of mAb2 (19), channel almost continuously open; 10 s after adding mAb2 (2.5 μg/ml), closures to different levels with identical voltage dependence indicate presence of two substates and partial block by mAb2; 2 min after adding mAb2, continuous closure. Outside-out patch and holding potential of +10 mV. Solutions: outside (NaCl solution), 134 mM NaCl/6 mM KCl/10 mM Hepes·NaOH/1.2 mM CaCl/1.2 mM MgCl/11 mM glucose; inside, 139 mM KF/4 mM KCl/5 mM EGTA/10 Hepes·KOH.

fused with 0.1 M Hepes plus antibody at ≈2.5 μg/ml (Fig. 5*C*). Consistent with the suggested topological orientation of the HL-VDAC (15, 24) is the observation that blocking was only achieved in the outside-out configuration. Ten seconds after adding the antibody to the patch solution, closures to different levels with identical voltage dependence occurred, indicating the presence of two substates and partial blockage by mAb2. Continuous closure was achieved 2 min after antibody exposure. The blockage was stable over a 10-min period (maximal measuring time) without spontaneous recovery. Specificity of the block by mAb2 was verified by preabsorbing the antibody with an N-terminal peptide [aa 1–19 of the HL-VDAC (19)]. Preabsorption completely prevented the blocking effect of mAb2 (data not shown). mAb1, which was raised to the entire electroeluted BR1-VDAC protein and was, therefore, not further characterized with respect to its epitope specificity, did not show blocking effects comparable with mAb2. pAb1, which recognizes the 19-aa internal sequence of BR1-VDAC (see above), was also negative in the blocking experiments. The efficiency of mAb2 strongly suggests that the N terminus is essential for the gating mechanism of the BR1-VDAC.

Cloning of the BR1-VDAC from bovine brain, which according to its predicted amino acid sequence is identical to the human lymphocyte porin (HL-VDAC), and identification of the BR1-VDAC in astrocytic plasma membranes help to explain the cryptic behavior of the astrocytic membrane. A large-conductance anion channel has been frequently described in astrocytes and we have stressed the strong similarity of the basic functional properties of this channel and that of mi-VDAC-type channels (see Introduction). By immunocytochemical studies, HL-VDAC has been localized in the plasma membrane of human lymphocytes (22). The same antibody (mAb2) was exploited in our blocking experiments. These data and our immunolocalization experiments strongly suggest that the molecular basis of the large-conductance anion channel of astrocytes is the plm-VDAC. The paradigm that VDACs are exclusively localized in the outer mitochondrial membrane has also been questioned by the recent cloning of another member of the VDAC family that copurifies with the $GABA_A$ receptor complex (16). This protein is 70% identical to our BR1-VDAC at the predicted amino acid level. Because of its Cl^- conductance, it was suggested that this brain-derived VDAC2 (BR2-VDAC) could modify the Cl^- gradient around its associated $GABA_A$ receptor and modulate the GABA-stimulated Cl^- transport. Although *in situ* localization of the BR2-VDAC is lacking, its firm association with the $GABA_A$ receptor complex suggests a plasmalemmal association. The finding that mAb1 showed intensive immunostaining of hippocampal granular cells and Purkinje cells in the cerebellum is consistent with this interpretation. The high degree of homology between BR1-VDAC and BR2-VDAC suggests that this labeling might result from cross-reactivity of mAb1 with the BR2-VDAC.

Our Northern blot data indicate that BR1-VDAC is also expressed at different levels in a variety of nonnervous tissues. These findings are in agreement with immunohistochemical data documenting HL-VDAC immunoreactivity in several peripheral tissues (19, 25). Consistent with this notion, numerous reports have shown the presence of a large-conductance anion channel in a variety of nonnervous cells—i.e., skeletal muscle (23), apical membranes of Cl^--secretory epithelium (26), and FO myeloma cells of mice (27). In view of this diversity, a uniform function of plm-VDACs seems unlikely. It has therefore been suggested that plm-VDACs are able to complex with different channel and/or receptor complexes (16, 28). The specific functional properties of such a complex may then depend on the particular molecular formula of its constituents. The presence of BR1-VDAC in the astrocytic membrane may be of exceptional importance regarding volume regulation in the brain. In this context, the observation of an increased large nonspecific conductance in swollen astrocytes treated with hypotonic solutions reported by Kimmelberg *et al.* (29) is relevant. It seems reasonable to propose that the swelling process changes the concentration and/or association of regulatory molecules that keep the high-conductance state of BR1-VDAC suppressed, thereby allowing the uncontrolled exchange of water and electrolytes. Further identification of the molecular background of this hypothesized regulative mechanism of BR1-VDAC should yield insights into the physiological channel-gating mechanism and related malfunctioning of this class of channels in the brain and elsewhere.

Note Added in Proof. While this manuscript was in preparation Blachly-Dyson *et al.* (30) reported on the cloning of two isoforms of human voltage-dependent anion channels and their expression in yeast mutants. Their clone HVDAC1 is the human homologue of our BR1-VDAC.

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