Molecular cloning and expression of a cyclic AMP-activated chloride conductance regulator: A novel ATP-binding cassette transporter

(basolateral membrane/chloride channel/kidney)

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ABSTRACT Cystic fibrosis transmembrane conductance regulator (CFTR) is an ATP-regulated, cAMP-activated chloride channel located in the apical membrane of many epithelial secretory cells. Here we report cloning of a cAMP-activated epithelial basolateral chloride conductance regulator (EBCR) that appears to be a basolateral CFTR counterpart. This novel chloride channel or regulator shows 49% identity with multidrug resistance-associated protein (MRP) and 29% identity with CFTR. On expression in *Xenopus* oocytes, EBCR confers a cAMP-activated chloride conductance that is inhibited by the chloride channel blockers niflumic acid, 5-nitro-2-(3-phenylpropylamine)benzoic acid, and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid. Northern blot analysis reveals high expression in small intestine, kidney, and liver. In kidney, immunohistochemistry shows a conspicuous basolateral localization mainly in the thick ascending limb of Henle's loop, distal convoluted tubules and to a lesser extent connecting tubules. These data suggest that in the kidney EBCR is involved in hormone-regulated chloride reabsorption.

Chloride reabsorption in the renal thick ascending limb of Henle's loop (TAL) is regulated by various hormones that in general act by means of a cAMP-dependent pathway (1). During this process, the $Na^+/K^+/2Cl^-$ cotransporter in the apical membrane is activated, which elevates the intracellular chloride concentration above equilibrium values. The channels involved in basolateral chloride efflux are not yet identified at the molecular level. Planar lipid bilayer fusion experiments, using membrane vesicles from outer medulla, have shown that basolateral chloride channels can be activated by increased intracellular chloride concentration or more directly, by means of a cAMP-dependent action (2, 3). In patch-clamp experiments on cells from cortical TAL, two chloride conductances of 45 and 7–9 pS have been detected (4, 5) that are both activated by protein kinase A. The smallest channel has characteristics that are similar to those described for the cystic fibrosis transmembrane conductance regulator (CFTR), which is a cAMP-activated chloride channel (6, 7) in the apical membrane of many chloride-secreting cells (8).

Recently, several renal voltage-gated chloride channels of the ClC gene family (9, 10) have been identified at the molecular level. Whereas ClC-K1 is restricted to thin ascending limb of Henle's loop and the inner medulla collecting duct (11), highly homologous rClC-K1 and rClC-K2 show different expression profiles (12). The latter is expressed in every segment of the nephron, but rClC-K1 is specifically expressed in late proximal tubules (S3), cortical TAL, and distal convoluted tubules. In humans two related chloride channels have

been cloned, hClC-ka and hClC-kb (12), which are, based on their identity, not considered strict homologues of either rClC-K1 or rClC-K2 (12). By a different approach two chloride channels have been cloned, rat ClC-K2L and ClC-K2S, which are expressed in TAL and collecting ducts (13). However, none of these chloride channels has been shown to be activated by cAMP.

In this study we report the cloning, expression, and functional characterization of an epithelial basolateral chloride conductance regulator (EBCR), a novel protein belonging to the superfamily of ATP-binding cassette (ABC) transporters. This protein shows significant identity to CFTR. The functional activation by cAMP and its conspicuous localization to the basolateral domain in epithelial cells of nephron segments involved in cAMP-dependent chloride reabsorption, suggest that EBCR is involved in chloride reabsorption and could well be a basolateral counterpart of CFTR.

EXPERIMENTAL PROCEDURES

Poly(A)$^+$ RNA Isolation. From rabbit skeletal muscle, lung, brain, heart, spleen, stomach, and epithelial cells scraped from colon, duodenum, jejunum, and ileum, total RNA was isolated by guanidinium isothiocyanate/phenol/chlorofrom extraction (14). Subsequently, poly(A)$^+$ RNA was isolated using an oligo(dT)- cellulose column (type 3; Collaborative Biomedical Products, Bedford, MA) as described (15).

Northern Blot Analysis. Equal amounts of poly(A)$^+$ RNA were run on a formaldehyde/agarose gel and blotted onto a nylon membrane (Biotrace, Gelman). The Northern blot was hybridized with a DNA probe containing 346 bp of 5′ untranslated region and 810 bp of the EBCR coding sequence. For normalization, the blot was hybridized with a ^{32}P-labeled 1250-bp *Pst*I fragment from rat glyceraldehyde-3-phosphate dehydrogenase DNA (16). DNA fragments used as probes were labeled with $[\alpha^{32}P]dCTP$ by random priming (17). Hybridization conditions were 250 mM PO_4^{3-}, pH 7.2, 7% SDS, 1 mM EDTA at 65°C and final washing was performed twice in 40 mM PO_4^{3-}, pH 7.2, 0.1% SDS, 1m MEDTA at 65°C for 20 min.

Reverse Transcription (RT)–PCR and Subcloning. RT-PCR was performed according to standard procedures (15). First-strand cDNA synthesis was carried out with 500 ng of

poly(A)$^+$ RNA from skeletal muscle or duodenum epithelial cells and 100 pmol of random hexamers (Promega). PCR was performed on 1 μl of RT-mix in 50 μl containing 1× PCR buffer (Promega; 50 mM KCl/10 mM Tris·HCl, pH 9.0 at 25°C, 0.1% Triton X-100, 250 μM dNTPs, 2.5 mM MgCl$_2$, and 100 pmol of PCR primers Cc1 and Cc2 (18). Two rounds of 35 PCR cycles were done as follows: 1 min at 94°C, 2 min at 50°C, and 3 min at 72°C. After completion fragment obtained by RT-PCR was phosphorylated and subcloned into pBluescript KS (Stratagene) digested with EcoRV.

Isolation of EBCR cDNA. A rabbit ileum mucosal Lambda ZAP II cDNA library was screened using the obtained PCR fragment, labeled with [α^{32}P]dCTP by random priming (17). Hybridization was performed in 250 mM PO$_4^{3-}$, pH 7.2, 7% SDS, 1 mM EDTA at 55°C. Final washing conditions were 100 mM PO$_4^{3-}$, pH 7.2, 0.1% SDS, 1 mM EDTA at 55°C. From 12 positive clones the inserts were excised using the R408 helper phage. One positive clone, containing an insert of approximately 3.8 kb, was selected (kim-d41). A 562-bp fragment from the 3' end of that clone was used to rescreen the same library. Nine positives were picked and one clone containing an insert of approximately 3.1 kb was isolated (kim-m1). The coding sequence of the two inserts and some 5' and 3' untranslated regions were sequenced on both strands (19). From the obtained open reading frame, a protein of 1564 aa, which we named EBCR, could be deduced. The nucleotide sequence reported in this paper has been deposited in the GenBank database under accession no. Z49144.

Immunohistochemistry. Rabbits were immunized with a synthetic peptide corresponding to amino acid residues 2–16 of the amino terminus of EBCR conjugated to keyhole limpet hemocyanin (Calbiochem) (20). Antisera were screened by ELISA and affinity purified. Although similar results were obtained using rabbit tissue sections, due to background staining from peroxidase-conjugated goat anti-rabbit Ig, results from rat tissues are presented. A kidney was removed from a 200-g male Wistar rat and prepared for immunoperoxidase staining as described previously, with minor modifications (21). Briefly, 5-μm sections were treated with 3% H$_2$O$_2$ and 100% methanol (1:1) for 15 min, washed, and incubated with 0.5% Triton X-100 for 15 min and washed again. Then sections were preincubated with 20% goat serum in Tris-buffered saline (TBS) for 30 min. Subsequently, sections were incubated overnight at 4°C with an 1:100 dilution of affinity-purified EBCR antibodies. After thorough washing with TBS, the sections were incubated with peroxidase-conjugated goat anti-rabbit Ig (1:100) for 60 min and rinsed three times with TBS. Peroxidase activity was revealed with diaminobenzidine in 0.03% H$_2$O$_2$ in TBS. The sections were counterstained as described (21). The immunolocalization was confirmed with double immunofluoresence staining (data not shown) using either antibodies against Tamm–Horsfall protein specific for the TAL (22) or R2G9, a monoclonal antibody specific for connecting tubules and cortical collecting ducts (23). Sections that were incubated with preimmune rabbit serum, EBCR antiserum preadsorbed with the EBCR synthetic peptide, or secondary antibody only were all negative (data not shown). All chemicals and conjugated antibodies were purchased from Sigma, except Tamm–Horsfall antibody, which was purchased from Organon Teknika–Cappel.

Voltage Clamp. For functional studies in Xenopus laevis oocytes an expression construct was made. First, from the kim-d41 clone a 2428-bp XmnI–XbaI fragment, including 2344 bp of the coding sequence containing the ATG start codon, was isolated and ligated into pBluescript KS (Stratagene) digested with SmaI and XbaI (clone pBS-EBCR-XXD41). Second, from the kim-m1 clone a 2747-bp XbaI fragment, containing the additional 2348 bp of the coding sequence, was isolated and ligated in frame into the XbaI site of pBS-EBCR-XXD41. The correct orientation was checked. The total

construct, named pBS-EBCR-T3, contained the complete 4692-bp open reading frame of EBCR with 84 bp and 369 bp of 5' and 3' untranslated sequences, respectively. For cRNA synthesis pBS-EBCR-T3 was digested with SmaI, isolated and transcribed with T3 RNA polymerase (Promega) as described (24).

Dissection of X. laevis ovaries, collection, and handling of oocytes have been described in detail (25). Voltage-clamp experiments were performed on oocytes injected with 10 ng of cRNA per oocyte. Two-electrode voltage-clamp recordings were done at room temperature, 3 to 8 days after injection, using a MacLab D/A converter and software for data acquisition and analysis (AD Instruments, Castle Hill, Australia). Currents induced by the expression of EBCR were recorded during voltage ramps (from −80 mV to 50 mV for 2 s) in the absence or after superfusion with 3-isobutyl-1-methylxanthine (IBMX; 1 mM) and forskolin (10 μM) for 15 min. The external control solution (ND 96) contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, 5 mM Hepes titrated with NaOH to pH 7.4. For some experiments all external chloride was replaced by iodide or bromide. The flow rate of the superfusion was 20 ml/min and a complete exchange of the bath solution was reached within about 10 s. Chemicals used were 5-nitro-2-(3-phenylpropylamine)benzoic acid (NPPB, gift from R. Greger, University, Freiburg, Germany), 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), niflumic acid, IBMX, and forskolin (Sigma). All data are given as means ± SEM where N indicates the number of experiments. All statistics were done using a paired Student's t test. The conductance in oocytes expressing EBCR varied from 2- to 10-fold, depending on the time period after cRNA injection and on the batch of oocytes (from different animals). All sets of experiments have been repeated at least once. In all repetitions, similar data were obtained.

Construction of Phylogeny Tree. A PILEUP multiple sequence alignment was performed with a gap weight of 3.0 and a gap weight length of 0.1. The alignment was used in a phylogeny program using the Fitch–Margoliash matrix calculation method using length-independent gap exclusion, no end gaps, gap penalty at 99.99, and no correction for underestimation of mutation distances. The final tree was made using global rearrangements and randomized input order and was rooted by using a prokaryotic ABC transporter (Escherichia coli hemolysin transporter: HlyB) as an outgroup.

RESULTS AND DISCUSSION

In renal epithelia, several α_1-subunit transcripts coding for L-type calcium channels have been successfully amplified using nondegenerate PCR primer sets (18). In the search for similar transcripts in the small intestine, an RT-PCR reaction was done on rabbit duodenum poly(A)$^+$ RNA with one of these primer sets. The only fragment obtained was a smaller one than anticipated (207 vs. 330 bp). Subsequent cloning and sequence analysis of this fragment, and comparison of the longest open reading frame with database sequences, revealed an approximately 49% identity with a human multidrug resistance-associated protein (MRP) (26, 27), a protein that belongs to the superfamily of ABC transporters (28).

To obtain a full-length cDNA clone, a rabbit ileum mucosal cDNA library was screened with the obtained PCR fragment and a clone was isolated containing only the 5' part of a cDNA. Rescreening of the same library with a labeled fragment, derived from the 3' end of this clone, identified another clone that contained additional 3' sequences. The cDNA inserts, which comprise approximately 6 kb, were subjected to sequence analysis. The longest open reading frame obtained starts with a Kozak consensus (29) sequence CCAGGAATCATGC and is preceded by several in-frame stop codons. It encodes a protein of 1564 aa with a predicted molecular mass

of 175 kDa (Fig. 1*A*). This protein, named EBCR, has several consensus sequences for N-glycosylation and protein kinase C dependent phosphorylation (Fig. 1), which suggests that EBCR is regulated by means of phosphorylation. Furthermore, it contains two nucleotide binding folds (NBFs; aa 648–825 and aa 1311–1497), each containing Walker A and B motifs (30) and a C domain (31), which are characteristics of ABC transporters (28).

Hydropathy analysis of EBCR predicts 12 transmembrane regions (TMRs; Fig. 1*A*), which is in agreement with other ABC transporters (28). Based on this analysis a proposed topology for EBCR is shown (Fig. 1*B*) in which the amino- and carboxyl-termini and both NBFs are located at the cytosolic face. This predicted topology for EBCR is similar to the one reported for MRP (26).

Northern blot analysis was performed to determine the tissues that express EBCR. High expression of a 6-kb transcript was detected in rabbit small intestine (duodenum, jejunum,

ileum), liver, and kidney, whereas EBCR transcript was undetectable in distal colon, stomach, spleen, heart, brain, lung, and skeletal muscle (Fig. 2*A*). It can be concluded from the size of the detected transcript that the combined inserts of the two clones represent a nearly full-length EBCR cDNA.

For immunohistochemistry, antibodies were raised against amino acid residues 2–16 of the amino terminus of EBCR and affinity-purified. In kidney, EBCR was mainly detected at the basolateral side of cells from the TAL, distal convoluted tubules, and connecting tubules, but a low expression was also detected in late proximal tubules (S3) (Fig. 2*B*). In duodenum, jejunum, and ileum, immunoreactivity was restricted to epithelial cells lining the villus tip, whereas crypt cells were negative (data not shown). In liver, the protein was present in hepatocytes predominantly near the central venules (data not shown).

According to the phylogeny tree (Fig. 3), the evolutionary distance provides conclusive evidence that EBCR is a novel
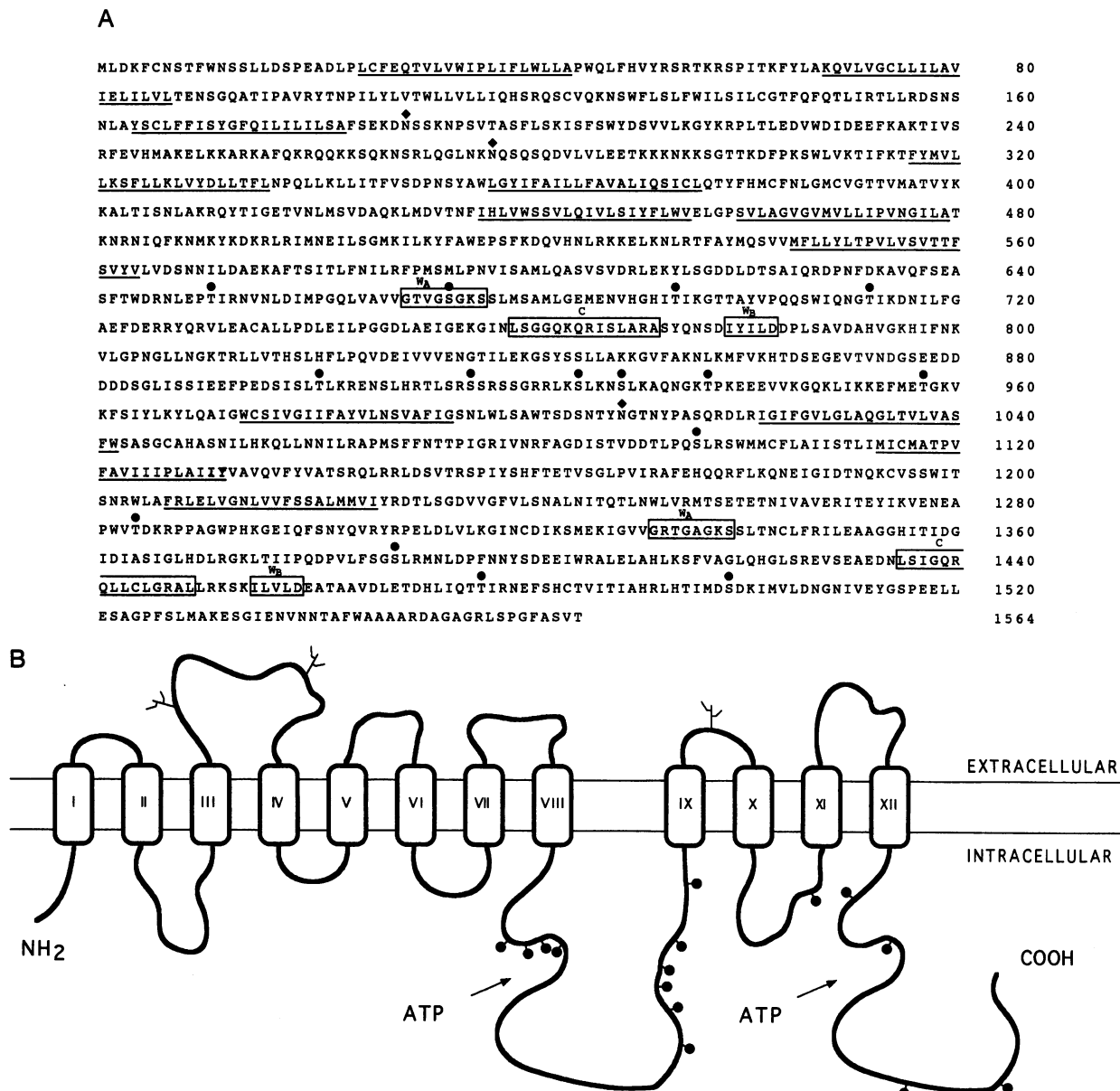
A

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MLDKFCNSTFWNSSLLDSPEADLPLCFEQTVLVWIPLIFLWLLAPWQLFHVYRSRTKRSPITKFYLAKQVLVGCLLILAV      80

IELILVLTENSGQATIPAVRYTNPILYLVTWLLVLLIQHSRQSCVQKNSWFLSLFWILSILCGTFQFQTLIRTLLRDSNS     160

NLAYSCLFFISYGFQILILILSAFSEKDNSSKNPSVTASFLSKISFSWYDSVVLKGYKRPLTLEDVWDIDEEFKAKTIVS     240

RFEVHMAKELKKARKAFQKRQQKKSQKNSRLQGLNKNQSQSQDVLVLEETKKKNKKSGTTKDFPKSWLVKTIFKTFYMVL     320

LKSFLLKLVYDLLTFLNPQLLKLLITFVSDPNSYAWLGYIFAILLFAVALIQSICLQTYFHMCFNLGMCVGTTVMATVYK     400

KALTISNLAKRQYTIGETVNLMSVDAQKLMDVTNFIHLVWSSVLQIVLSIYFLWVELGPSVLAGVGVMVLLIPVNGILAT     480

KNRNIQFKNMKYKDKRLRIMNEILSGMKILKYFAWEPSFKDQVHNLRKKELKNLRTFAYMQSVVMFLLYLTPVLVSVTTF     560

SVYVLVDSNNILDAEKAFTSITLFNILRFPMSMLPNVISAMLQASVSVDRLEKYLSGDDLDTSAIQRDPNFDKAVQFSEA     640

SFTWDRNLEPTIRNVNLDIMPGQLVAVVGTVGSGKSSLMSAMLGEMENVHGHITIKGTTAYVPQQSWIQNGTIKDNILFG     720

AEFDERRYQRVLEACALLPDLEILPGGDLAEIGEKGINLSGGQKQRISLARASYQNSDIYILDDPLSAVDAHVGKHIFNK     800

VLGPNGLLNGKTRLLVTHSLHFLPQVDEIVVVENGTILEKGSYSSLLAKKGVFAKNLKMFVKHTDSEGEVTVNDGSEEDD     880

DDDSGLISSIEEFPEDSISLTLKRENSLHRTLSRSSRSSGRRLKSLKNSLKAQNGKTPKEEEVVKGQKLIKKEFMETGKV     960

KFSIYLKYLQAIGWCSIVGIIFAYVLNSVAFIGSNLWLSAWTSDSNTYNGTNYPASQRDLRIGIFGVLGLAQGLTVLVAS    1040

FWSASGCAHASNILHKQLLNNILRAPMSFFNTTPIGRIVNRFAGDISTVDDTLPQSLRSWMMCFLAIISTLIMICMATPV    1120

FAVIIIPLAIIYVAVQVFYVATSRQLRRLDSVTRSPIYSHFTETVSGLPVIRAFEHQQRFLKQNEIGIDTNQKCVSSWIT    1200

SNRWLAFRLELVGNLVVFSSALMMVIYRDTLSGDVVGFVLSNALNITQTLNWLVRMTSETETNIVAVERITEYIKVENEA    1280

PWVTDKRPPAGWPHKGEIQFSNYQVRYRPELDLVLKGINCDIKSMEKIGVVGRTGAGKSSLTNCLFRILEAAGGHITIDG    1360

IDIASIGLHDLRGKLTIIPQDPVLFSGSLRMNLDPFNNYSDEEIWRALELAHLKSFVAGLQHGLSREVSEAEDNLSIGQR    1440

QLLCLGRALLRKSKILVLDEATAAVDLETDHLIQTTIRNEFSHCTVITIAHRLHTIMDSDKIMVLDNGNIVEYGSPEELL    1520

ESAGPFSLMAKESGIENVNNTAFWAAAARDAGAGRLSPGFASVT                                       1564
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B

FIG. 1. Sequence and proposed topology of rabbit EBCR. (*A*) Sequence of EBCR in which predicted TMRs are underlined. The Walker A and B domains (30) and C domain (31) are boxed and indicated (w_a, w_b, c). (*B*) Proposed topology of EBCR as determined according to the algorithm of Kyte and Doolittle (32). The TMRs are numbered I to XII. The two NBFs involved in ATP binding and hydrolysis (→ ATP) are indicated. Potential protein kinase C phosphorylation (●) and N-glycosylation (♦) sites are shown in *A* and *B*.
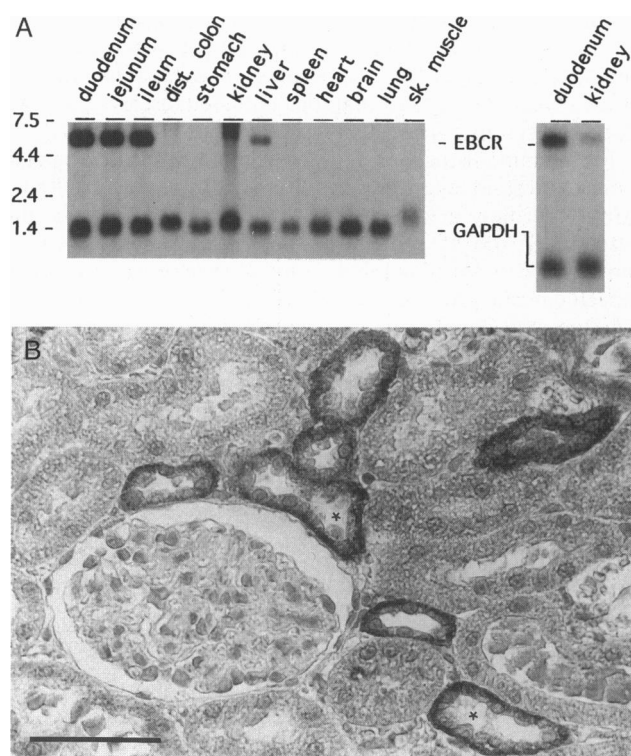
FIG. 2. Expression of EBCR. (*A*) Northern blot analysis of EBCR. In the duodenum lane, 3 μg of poly(A)⁺ RNA was loaded and other samples were adjusted to the glyceraldehyde-3-phosphate dehydrogenase signal of this sample. Sizes of RNA are indicated in kb. (*B*) Immunohistochemistry of EBCR in rat kidney. TAL is indicated by asterisks. (Bar = 50 μm.)

FIG. 3. Phylogeny tree of EBCR related sequences.

member of the ABC superfamily of transporters (28). In addition, EBCR appears more closely related to CFTR than to the multidrug resistance proteins.

In view of the conspicuous localization of EBCR in the basolateral domain of nephron segments involved in chloride reabsorption (1, 2), and the fact that CFTR is a chloride channel (6, 7), we hypothesize that EBCR has a function in chloride reabsorption. Therefore, *X. laevis* oocytes were injected with cRNA encoding EBCR. Under control conditions the conductances of oocytes injected with EBCR cRNA (2.69 ± 0.47 μS, $n = 12$) or water (2.55 ± 0.32 μS, $n = 5$) were not significantly different. Superfusion with forskolin and IBMX resulted, after subsequent washout, in a persistent increase of the conductance in EBCR-expressing oocytes, but not in water-injected oocytes (Fig. 4*A* and *B*). After forskolin–IBMX treatment the conductance of EBCR-expressing oocytes was 4.31 ± 0.64 μS ($n = 12$) and in water-injected controls 2.29 ± 0.26 μS ($n = 5$), which denotes an increase of 80 ± 27%. The reversal potential in EBCR-expressing oocytes was shifted from −45.3 ± 6.9 mV to −37.0 ± 7.1 mV after forskolin–IBMX treatment ($p < 0.01$, $n = 7$). This shift toward the equilibrium potential for chloride points to a forskolin–IBMX-induced chloride conductance. In addition, the forskolin–IBMX-induced conductance was also permeable for the halides iodide and bromide (Fig. 4*C*). Replacement of chloride with iodide resulted in a statistically significant increase in the conductance by 31.0 ± 4.4% ($n = 6$, Fig. 4*D*). The reversal potential of the conductance was significantly shifted from −31.7 ± 1.3 mV ($n = 6$) in ND96 solution to −36.0 ± 3.0 mV ($n = 3$) at 100 mM iodide. Replacement of chloride by bromide did not significantly alter the conductance or the reversal potential ($n = 3$). The chloride channel blockers niflumic acid, NPPB, and DIDS (all at 100 μM) inhibited the conductance in EBCR-expressing oocytes after forskolin–IBMX treatment by
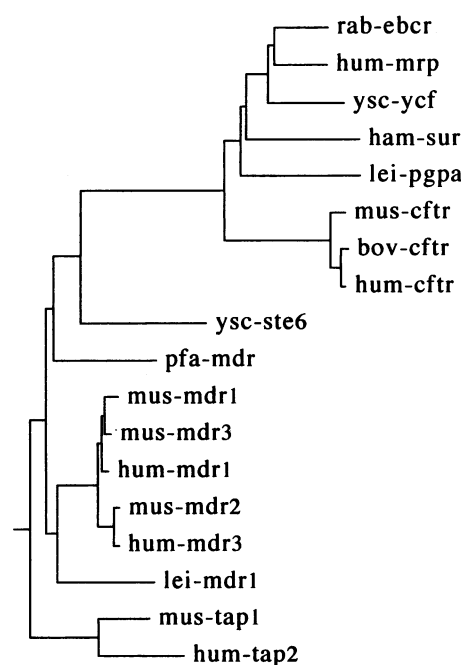
67.8 ± 12.5%, 53.1 ± 6.7%, and 68.2 ± 10.7%, respectively ($n = 4$, Fig. 4 *E* and *F*). Altogether, these oocyte experiments demonstrate that EBCR could function as a cAMP-activated chloride conductance regulator. However, it must be pointed out that the conductance induced by forskolin–IBMX varied considerably amoung different batches of EBCR-expressing oocytes, from 0.2 to 30 μS. The large variance in the forskolin–IBMX- induced conductance indicates that not only EBCR expression but also endogenous factors may be responsible for the induction of this conductance.

The characteristics of the chloride channel conductance in EBCR-expressing oocytes are analogous to those of the smallest conductance described (7–9 pS) in cortical TAL (5), e.g., inhibition by NPPB and DIDS and activation by forskolin. However, two important differences are also noted. The smallest conductance in cortical TAL shows a linear I-V relation and can be blocked by iodide, whereas EBCR shows an outward rectification and is highly permeable for iodide.

Remarkably, EBCR shows a 29% identity to CFTR, whereas voltage-gated chloride channels of the ClC gene family are not related. Nevertheless, structural differences between CFTR and EBCR are striking. (*i*) While hydropathy analysis of EBCR suggests eight TMRs before the first NBF and four TMRs before the second NBF, CFTR is supposed to contain twice six TMRs each followed by a NBF (28). (*ii*) CFTR contains a large regulatory domain involved in cAMP-dependent gating activity (33) that is absent in EBCR. (*iii*) Alignment of EBCR with CFTR (data not shown) demonstrates other regions only present in CFTR that may be involved in channel function. Besides being a chloride channel, recent evidence suggests that CFTR also activates an outward rectifying chloride channel in an autocrine fashion by extrusion of ATP (34). CFTR even regulates sodium channels in a cAMP-dependent manner (35). Whether EBCR is a channel in itself or whether it activates other ion channels remains to be clarified. Surprisingly, EBCR shows no protein kinase A consensus sequences. This could mean that cryptic sites are used, a phenomenon that has also been described for CFTR (36, 37). More detailed immunohistochemistry, including electronmicroscopy, must be performed to clarify whether EBCR is also present in intracellular organelles, as shown for CFTR (38).
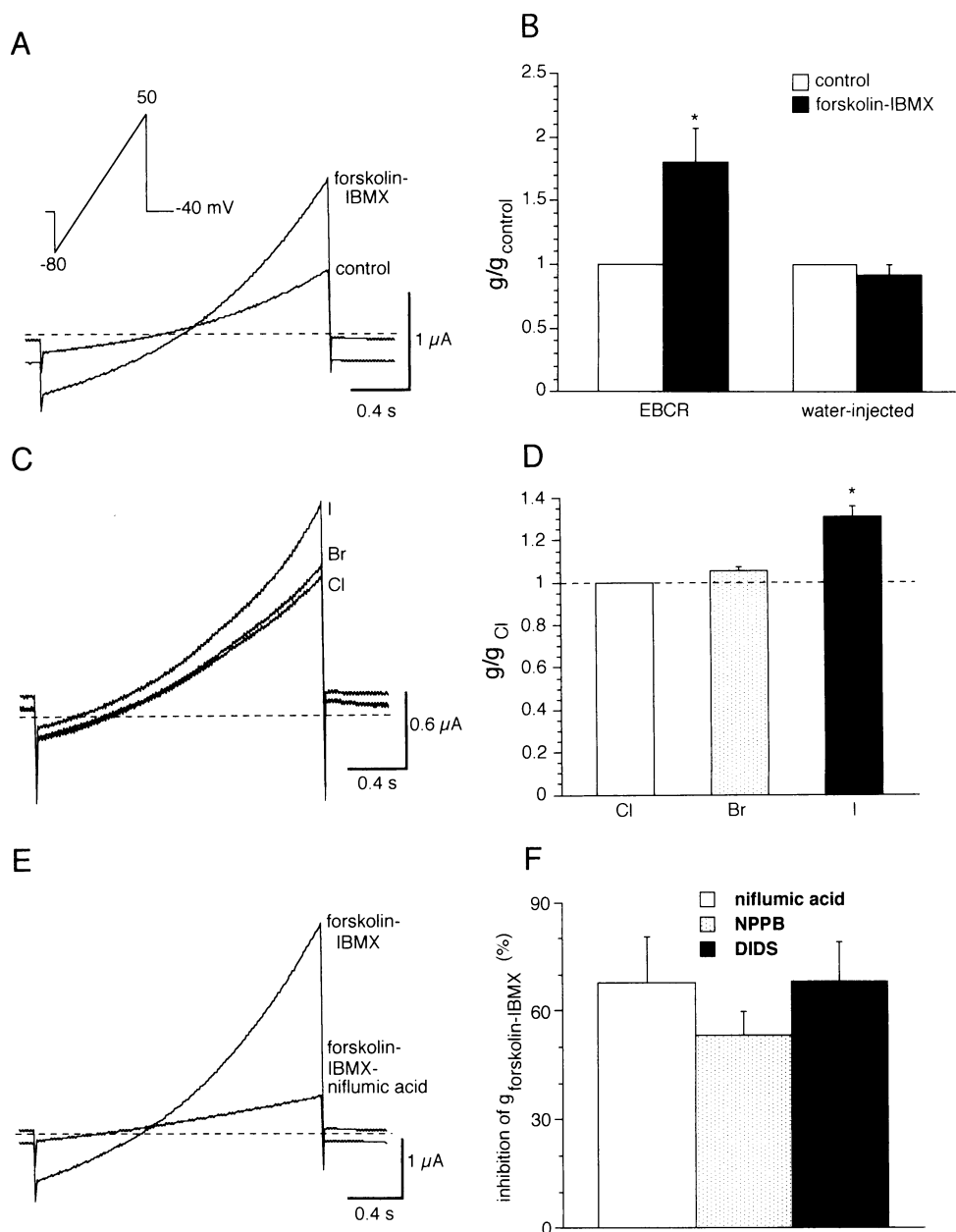
FIG. 4. Voltage-clamp experiments on EBCR–cRNA-injected oocytes. (*A*) Current trace of an EBCR–cRNA- injected oocyte treated with forskolin–IBMX and a corresponding control. The dashed line represents the zero-current trace. (*B*) Forskolin–IBMX-induced increase of conductance (g) in EBCR expressing oocytes compared to unstimulated injected oocytes (∗, $P < 0.05$). No increase of conductance was detected in water-injected oocytes. (*C*) Comparison of conductances of EBCR–cRNA-injected oocytes treated with forskolin–IBMX under conditions in which all external chloride (Cl) was substituted by iodide (I) or bromide (Br). (*D*) Calculation of halide specific conductance divided by chloride conductance (∗, $P < 0.05$). (*E*) Control current trace of an EBCR–cRNA-injected oocyte treated with forskolin–IBMX and experimental trace with the chloride channel blocker niflumic acid. (*F*) Percent of inhibition of chloride conductance by different chloride channel blockers, niflumic acid, NPPB, and DIDS.

EBCR shows highest identity to MRP (26, 27), originally cloned from a doxorubicin-selected multidrug-resistant human small cell lung cancer cell line H69AR (39). Because MRP confers multidrug resistance to cells after stable transfection (40–42), a potential role of EBCR in multidrug resistance warrants further study. MRP is linked to the glutathione system for efflux of cationic drugs (43), but not for anionic compounds (44). Whether EBCR also exhibits drug transport activity remains to be determined.

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