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An Anion Channel in Guinea Pig Gallbladder Epithelial Cells Is Highly Permeable to HCO_3^-

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In guinea pig gallbladder epithelium, a secretion of fluid, secondary to an electrogenic secretion of Cl^- and HCO_3^-, is elicited in the presence of a high intracellular concentration of adenosine 3′-5′-cyclic monophosphate (cAMP). The aim of this study was to analyze the effects of secretagogues on the activity of anionic channels in isolated epithelial cells using the patch-clamp technique and measuring the electrical potential difference of the cellular membrane (pd_{cm}). In cell-attached configuration, with the microelectrode filled with a solution of *N*-methylglucamine–Cl, or in inside-out configuration (symmetrical solution), it was possible to demonstrate the presence of an 18-pS Cl^- channel with linear current/voltage (I/V) relationship and voltage independence; this channel is not activated by cAMP (cell-attached configuration). In inside-out configuration (symmetrical solution), another anionic channel with a conductance of 2.8 pS, voltage independence, and a linear I/V relationship was also identified. This channel was stimulated by cAMP (cell-attached configuration) and by PKA + ATP + cAMP (inside-out configuration). The channel was inhibited by NPPB (10^{-5} M), but not by other anionic inhibitors. Measurements of the pd_{cm} value suggested that in isolated cells, as in whole tissue, cAMP activates conductance for both Cl^- and HCO_3^-. The selectivity of the channel was gluconate < SO_4^{2-} < Cl^- < Br^- < I^- < HCO_3^- < SCN^- and the P_{HCO_3}/P_{Cl} was 2.6. Some features of the channel resemble those of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel and RT-PCR performed on mRNA from isolated epithelial cells detected the presence of a CFTR homologue mRNA. The results obtained indicate that this channel is responsible for the HCO_3^- conductance activated by cAMP.

Key Words: **HCO_3^- channel; Cl^- channel; CFTR; cAMP; gallbladder epithelium**.

Epithelia are usually mainly or completely absorbing or secreting in function. When both functions are present, they can be performed by different cells (e.g., small intestine) (1). Guinea pig gallbladder epithelium, like human epithelium (2, 3), represents an interesting model, as it is able to absorb Na^+ salts and water, and this can be turned into a secreting function in the presence of secretagogues (4). This property makes it similar to respiratory epithelium, in which the secreting function can replace the absorbing function, although both are carried out by the same type of cell (5).

The secreting function of guinea pig gallbladder epithelium is stimulated by an increase in the intracellular cAMP level (6), and seems to prevail during digestion or in cholecystitis (3, 7, 8). Under secreting conditions, not only is Na^+ absorption inhibited due to inhibition of the apical Na^+/H^+ antiporter; in addition, both Cl^-/HCO_3^- exchange and conductance for Cl^- and HCO_3^- (the latter being able to determine a net HCO_3^- electrogenic secretion) are apically enhanced (6, 9). This HCO_3^- secretion may play a part in bile alkalization. Other kinds of epithelia are characterized by HCO_3^- secretion (e.g., jejunum, pancreatic duct, salivary ducts), but no specific HCO_3^- channels have ever been detected. The Cl^- channels observed in cells derived from sweat ducts (10), submandibular acinar cells (11), choroid plexus epithelial cells (12) and cystic fibrosis transmembrane conductance regulator (CFTR) type channels from different epithelia (13–15) present partial permeability to HCO_3^-. In mouse and Necturus gallbladders and in cells isolated from human gallbladder adenocarcinoma, different Cl^- channels have been observed, some of them activated by cell swelling and by Ca^{2+} (16–18), while others are similar to CFTR (19, 20). The possibility that these channels take part in HCO_3^- conductance, has been considered in mouse gallbladder (21, 22). In Necturus, this possibility has been rejected, as the intact epithelium does not display any conductance for HCO_3^-, even in the presence of cAMP (23, 24).

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The aim of our study was to identify the responsibility of cAMP-activated anion conductances in guinea pig gallbladder, with special reference to the origin of HCO_3^- conductance. We performed patch-clamp experiments in cell-attached and inside-out configurations on isolated epithelial cells and cultured cells. We also determined the membrane electric potential differences under different experimental conditions using conventional microelectrodes, and tested for the presence of CFTR mRNA.

MATERIALS AND METHODS

Cell isolation and primary cultures. The method used to isolate cells was similar to the one previously reported (25). Briefly, guinea pigs were killed by a blow on the head, and the gallbladders were removed and washed free of bile. The gallbladder, after being cannulated, was maintained (10 min at 37°C) in a solution containing a high concentration of K^+ and glucose (Sol A). The filling solution was then replaced with the same solution (37°C, 5 min) containing 0.5 mM dithiothreitol and 0.2 EGTA. The gallbladder was then palpated, the filling liquid was filtered and diluted (ratio 1:9, 4°C), with a minimum essential medium with Hanks' salts (HMEM) and buffered with Tes (pH 7.4). The suspension was centrifuged (4°C) and the pellet was re-suspended in the same culture medium containing 0.4 mg/ml collagenase–dispase (0.1–0.8 U mg^{-1}, Boehringer) and incubated at 37°C (5 min). The suspension was then centrifuged (150 g, 10 min, 4°C), and the pellet was re-suspended in HMEM; these two operations were repeated. When required, a primary culture was obtained as described in a previous paper (26).

Solutions. Solution A contained (mM): 7 K_2SO_4; 44 K_2HPO_4; 9 $NaHCO_3$; 10 Hepes and 180 glucose (pH 7.4). In the impalement and patch-clamp experiments (cell-attached configuration), the cell incubation solution was HMEM without bicarbonate, buffered to pH 7.4 with 10 mM Tes/NaOH. In some cases a bicarbonate Krebs–Henseleit solution was used as incubation medium. It included (mM): 118 NaCl; 24.9 $NaHCO_3$; 4.7 KCl; 1.2 $MgSO_4$; 1.2 KH_2PO_4; 2.5 $CaCl_2$ (pH 7.4); the solution was bubbled with O_2/CO_2 at 95/5%. The microelectrode filling solution contained (mM): 137.4 *N*-methyl-glucamine–Cl (NMGCl); 5 $BaCl_2$; 1.8 $CaCl_2$; 1 $MgCl_2$; 10 TES/NMG (pH 7.4). In inside-out configuration experiments, the solution on the cytoplasmic side was (mM): 146 NMGCl; 1 $MgCl_2$; 10 Tes/NMG (pH 7.3); 3 EGTA and 2.5 $CaCl_2$ in order to obtain a free [Ca^{2+}] of 1 μM.

In selectivity experiments, the solutions used differed from the one described due to the replacement of 146 mM NMGCl with 15 mM NMGCl and 131 mM of: Na_2SO_4; NaI; NaBr; Na-gluconate and NaSCN. In the case of experiments designed to establish HCO_3^- selectivity, two solutions were used: in the first one, 50 mM of NMGCl was replaced by $NaHCO_3$ (the solution was bubbled with a 90/10% mixture of O_2/CO_2), and in the second one, 100 mM of NMGCl was replaced by the same concentration of $NaHCO_3$ (the solution was bubbled with an 80/20% mixture of O_2/CO_2). The two solutions were used to ascertain that the result was independent of the experimental conditions chosen.

Impalements using conventional microelectrodes. The conventional microelectrodes, measurements set up and acceptance criteria for impalements were similar to those previously described (27, 28). The microelectrodes had a resistance of 40–70 MΩ, and were connected to a very high input resistance (2×10^{14} Ω) electrometer (Ketley 617, Cleveland, OH) and a strip chart recorder (Linear 1200, Reno, NV).

Patch-clamp technique. The patch-clamp technique and data analysis were applied as reported (25, 29). We used microelectrodes with a resistance of 14–18 MΩ. The seals had a resistance in the 20–40 GΩ range. The signals were filtered at 100–200 Hz with an eight-pole Bessel filter, digitized (DigiData 1200 interface, Axon Instruments, Foster City, CA), collected at 5 kHz with a computer and analyzed with a patch-clamp program (Pclamp 7, Axon Instruments). The bath was earthed with an Ag/AgCl electrode immersed in an agar bridge with either the same ion composition as the cell incubation medium or 3 M KCl. The junction potentials were taken into account. In the presence of multiple channel openings, the open probability of the single channel (P_o) was calculated as previously reported (25). In the cell-attached configuration, the potentials were expressed as overall potential, considering the cell membrane electrical potential difference (pd_{cm}) and the holding potential. The potential sign was that of the cytoplasmic side with respect to the pipette solution; the inward currents (positive charges from microelectrode to cytoplasmic side) were reported as negative.

Reverse transcriptase (RT)-PCR. Gallbladder epithelial cells were obtained in accordance with the same isolation procedure as described in this paper, omitting the steps after the first centrifugation. Total RNA was isolated using the Promega SV Total RNA Isolation System. Poly(A)$^+$ was purified from total RNA using the OligotexmRNA Purification System (Qiagen, Germany). Reverse transcription was performed with the Sigma (Sigma-Aldrich) Enhanced Avian RT-PCR kit (one-step reaction), according to the manufacturer's instructions. The first strand of cDNA was synthesized using random hexamers in the reverse transcription reaction.

The pair of primers used in the PCR(sense 5′-CATCTTTGGTGTTTCCTA-3′; antisense 5′-AGTCTTCTCTCCACAGGG-3′) were synthesized by Gibco Life Technologies (Glasgow, UK), and were designed on the basis of the guinea pig partial cds sequences found in the databank (GenBank M96679.1 and AF133216). Aliquots of the PCR products were run on 1.5% agarose gel in 0.5× TAE Buffer [32] and stained with an 0.2% ethidium bromide solution. The PCR fragments of interest were extracted from agarose gels (Gel Extraction kit, Qiagen Gmbh, Hilden, Germany), ligated into pSTblue1 vector (Perfectly Blunt Cloning kit, Novagen Inc., Mannheim Germany), and sent to MWG-Biotech (Ebesberg, Germany) for double strand sequencing.

The BioEdit sequence alignment editor and the BLAST protein sequencing database search program were used to analyze sequence similarities.

Drugs and chemicals. 8-Br-cAMP (8-bromoadenosine 3′:5′-cyclic monophosphate), ATP (adenosine 5′-triphosphate), PKA (protein kinase A), EGTA (ethylene glycol-bis (β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid), Tes (*N*-Tris [hydroxymethyl] methyl-2-aminoethane sulfonic acid), HMEM (minimum essential medium with Hanks' salts), SITS (4-acetamido-4′-isothiocynostilbene-2,2′-disulphonate), and NPPB (5-nitro-2-(3-phenylpropylamino)-benzoic acid) were purchased from Sigma (St. Louis, MO); DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid) and DPC (diphenylamine-2-carboxilic acid) from Aldrich Chemical (Milwaukee, WI); NMG (*N*-methyl-D-glucamine) from Fluka (Buchs, Switzerland).

Statistics. Unless otherwise specified, the experimental values are expressed as mean ± SEM of *n* experiments. Student's *t* test was used (with unpaired data unless otherwise specified) for the statistical analysis. The best fits were performed by linear and non-linear regression analysis (program: Graphpad Prism, version 2.01, Graphpad Software Inc. San Diego, CA).

RESULTS

Effect of cAMP on membrane potential. It was established that isolated cells (round-shaped and apparently without morphological polarity) behaved similarly to intact epithelium (6) as they presented a pd_{cm} sensitive to the presence of cAMP in the incubation medium. Figure 1A shows pd_{cm}, before and after the addition of 8-Br-cAMP (250 μM), obtained under a

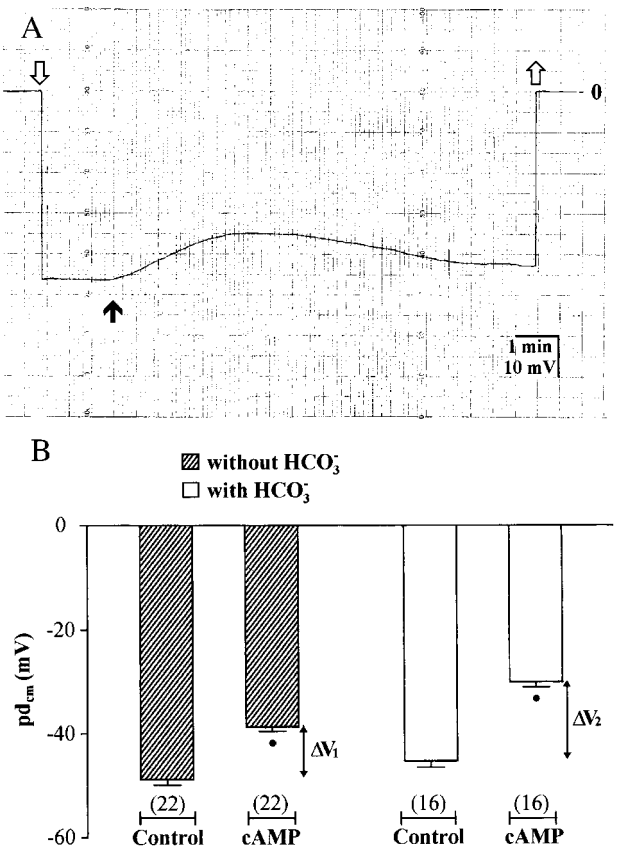

FIG. 1. Effect on the membrane electric potential difference (pd_{cm}) of the presence of 8-Br-cAMP (250 μM) in the incubation medium. (A) Recording of a pd_{cm} measurement. ⇩ and ⇧ relate to the beginning and end of the impalement. ↑ relates to the substitution of the bath solution with a solution containing 8-Br-cAMP. (B) pd_{cm} measured in the presence or absence of HCO_3^-, before or after the addition of 8-Br-cAMP. Means ± SEM, number of data in parentheses. •$P < 0.05$ compared with control obtained under the same experimental conditions.

single impalement. The average value of pd_{cm}, obtained under control conditions, is shown in Fig. 1B. The addition of 8-Br-cAMP to the incubation medium caused depolarization (Figs. 1A and 1B) of about 10 mV ($\triangle V_1$, 10.1 ± 1.4 mV; $n = 22$) in about 2–3 min. This depolarization is in accordance with the activation of anion channels. In a longer time we observed partial repolarization of the membrane, so that after 7–10 min the depolarization compared with the initial value was reduced to 4.3 ± 0.5 mV, $n = 22$. The partial repolarization is in accordance with the activation of K^+ channels. Similar experiments have also been performed using incubation solutions that contained HCO_3^- (25 mM)/CO_2 (5%). The initial pd_{cm} (Fig. 1B) was not significantly different from the value obtained in the absence of HCO_3^-, but the maximum depolarization observed in the presence of 8-Br-cAMP ($\triangle V_2$, 15.2 ± 1.6 mV; $n = 16$) was significantly higher ($P < 0.05$) than the value obtained in the absence of HCO_3^-.

Medium conductance Cl^- channel. Figure 2A shows single-channel currents recorded (on isolated cells) in cell-attached configuration and with NMGCl filling solution in the microelectrode (incubation solution: HMEM). The single-channel current/voltage relationship (I/V) obtained (Fig. 2B) revealed a single-channel conductance of 17.7 ± 1.1 pS ($n = 6$). The intercept point with the voltage axis was −39.2 ± 1.6 mV ($n = 6$), in accordance with a possible reversal potential of a Cl^- current. This channel appeared with low frequency, in about 2–4% of the patches. The open probability (P_o) was approximately constant (mean value 0.568 ± 0.040; $n = 4$) at different voltages (from −100 to 20 mV) and was not modified by the presence of 8-Br-cAMP (250 μM) or theophylline (3 mM) and forskolin (2.5 μM) in the incubation bath.

Further confirmation of the nature of the channel is derived from experiments performed in inside-out configurations. In the presence of NMGCl symmetrical solutions the I/V relationship (Fig. 2C) was linear and the reversal potential was 0.3 ± 0.4 mV ($n = 4$), not significantly different from 0. Replacement of NMGCl the cytoplasmic side with a solution containing NaCl or KCl did not significantly modify the reversal potential, which shifted to a negative value (−38.6 ± 2.4 mV; $n = 4$) when the replacement substance was NMG-gluconate ($P_{gluconate}/P_{Cl} = 0.09$).

Low-conductance anion channel. In cell-attached configuration experiments, tracings similar to those reported in Fig. 3A have also been observed; 2-3 channels of the same type are frequently present. The corresponding I/V relationship (Fig. 3B) displays a reversal potential at negative values (−41.3 ± 1.9 mV; $n = 12$), which agrees with the behavior of a Cl^- channel.

In inside-out configuration (symmetrical NMGCl solutions), the I/V relationship (Fig. 3C) was linear, and exhibited a reversal potential (−0.5 ± 0.5 mV; $n = 12$) which did not differ from 0 to a statistically significant extent. The channel elicited conductance of 2.8 ± 0.4 pS ($n = 12$), corresponding to the value obtained in the cell-attached configuration. Replacement on the cytoplasmic side with a solution containing KCl or NaCl did not cause any statistically significant shift of the reversal potential, which otherwise shifted to negative values in the presence of NMG-gluconate. The sum of these data confirmed the anion nature of the channel.

To obtain information about the possible involvement of the channel in secreting events, we considered the effects of PKA, ATP and cAMP in inside-out configuration. Figure 4A shows single-channel tracings obtained both in the absence and in the presence of PKA (1 mg/ml, 150 U/ml), ATP (1 mM), and cAMP (250 μM) on the cytoplasmic side. Figure 4B reports the P_o relating to the voltage. Under control conditions, the value of P_o is very low, and does not elicit voltage dependence. After treatment, this value increases sig-

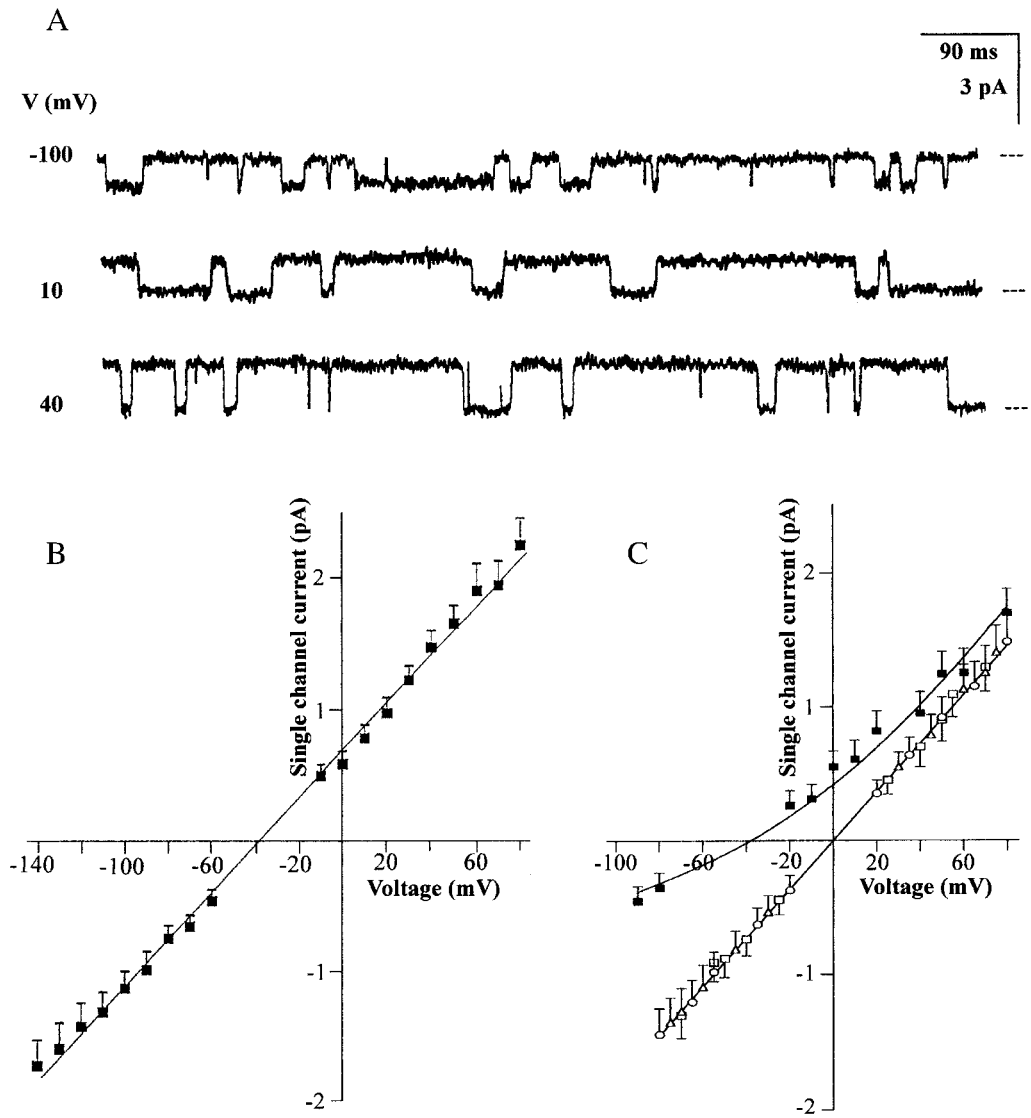

FIG. 2. Medium-conductance Cl^- channel. (A) Tracings obtained in cell-attached configuration at the voltages indicated when cells were bathed in HMEM and the microelectrode filling solution contained NMGCl. — - , zero current level. (B) Single-channel current/voltage (I/V) relationship obtained in cell-attached configuration. Means ± SEM ($n = 6$). (C) Single-channel current/voltage (I/V) relationship obtained in inside-out configuration: ○, symmetrical solutions containing NMGCl; △, NMGCl in the pipette, KCl on the cytoplasmic side; □, NMGCl in the pipette, NaCl on the cytoplasmic side; ■, NMGCl in the pipette, NMG-gluconate on the cytoplasmic side. Means ± SEM ($n = 4$).

nificantly at every voltage (P_o about 0.3). The presence of ATP only (1 mM) on the cytoplasmic side did not significantly modify the P_o at any voltage tested. To confirm the role of this channel in secreting events, we analyzed the P_o in cell-attached configuration experiments. The P_o was not dependent on the voltage (from −150 to 70 mV) and increased (from 0.003 ± 0.002 to 0.274 ± 0.033; $n = 5$; $P < 0.01$, paired data) when 8-Br-cAMP (250 μM) was used in the incubation bath (HMEM). During the first few minutes in the presence of 8-Br-cAMP, the P_o increased gradually, reaching a maximum after 3 min.

To define the characteristics of the channel, we switched to inside-out configuration (NMGCl symmetrical solution, with 3 mM theophylline on the cytoplasmic side to inhibit dephosphorylation of the channel) from cells pre-treated with 8-Br-cAMP. We tested the P_o at different voltages, under control conditions (P_o = 0.322 ± 0.043; $n = 28$) and subsequently, in the presence of different inhibitors on the cytoplasmic side. SITS (10^{-5} M), DIDS (10^{-5}, 2×10^{-4} M) and DPC (10^{-5}, 5×10^{-4} M) did not determine any variations in the channel activity ($n = 4$, for each inhibitor); however, NPPB elicited an inhibition which increased from a concentration of 10^{-5} M to 10^{-4} M (mean value of P_o, at voltages between −100 and 100 mV, 0.198 ± 0.063 at 10^{-5} M, $n = 4$, $P < 0.05$; 0.052 ± 0.055 at 10^{-4} M, $n = 4$, $P < 0.05$, paired data).

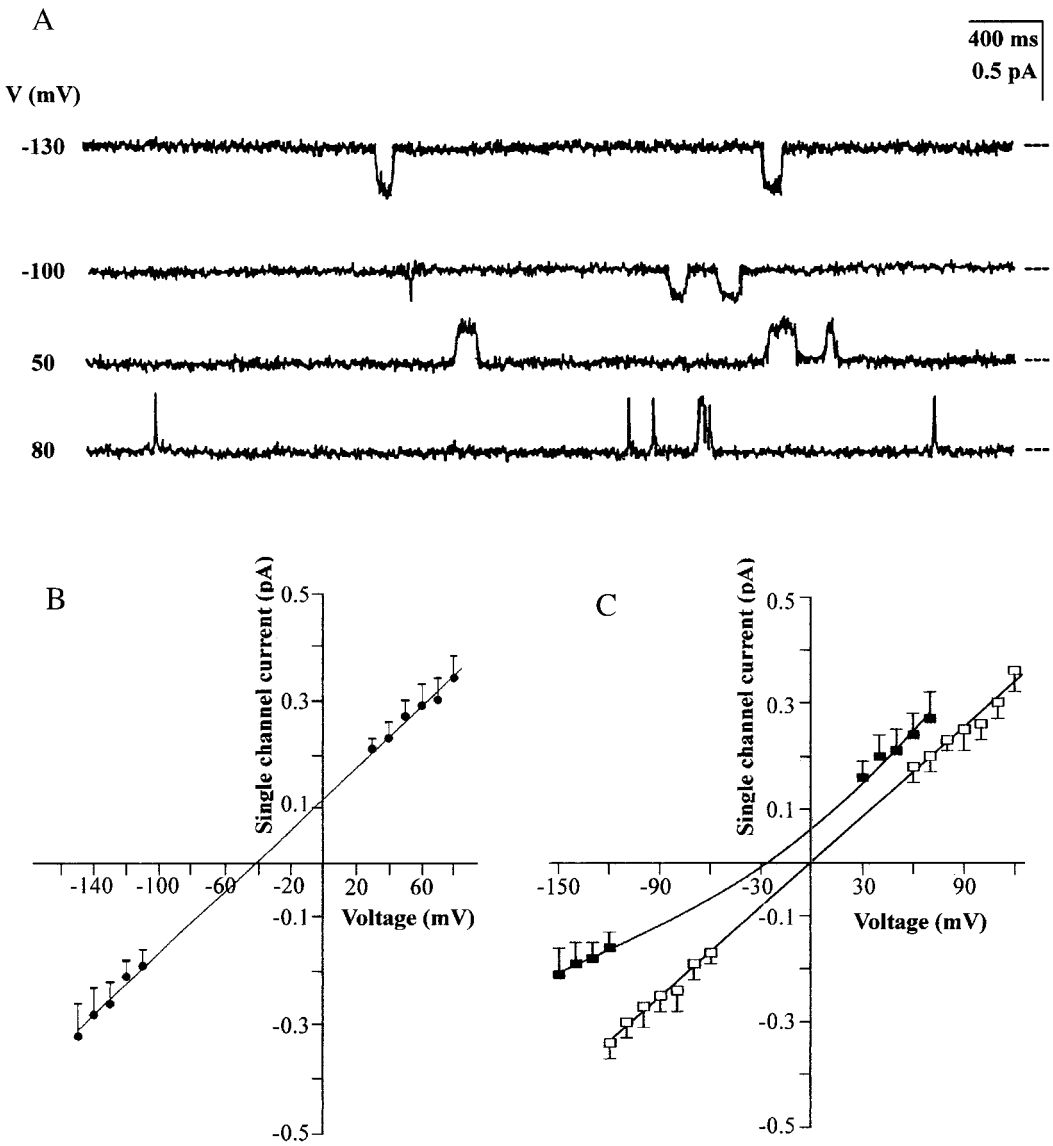

FIG. 3. Low-conductance anion channel. (A) Tracings obtained in cell-attached configuration at the voltages indicated when cells were bathed in HMEM and the microelectrode filling solution contained NMGCl. (B) Single-channel current/voltage (I/V) relationship obtained in cell-attached configuration. Means ± SEM (n = 12). (C) Single-channel current/voltage (I/V) relationship obtained in inside-out configuration: □, symmetrical solutions containing NMGCl. Means ± SEM (n = 12). ■, NMGCl in the pipette, NMG-gluconate on the cytoplasmic side. Means ± SEM (n = 6)

To confirm the location of these channels on the apical membrane, we also performed experiments on primary cultures of guinea pig gallbladder epithelial cells grown to a confluent monolayer (so that the cells are polarized). On the apical membrane we observed the presence of low-conductance anion channels with characteristics (conductance, linear I/V relationship, inhibition by NPPB) similar to those exhibited by isolated cells.

We also performed experiments in inside-out configuration (using cells pretreated with 8-Br-cAMP) in which, after observing a channel with results corresponding to those already reported, in the presence of symmetric solutions of Cl^-, the solution on the cytoplasmic side was replaced with a solution containing HCO_3^- and CO_2. We used two different HCO_3^- concentrations and CO_2 partial pressures, but always we maintained the same pH. After the solution was changed, the number of observable channels did not alter, but the reversal potential determined by the I/V relationship, shifted to positive values. Similar results, but with different variations on the reversal potential (Vr), were obtained if Cl^- was partially replaced with different anions (gluconate, Vr = −26.1 ± 3.0 mV, n = 6; SO_4^{2-}, Vr = −16.8 ± 1.7 mV, n = 3; Cl^-, Vr = −0.2 ± 0.5, n = 18; Br^-, Vr = 10.3 ± 0.7, n = 3; I^-, Vr = 14.7 ±

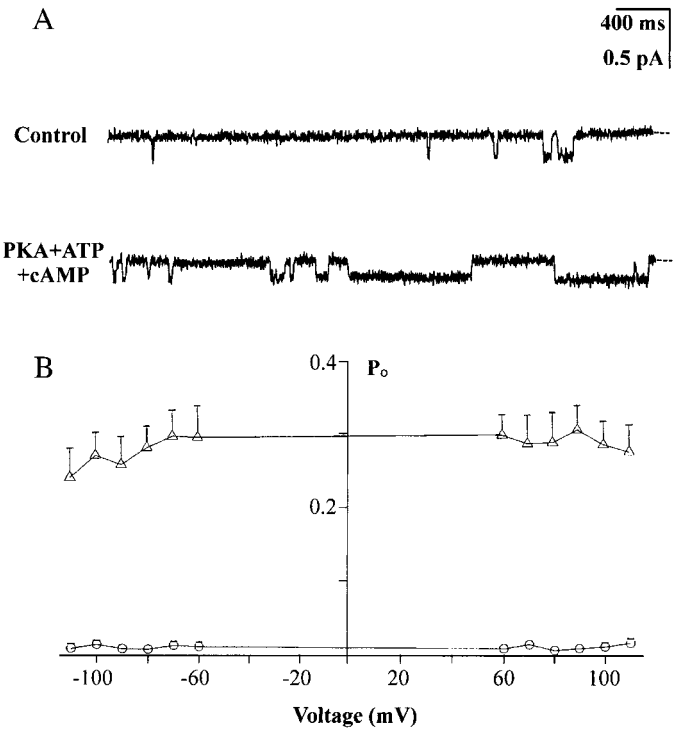

FIG. 4. Effect on the activity of the low-conductance anion channel of PKA (1 mg/ml, 150 U/ml), ATP (1 mM), and cAMP (250 μM) on the cytoplasmic side (inside-out configuration). (A) Tracings obtained under the two different conditions at −60 mV. (B) Open probability (P_o) as a function of voltage before (○) and after (△) treatment. Means ± SEM ($n = 4$) ($P < 0.01$ compared to control at all voltages, paired data).

1.7, $n = 4$; 100 mM HCO_3^-, Vr = 16.0 ± 2.3, $n = 3$; 50 mM HCO_3^-, Vr = 11.6 ± 2.5 mV, $n = 7$; SCN^-, Vr = 26.7 ± 2.7, $n = 3$). The permeabilities relative to Cl^- which can be deduced from these experiments are shown in Fig. 5. This result in the following selectivity scale: gluconate $< SO_4^{2-} < Cl^- < Br^- < I^- < HCO_3^- < SCN^-$.

Finally, we performed some experiments in cell-attached configuration where the incubation solution (HMEM) was replaced with a solution containing HCO_3^- (25 mM) and CO_2 (5%), to which 8-Br-cAMP (250 μM) was then added. It was predictable that this substitution would increase the HCO_3^- concentration in the cell, probably reducing, or at least not increasing, the Cl^- concentration. Under these conditions we measured a pd_{cm} of −40.8 ± 1.4 mV ($n = 16$) with conventional microelectrodes. Considering the I/V relationship of an experiment of this kind, we observed a shift to the right of the reversal potential (from −41.3 ± 1.9 mV ($n = 12$) obtained in HMEM to −26.1 ± 3.1 mV ($n = 4$), obtained using a cell medium containing HCO_3^-/CO_2 and 8-Br-cAMP ($P < 0.05$). This suggests channel behavior in cell attached configuration similar to that observed in inside-out configuration (shift to the right of the reversal potential in the presence of HCO_3^-/CO_2) as a consequence of high permeability to HCO_3^-.

RT-PCR analysis. The 3 pS channel shares some similarities with the CFTR anion channel (i.e., linear I/V relationship and cAMP activation), but some features, especially as regards the selectivity for HCO_3, seem to be typical of this guinea pig channel. The presence of CFTR has also been reported in the gallbladder epithelium of other species (20, 22, 30–32).

On this basis we decided to investigate the expression of CFTR in guinea pig gallbladder epithelial cells by RT-PCR, in order to elucidate a possible relationship between CFTR and the channel observed in the patch-clamp experiments. A specific pair of primers was designed on the basis of the known guinea pig regulatory domain sequence (Accession No. M96679) and of the first nucleotide binding domain partial sequence (Accession No. AF133216). The primers were designed to span the two fragments, as shown in Fig. 6. RT-PCR was performed using poly-A extracted from freshly-isolated guinea pig cells, and a negative control without reverse transcriptase was also set up, in order to exclude genomic DNA contamination. As shown in Fig. 6B, a product of the expected molecular weight (690 bp) was obtained in the liver reaction, and its identity was confirmed by sequencing. The cDNA fragment is almost 100% identical to the sequence resulting from the sum of the two known partial sequences. Interestingly, in the region between nucleotide 600 and 615, where the two previously known sequences exhibit more differences, the gallbladder sequence is identical to the small intestine sequence (AF133216).

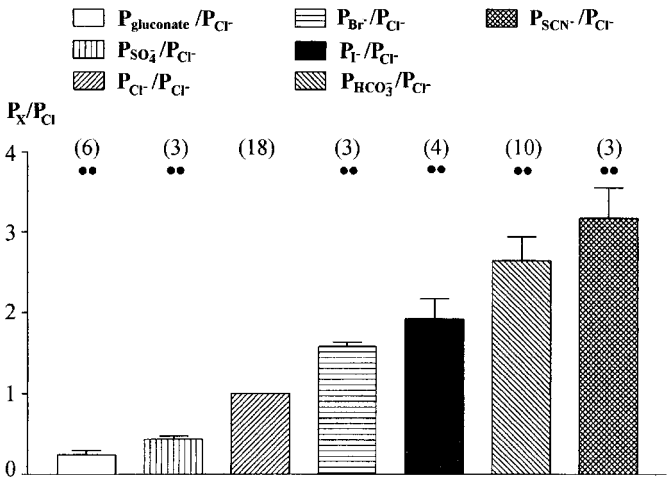

FIG. 5. Anion permeabilities relative to chloride (P_A^-/P_{Cl}^-) of the low-conductance anion channel. The P_A^-/P_{Cl}^- values were calculated from the best fit to the Goldman–Hodgkin–Katz equation. Means ± SEM; number of data in parentheses; ●●$P < 0.01$.

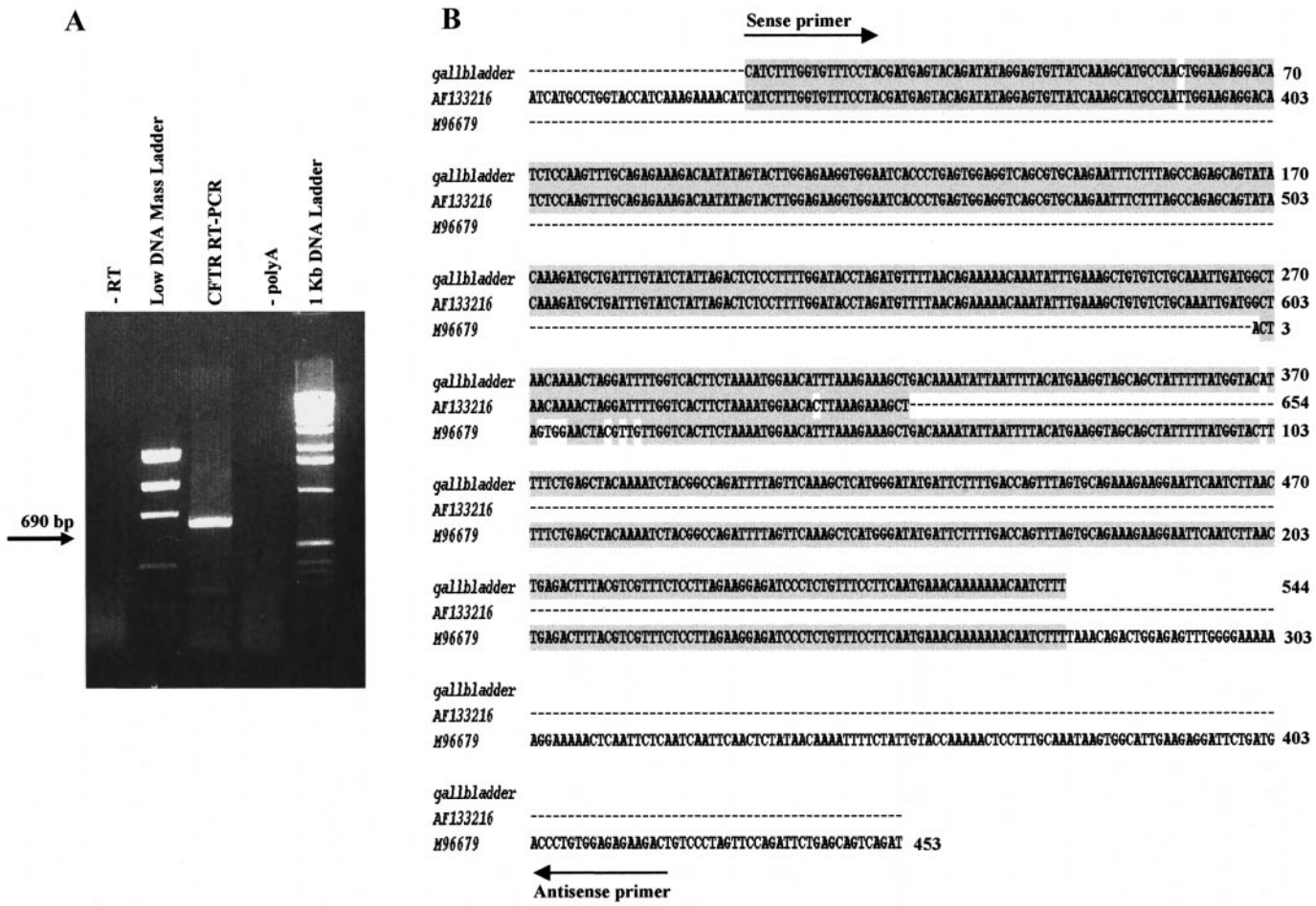

FIG. 6. Detection of CFTR mRNA in freshly-isolated guinea pig gallbladder epithelial cells. Aliquots of the PCR and of the two negative controls [−RT and −poly(A)] were run on 1.5% agarose gel (A). The band of the expected molecular size is indicated by the arrow. The cDNA fragment sequence was aligned with that of two already cloned guinea pig CFTR fragments (accession numbers in the figure) to analyze sequence similarities (B) Identical nucleotides are shadowed in gray. The primers employed in the PCR are shown in the figure by arrows.

DISCUSSION

The 18 pS anion channel was not activated by cAMP, forskolin or theophylline, and therefore does not seem to be involved in secreting events. Apical Cl^- conductive pathways not activated by cAMP have also been reported in other gallbladder epithelia (23, 31). In any event the low percentage of appearance of this channel suggests that it plays little part in the normal secretory condition.

Conversely, the 2.8 pS channel was seen with high frequency; it was activated by treatment with cAMP, forskolin and theophylline, and in inside-out configuration by phosphorylation due to PKA, cAMP and ATP. It lacked direct activation by ATP only. In Necturus gallbladder, which has been studied for a long time (33), two types of anion channel have been observed: one of these showed a conductance of about 10 pS (20, 24), linear I/V relationship, voltage and Ca^{2+} independence, activation by PKA, and no inhibition by several anion channel inhibitors including NPPB. The channel identified has similar but not identical characteristics; in fact, its conductance is much lower (2.8 pS), NPPB is effective, and the channel exhibits high permeability to HCO_3^-.

In some respects, this channel is similar to the CFTR channel which has been found not only in respiratory epithelium, but also in intestinal epithelium (34), pancreatic duct (35), biliary duct (36) and cultured mouse epithelial cells (19).

According to tissue and animal species, CFTR presents a conductance of between 4 and 10 pS, activation by cAMP and phosphorylation, a linear I/V relationship, voltage independence, inhibition by NPPB and usually by DPC, but never by DIDS, a selectivity sequence normally with $Br^- \geq Cl^- > I^-$ (34, 37), and low activation by ATP only (38). The channel we described has lower conductance; it is not activated at all by ATP alone, is not inhibited by DPC, and exhibits selectivity

with $I^- > Br^- > Cl^-$. The same selective sequence has been observed for cAMP-activated apical Cl^- conductance in Necturus gallbladder (23, 39), but not in mouse gallbladder (19).

The hypothesis that the protein channel supporting this chloride current is a CFTR homologue is strongly supported by the results of the RT-PCR experiments. In particular, the fragment we obtained is almost identical to the partial small intestine sequence (AF133216). The sequence we amplified corresponds to part of the first nucleotide binding domain and of the regulatory region, so differences may conceivably exist in other regions of the protein (i.e., the transmembrane domains), resulting in anomalous anion selectivity and pharmacological profile. Interestingly, the expression of CFTR has also been reported in human (32, 40), mouse (30) and Necturus gallbladder (20), and a sequence corresponding to the transmembrane region of a CFTR-like channel of the prairie dog has been retrieved from the GenBank database (Accession No. AF012893). In more than one case it has been suggested that a relationship exists between the CFTR channel and gallbladder bicarbonate secretion (22, 31), but unfortunately, no patch-clamp characterization exists for most of these channels.

The pd_{cm} depolarization observed with conventional microelectrodes after addition of 8-Br-cAMP to the incubation medium agrees with the findings made in intact epithelium (6), and also with an increase in anion conductance stimulated by cAMP, to which the 2.8 pS anion channel contributes. In intact epithelium, this depolarization is due to the apical membrane, and this is in accordance with the apical location of the low-conductance anion channel, which can be deduced from experiments on primary cultures in which cells have reached confluence. Partial pd_{cm} repolarization, which takes longer to occur after the addition of cAMP, could be due to the appearance of other conductances, e.g., for K^+; the validity of this hypothesis is discussed separately.

The experiments performed on intact guinea pig gallbladder epithelium show HCO_3^- conductance on the apical membrane activated by the presence of secretagogues (6). In different epithelial cells, the CFTR channel is considered to be one of the possible causes of apical HCO_3^- secretion (13–15, 21, 22). By contrast with our observations, when CFTR permeability to HCO_3^- was tested (15, 35), it was always lower than permeability to Cl^- (P_{HCO_3}/P_{Cl} from 0.6 to 0.14) (15, 35, 41). Other anion channels not comparable with CFTR which were tested using the whole-cell patch-clamp technique demonstrated permeability to HCO_3^- which, however, was not higher than the permeability to Cl^- (11, 12). Single outwardly rectifying anion channels which present HCO_3^- permeability equal to 50% of Cl^- permeability have been identified in different human cells (10). HCO_3^- permeability becomes higher then the Cl^- one (P_{HCO_3}/P_{Cl} 1.2) under particular experimental conditions, e.g., highly basic extracellular pH. If it is assumed that in guinea pigs, the Cl^- channels actually correspond to the Necturus channels, it must be concluded that HCO_3^- conductance (which is absent in Necturus) is due to different specific HCO_3^- channels in guinea pigs. This conflicts with our data. The fact that HCO_3^- conductance is present in isolated cells is verifiable not only from excise-patch experiments, but also from those performed with intact cells. This last conclusion is suggested by the higher depolarization of pd_{cm} after cAMP treatment in relation to the presence or absence of HCO_3^-/CO_2 in the cell medium. The behavior of the I/V relationships observed in cell-attached configuration (in the presence of cAMP, and in the absence or presence of HCO_3^-) also agrees with greater selectivity of the channel for HCO_3^- by comparison with Cl^- selectivity, even under these conditions.

In conclusion, our data indicate the presence in guinea pig gallbladder epithelium of anion channels activated by cAMP which are more permeable to HCO_3^- than to Cl^- and explain the simultaneous presence of considerable apical conductance for both Cl^- and HCO_3^-, as already observed in intact tissue (6).

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