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Strong activation of bile acid-sensitive ion channel (BASIC) by ursodeoxycholic acid

Dominik Wiemuth,[1,*] Hacer Sahin,[2] Cathérine M.T. Lefèvre,[1] Hermann E. Wasmuth[2] and Stefan Gründer[1,*]

[1]Department of Physiology; RWTH Aachen University; Aachen, Germany; [2]Department of Medicine III; RWTH Aachen University; Aachen, Germany

Bile acid-sensitive ion channel (BASIC) is a member of the DEG/ENaC gene family of unknown function. Rat BASIC (rBASIC) is inactive at rest. We have recently shown that cholangiocytes, the epithelial cells lining the bile ducts, are the main site of BASIC expression in the liver and identified bile acids, in particular hyo- and chenodeoxycholic acid, as agonists of rBASIC. Moreover, it seems that extracellular divalent cations stabilize the resting state of rBASIC, because removal of extracellular divalent cations opens the channel. In this addendum, we demonstrate that removal of extracellular divalent cations potentiates the activation of rBASIC by bile acids, suggesting an allosteric mechanism. Furthermore, we show that rBASIC is strongly activated by the anticholestatic bile acid ursodeoxycholic acid (UDCA), suggesting that BASIC might mediate part of the therapeutic effects of UDCA.

Keywords: acid-sensing ion channel, ASIC, BASIC, bile acid, BLINaC, cholangiocyte, DEG/ENaC, ENaC, epithelial Na^+ channel

Abbreviations: ASIC, acid-sensing ion channel; BASIC, bile acid-sensitive ion channel; BLINaC, brain liver intestine Na^+ channel; β-MCA, β-muricholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; ENaC, epithelial Na^+ channel; HDCA, hyodeoxycholic acid; HyNaC, Hydra Na^+ channel; INaC, intestine Na^+ channel; UDCA, ursodeoxycholic acid

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*Correspondence to: Dominik Wiemuth and Stefan Gründer; Email: dwiemuth@ukaachen.de and sgruender@ukaachen.de

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Introduction

Bile acid-sensitive ion channel (BASIC) is a member of the DEG/ENaC family of cation channels. Other mammalian members of this gene family are the epithelial Na^+ channel (ENaC) and acid-sensing ion channels (ASICs). While the function of ENaC in epithelial Na^+ reabsorption and Na^+ homeostasis has been known for some time[1] and the role of ASICs in neuronal transmission and sensation of painful acidosis has been revealed over the last years,[2,3] the physiological role of BASIC has remained unknown. Cloned more than a decade ago from rat and mouse, it was originally named BLINaC, according to its predominant sites of expression, namely the brain, the liver and the intestinal tract.[4] The human homolog was cloned shortly after BASIC and originally named INaC (intestine Na^+ channel), because its expression was mainly restricted to the intestinal tract.[5] While BASIC from mouse (mBASIC) is a constitutively open, Na^+-selective channel, its ortholog from rat (rBASIC) is almost completely blocked by physiological concentrations of extracellular Ca^{2+}. The residual rBASIC current is unselective but removal of extracellular Ca^{2+} opens rBASIC and renders the channel more selective for Na^+ over K^+.[6]

A hallmark of DEG/ENaC channels is the block by the diuretic amiloride. While mBASIC is inhibited by micromolar concentrations of amiloride, rBASIC is only partially inhibited by millimolar concentrations of the drug.[6] Additional pharmacological tools to investigate the physiological function of BASIC were identified recently.[7] The anti-protozoal diarylamidines, in particular diminazene and the related compound nafamostat, inhibit BASIC at micromolar concentrations. Because diarylamidines do not inhibit ENaC,[8] they are well suited to distinguish between ENaC and BASIC currents in tissues and cells.

Since its cloning, it was hypothesized that BASIC might be a ligand-gated channel, like other members of the DEG/ENaC family, for example the Hydra Na^+ channel (HyNaC) from the freshwater polyp *Hydra magnapapillata* and FaNaC, the FMRFamide-activated Na^+ channel from snails, which have neuropeptides as ligands.[9-11] The ligand-hypothesis gained further support when flufenamic acid

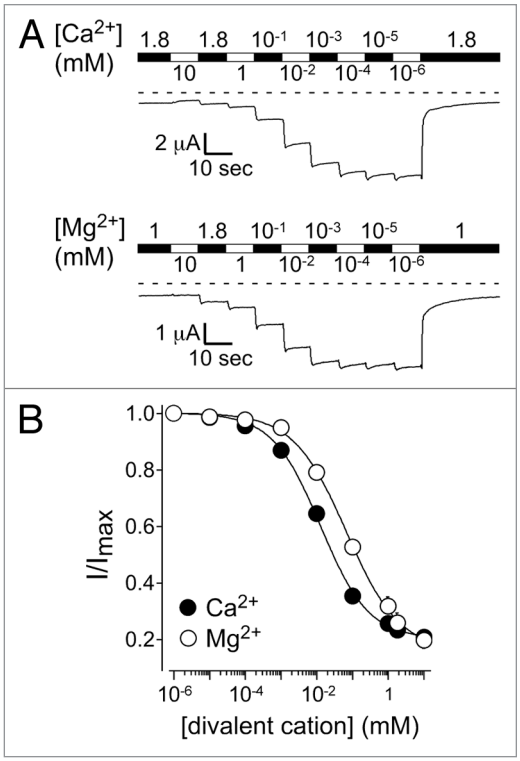

Figure 1. rBASIC is inhibited by physiological concentrations of extracellular Ca^{2+} and Mg^{2+}. (**A**) Upper panel, representative current trace from rBASIC-expressing oocytes recorded in the absence of Mg^{2+} and decreasing $[Ca^{2+}]_e$. Lower panel, representative current trace recorded in the absence of Ca^{2+} and decreasing $[Mg^{2+}]_e$. The dotted lines represent the 0 current level. (**B**) Concentration-dependent inhibition of rBASIC by extracellular Ca^{2+} (closed circles) and Mg^{2+} (open circles). Currents were normalized to the current in the presence of 10 nM Ca^{2+} or Mg^{2+}, respectively. Error bars = S.E.M., n = 8. Curves represent fits to the Hill-equation. Holding potential was –70 mV.

(FFA) was identified as an artificial agonist of rBASIC.[7] Micro- to millimolar concentrations of FFA rapidly activate the channel, inducing Na^+-selective currents.

Recently we identified cholangiocytes, the epithelial cells lining the bile ducts, as the main site of BASIC protein expression in the liver.[12] This in turn led to the identification of bile acids as agonists of rBASIC, confirming that rBASIC requires a ligand for activation. Based on the sensitivity of the channel for bile acids we changed the original name BLINaC[4] to BASIC.[12] Various bile acids naturally occurring in mouse, rat and pig bile, in particular hyodeoxycholic acid (HDCA) and chenodeoxycholic acid (CDCA), activate rBASIC when applied individually. When applied together, HDCA and CDCA activate rBASIC synergistically.[12]

Bile acids are required to emulsify dietary fat in order to facilitate efficient lipolysis. They are synthesized by hepatocytes and then transported via the bile ducts to the gallbladder, where they are stored and from which they are released into the small intestine when required. Rats lack a gallbladder; in these animals the bile is directly released from the major extrahepatic bile duct into the duodenum. Hundreds of different bile acids are known to date, the structure of their side chain, their stereochemistry and the number and position of their hydroxylgroups varies and determines their chemical properties. Furthermore the bile acid composition is highly variable between different species. In humans for example, the major bile acids are CDCA and cholic acid (CA) whereas in rodents, CA, β-muricholic acid (β-MCA) and HDCA are the major bile acids.

Ursodeoxycholic acid (UDCA) is the major physiological constituent of bear bile but it is also present in trace amounts in human and rodent bile.[13] In traditional chinese medicine, UDCA isolated from bear bile has been administered as a remedy for liver diseases for almost 3,000 years[14] In the 20th century, UDCA was discovered by Western medicine as a compound capable of dissolving gallstones and inducing choleresis, an increased bile flow.[15] Today UDCA is used to treat various cholestatic liver diseases, for example primary biliary cirrhosis, primary sclerosing cholangitis or intrahepatic cholestasis of pregnancy.[14] UDCA exerts its beneficial anti-cholestatic effect by several different but possibly linked mechanisms in hepatocytes and cholangiocytes. In hepatocytes, UDCA increases secretion by stimulating the expression of transporter proteins required for secretory processes, for example the bile salt export pump (BSEP) and the multidrug resistance-associated protein 2 (MRP2),[16] and by increasing the insertion rate of these proteins into the apical membrane.[17] Furthermore, UDCA was shown to have anti-apoptotic effects in hepatocytes.[18] Cholangiocytes are constantly exposed to high concentrations of hydrophobic bile acids, which can damage the plasma membrane leading to cholangiocyte malfunction.[19] UDCA, a relatively hydrophilic bile acid counteracts this membrane-damaging effect in vitro.[20,21] In addition, UDCA can stimulate secretion of cholangiocytes indirectly via intracellular signaling cascades that affect apically located proteins involved in secretion, e.g., purinergic PY receptors and the chloride channel CFTR (cystic fibrosis transmembrane conductance regulator).[22]

In this addendum to our previous study,[12] we demonstrate that the removal of extracellular divalent cations potentiates the activation of BASIC by bile acids. Furthermore, we demonstrate that UDCA robustly activates rBASIC.

Results and Discussion

rBASIC is strongly inhibited by extracellular Mg^{2+}. We had previously shown that removal of extracellular Ca^{2+} opens rBASIC, suggesting that Ca^{2+} strongly stabilizes the inactive, resting state of rBASIC.[6] Here we determined the current amplitude of rBASIC with different concentrations of extracellular Mg^{2+} in the absence of Ca^{2+}, revealing that Mg^{2+} also strongly inhibited rBASIC with an IC_{50} of 79 ± 10 μM (n = 8; **Fig. 1**). Thus,

apparent affinity of Mg^{2+}-inhibition was 6-fold lower than of Ca^{2+}-inhibition (13 ± 2 μM, n = 8, p < 0.01; **Fig. 1**), showing that both, Ca^{2+} and Mg^{2+}, tightly control rBASIC activity and stabilize its resting state.

rBASIC activation by bile acids is potentiated by removal of divalent cations. Next, we tested whether removal of extracellular divalent cations affects the activation of BASIC by HDCA and whether it can further increase BASIC activity in the presence of a maximal concentration of HDCA. We used Mg^{2+}-free solutions and determined the EC_{50} of BASIC for HDCA both in the presence (1.8 mM) and the absence (10 nM) of extracellular Ca^{2+} (**Fig. 2**). Similar to our previous study,[12] we found that 2 mM HDCA did not change the concentration of free Ca^{2+} compared with standard bath (not shown), ruling out an unspecific effect via chelation of Ca^{2+}. EC_{50} for HDCA was modestly increased from 2.1 ± 0.04 mM in the presence of Ca^{2+} to 1.6 ± 0.05 mM in its absence (n = 8, p < 0.01; **Fig. 2B**). Moreover, at any concentration of HDCA, the current amplitudes induced by HDCA were three- to four-fold higher in the absence of extracellular Ca^{2+} than in its presence (**Fig. 2**). Thus, removal of Ca^{2+} potentiated activation by HDCA.

Removal of divalent cations not only opens BASIC but also changes its ion selectivity.[6] Therefore, we previously proposed that removal of divalent cations does not simply unblock the BASIC pore but induces a conformational change that is associated with open gating of the channel.[6] Assuming a pure effect of divalent cations on gating, potentiation of the BASIC current by removal of Ca^{2+} at a maximal concentration of HDCA suggests that HDCA is a partial agonist that does not induce full BASIC activity. Likewise, the similar HDCA concentration-response relationship in the presence and absence of Ca^{2+} suggests that HDCA further increases BASIC activity even when Ca^{2+} was not bound to the channel. These results, thus, argue that removal of Ca^{2+} and HDCA gate BASIC by an allosteric mechanism, meaning that removal of Ca^{2+} and bile acids use two independent molecular mechanisms

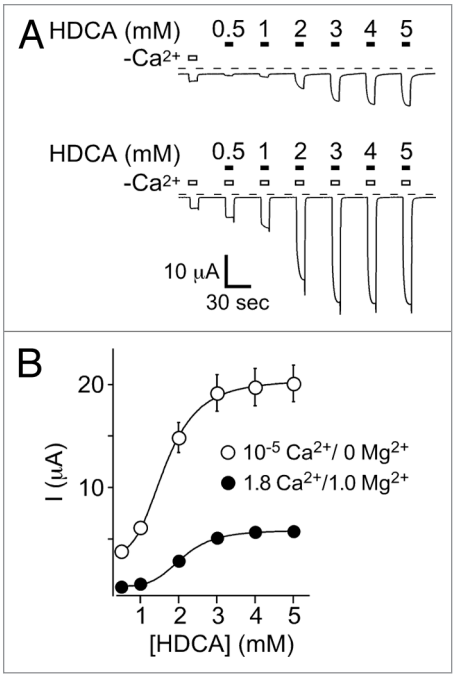

Figure 2. rBASIC activation by bile acids is potentiated by removal of extracellular divalent cations. (**A**) Representative current traces from rBASIC-expressing oocytes showing the concentration-dependent activation of rBASIC by HDCA in the presence of 1.8 mM extracellular Ca^{2+} and 1.0 mM extracellular Mg^{2+} (upper panel) or of 10 nM Ca^{2+} and 0 Mg^{2+} ("-Ca^{2+}") (lower panel). Dotted lines represent the 0 current level. (**B**) Concentration-response curves for HDCA in the presence of 1.8 mM extracellular Ca^{2+} and 1.0 mM extracellular Mg^{2+} (closed circles) or of 10 nM Ca^{2+} and 0 Mg^{2+} (open circles). Error bars = S.E.M., n = 8. Curves represent fits to the Hill-equation.

to synergistically influence the activity of BASIC. At present we cannot exclude, however, that a block by Ca^{2+} of the open BASIC pore is the reason of the increased current amplitude after removal of Ca^{2+}. Future studies determining the dependence of the open channel amplitude on Ca^{2+} will show whether Ca^{2+} has a pure gating effect on BASIC or a combined effect on gating and single channel amplitude.

rBASIC is activated by ursodeoxycholic acid. UDCA is structurally very similar to CDCA and HDCA, which strongly activate BASIC.[12] UDCA and HDCA differ only in the position of one hydroxyl group: in UDCA, C-7 is hydroxylated, whereas in HDCA, C-6 is hydroxylated. The only difference between UDCA and CDCA is the steric orientation of the hydroxyl group at position C-7: in UDCA it is in the β-position, whereas in CDCA it is in the α-position (**Fig. 3A**). Because of this high similarity we reasoned that UDCA might also activate rBASIC. Indeed, UDCA robustly activated rBASIC when applied at a concentration of 2 mM. The current amplitude induced by UDCA was even significantly higher (3.0 ± 0.5 μA, n = 10) than the amplitude induced by the same concentration of HDCA (1.9 ± 0.3 μA, n = 10, p < 0.05; **Fig. 3B**). Activation of rBASIC by UDCA was concentration dependent, similar to activation by HDCA (**Fig. 3C**). The EC_{50} value for UDCA was 2.5 ± 0.04 mM (n = 10), similar to the EC_{50} for HDCA (2.1 ± 0.04 mM, n = 8). We have shown previously that when HDCA and CDCA are applied together at a concentration of 1.0 and 0.5 mM, respectively, they induce a significantly larger current amplitude than when applied individually, suggesting that both bile acids activate the channel synergisitically.[12] The same applies for UDCA and CDCA (**Fig. 3D**). UDCA at a concentration of 1 mM together with 0.5 mM CDCA induced a 3-fold larger current (2.4 ± 0.3 μA, n = 10) than UDCA applied alone at a concentration of 1.5 mM (0.7 ± 0.1 μA, n = 10, p < 0.01), suggesting that CDCA and UDCA synergistically activate rBASIC.

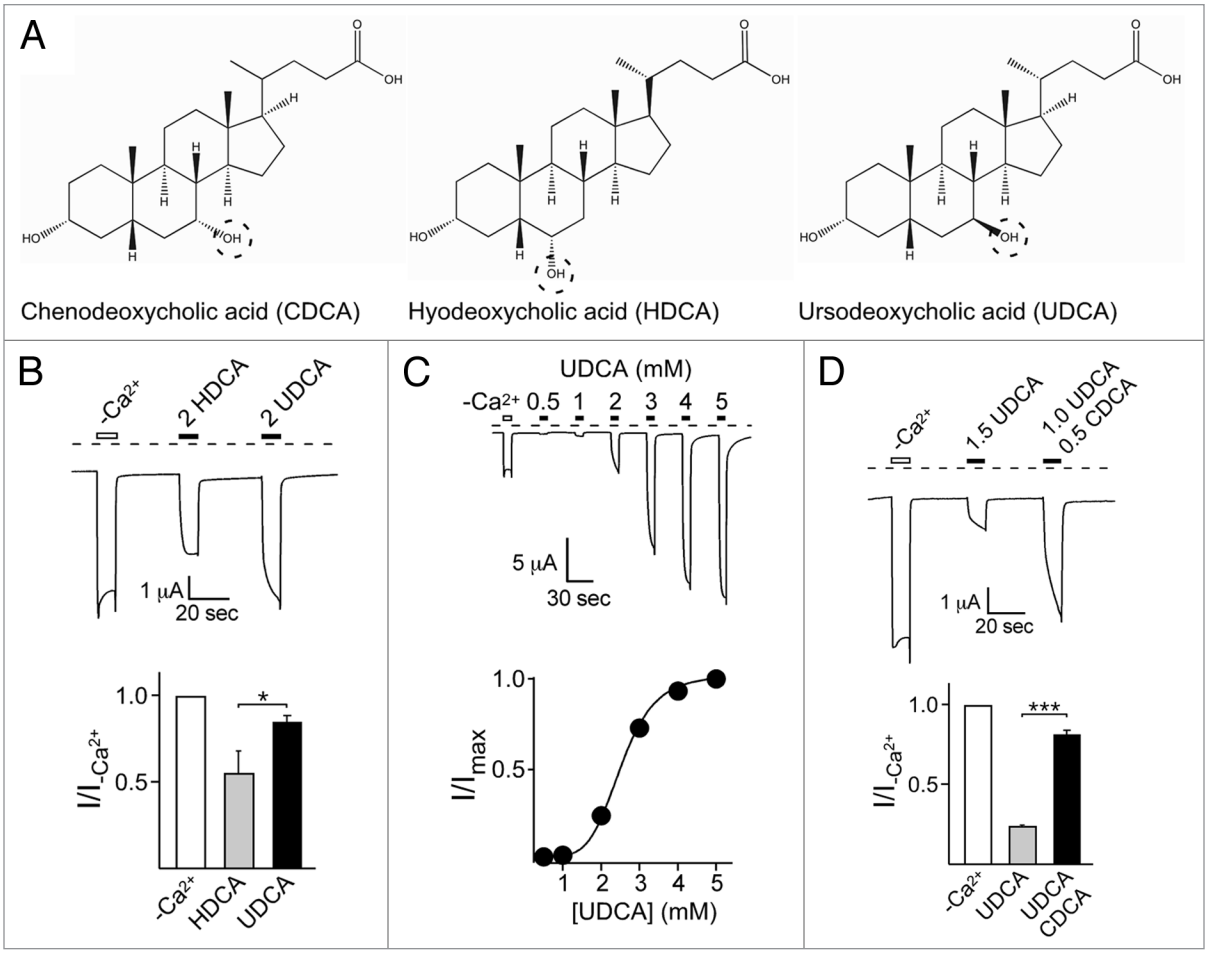

Figure 3. rBASIC is activated by ursodeoxycholic acid (UDCA). (**A**) Structures of cheno-, hyo- and ursodeoxycholic acid. The different hydroxylgroups are highlighted with dashed circles. Structures were drawn using CS ChemDraw Ultra (CambridgeSoft) based on structures available from PubChem (http://pubchem.ncbi.nlm.nih.gov/). (**B**) rBASIC activation by UDCA is stronger than by HDCA. Upper panel, representative current trace from an rBASIC-expressing oocyte showing the activation of rBASIC by individual application of 2 mM HDCA or UDCA. Dotted line represents the 0 current level. Lower panel, quantitative comparison of current amplitudes induced by HDCA (gray bar) and UDCA (black bar). Currents were normalized to the current induced by the removal of extracellular divalent cations (10 nM Ca^{2+}, 0 Mg^{2+}; "-Ca^{2+}") which was 3.6 ± 0.5 µA (n = 10). Error bars = SEM (**C**) Upper panel, representative current trace from an rBASIC-expressing oocyte showing the concentration-dependent activation of rBASIC by UDCA. Lower panel, concentration-response curve for UDCA. Currents were normalized to the maximum current in the presence of 5 mM UDCA, which was 13.2 ± 1.1 µA (n = 8). Error bars = SEM. Curve represents a fit to the Hill-equation. (**D**) CDCA potentiates the activation of rBASIC by UDCA. Upper panel, representative current trace showing the activation of rBASIC by individual application of 1.5 mM UDCA and by co-application of 1.0 mM UDCA and 0.5 mM CDCA, respectively. Lower panel, quantitative comparison of current amplitudes induced by UDCA (gray bar) and UDCA/CDCA (black bar). Currents were normalized to the current induced by the removal of extracellular divalent cations ("-Ca^{2+}") which was 2.9 ± 0.4 µA (n = 10). Error bars = SEM.

Whether the effect of UDCA on rBASIC activity is of any physiological or clinical relevance remains to be elucidated but it is tempting to speculate that rBASIC is involved in absorptive and/or secretory processes in cholangiocytes and that it might represent an hitherto unknown molecular target of UDCA. BASIC is a cation channel, however, and cation reabsorption by cholangiocytes would lead to a net fluid absorption and thus reduced bile flow, which contradicts the anticholestatic effect of UDCA. Future results will show whether BASIC contributes to cation transport in bile ducts and whether it promotes secretion or absorption by the bile duct epithelium.

Outlook

Despite our recent progress regarding the expression pattern of BASIC in cholangiocytes and regarding its activation by bile acids, we are still far away from understanding the physiological role of BASIC in these cells, let alone its role in other tissues such as the intestinal tract or the brain. In future studies it will therefore be important to confirm expression of BASIC in cholangiocytes and to discover its cellular and subcellular expression pattern in other tissues. Furthermore it will be necessary to study BASIC in a native cell or a native epithelium or both, to unravel its role in ion transport mediated by these cells and epithelia.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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