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

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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.

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 and the role of ASICs in neuronal transmission and sensation of painful acidosis has been revealed over the last years, 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. 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. 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 Ca2+. The residual rBASIC current is unselective but removal of extracellular Ca2+ opens rBASIC and renders the channel more selective for Na+ over K+.

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. Additional pharmacological tools to investigate the physiological function of BASIC were identified recently. The anti-protozoal diarylamidines, in particular diminazene and the related compound nafamostat, inhibit BASIC at micromolar concentrations of the drug. Additional pharmacological tools to investigate the physiological function of BASIC were identified recently. The anti-protozoal diarylamidines, in particular diminazene and the related compound nafamostat, inhibit BASIC at micromolar concentrations of the drug. Additional pharmacological tools to investigate the physiological function of BASIC were identified recently. The anti-protozoal diarylamidines, in particular diminazene and the related compound nafamostat, inhibit BASIC at micromolar concentrations of the drug. Additional pharmacological tools to investigate the physiological function of BASIC were identified recently.

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. The ligand-hypothesis gained further support when flufenamic acid
(FFA) was identified as an artificial agonist of rBASIC. Micro- to millimolar concentrations of FFA rapidly activate the channel, inducing Na+-selective currents. Various bile acids naturally occurring in mouse, rat and pig bile, in particular hyocholic acid (CA), β-MCA and HDCA are the major bile acids. Hundreds of different bile acids are known to date, the structure of their side chain, their stereochemistry and the number and position of their hydroxyl groups 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. In traditional chinese medicine, UDCA isolated from bear bile has been administered as a remedy for liver diseases for almost 3,000 years. In the 20th century, UDCA was discovered by Western medicine as a compound capable of dissolving gallstones and inducing choleresis, an increased bile flow. Today UDCA is used to treat various cholestatic liver diseases, for example primary biliary cirrhosis, primary sclerosing cholangitis or intrahepatic cholestasis of pregnancy. 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), and by increasing the insertion rate of these proteins into the apical membrane. Furthermore, UDCA was shown to have anti-apoptotic effects in hepatocytes. Cholangiocytes are constantly exposed to high concentrations of hydrophobic bile acids, which can damage the plasma membrane leading to cholangiocyte malfunction. UDCA, a relatively hydrophilic bile acid counteracts this membrane-damaging effect in vitro. 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).

In this addendum to our previous study, 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 Mg2+. We had previously shown that removal of extracellular Ca2+ opens rBASIC, suggesting that Ca2+ strongly stabilizes the inactive, resting state of rBASIC. Here we determined the current amplitude of rBASIC with different concentrations of extracellular Mg2+ in the absence of Ca2+, revealing that Mg2+ also strongly inhibited rBASIC with an IC50 of 79 ± 10 μM (n = 8; Fig. 1). Thus,
apparent affinity of Mg\textsuperscript{2+}-inhibition was 6-fold lower than of Ca\textsuperscript{2+}-inhibition (13 ± 2 μM, n = 8, p < 0.01; Fig. 1), showing that both, Ca\textsuperscript{2+} and Mg\textsuperscript{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\textsuperscript{2+}-free solutions and determined the EC\textsubscript{50} of BASIC for HDCA both in the presence (1.8 mM) and the absence (10 nM) of extracellular Ca\textsuperscript{2+} (Fig. 2). Similar to our previous study, we found that 2 mM HDCA did not change the concentration of free Ca\textsuperscript{2+} compared with standard bath (not shown), ruling out an unspecific effect via chelation of Ca\textsuperscript{2+}. EC\textsubscript{50} for HDCA was modestly increased from 2.1 ± 0.04 mM in the presence of Ca\textsuperscript{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\textsuperscript{2+} than in its presence (Fig. 2). Thus, removal of Ca\textsuperscript{2+} potentiated activation by HDCA.

Removal of divalent cations not only opens BASIC but also changes its ion selectivity. 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. Assuming a pure effect of divalent cations on gating, potentiation of the BASIC current by removal of Ca\textsuperscript{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\textsuperscript{2+} suggests that HDCA further increases BASIC activity even when Ca\textsuperscript{2+} was not bound to the channel. These results, thus, argue that removal of Ca\textsuperscript{2+} and HDCA gate BASIC by an allosteric mechanism, meaning that removal of Ca\textsuperscript{2+} and bile acids use two independent molecular mechanisms to synergistically influence the activity of BASIC. At present we cannot exclude, however, that a block by Ca\textsuperscript{2+} of the open BASIC pore is the reason of the increased current amplitude after removal of Ca\textsuperscript{2+}. Future studies determining the dependence of the open channel amplitude on Ca\textsuperscript{2+} will show whether Ca\textsuperscript{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. 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 stereic 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\textsubscript{50} value for UDCA was 2.5 ± 0.04 mM (n = 10), similar to the EC\textsubscript{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 synergistically. 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 2](image.png)

**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\textsuperscript{2+} and 1.0 mM extracellular Mg\textsuperscript{2+} (upper panel) or of 10 nM Ca\textsuperscript{2+} and 0 Mg\textsuperscript{2+} (‘-Ca\textsuperscript{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\textsuperscript{2+} and 1.0 mM extracellular Mg\textsuperscript{2+} (closed circles) or of 10 nM Ca\textsuperscript{2+} and 0 Mg\textsuperscript{2+} (open circles). Error bars = S.E.M., n = 8. Curves represent fits to the Hill-equation.
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.

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 important 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.

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No potential conflicts of interest were disclosed.
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