



# Comparative electrophysiological analysis of the bile acid-sensitive ion channel (BASIC) from different species suggests similar physiological functions

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## Abstract

Despite the identification of cholangiocytes in the liver and unipolar brush cells in the cerebellum as sites of expression, the physiological function of the bile acid-sensitive ion channel (BASIC) remains unknown. Rat BASIC (rBASIC) and mouse BASIC (mBASIC) share 97% of their amino acid sequence but show strikingly different biophysical properties. rBASIC is inactive at rest while mBASIC is constitutively active, when expressed in *Xenopus* oocytes. This conundrum rendered the identification of the physiological function even more difficult. In this study, we investigated the electrophysiological and pharmacological properties of BASIC from rat, mouse, and human in Hek293 cells using the patch clamp technique. Surprisingly, in Hek293 cells, rBASIC and mBASIC showed almost completely identical properties. Both are blocked by extracellular  $\text{Ca}^{2+}$  and thus are inactive at rest; both are selective for  $\text{Na}^+$ , show similar affinities for extracellular  $\text{Ca}^{2+}$ , were inhibited by diminazene, and activated by various bile acids. This is in contrast to previous results derived from *Xenopus* oocytes as expression system and suggests that the cell type is important for shaping the biophysical properties of BASIC. Furthermore, we compared hBASIC with rBASIC and mBASIC and observed similar properties between these channels with one exception: the bile acid sensitivity profile of hBASIC is different from rBASIC and mBASIC; hBASIC is more sensitive to bile acids which are abundant in human bile but not in rodent bile. Taken together, these results suggest similar physiological roles for BASIC in different species.

**Keywords** BASIC · Bile acid · Cation channel · ENaC · ASIC

## Abbreviations

ASIC	Acid-sensing ion channel
BASIC	Bile acid-sensitive ion channel
CA	Cholic acid
CDCA	Chenodeoxycholic acid
DCA	Deoxycholic acid
ENaC	Epithelial $\text{Na}^+$ channel
HDCA	Hyodeoxycholic acid
LCA	Lithocholic acid
UDCA	Ursodeoxycholic acid

## Introduction

The bile acid-sensitive ion channel (BASIC) is a member of the DEG/ENaC family of ion channels [13, 14, 25]. The family is comprised of several subfamilies from different species with different expression patterns and a large variety of function ranging from mechanosensation in *C. elegans* [2, 6], and peptide-driven neuronal signal transmission in mollusks [16] and Hydra [1, 7] to epithelial reabsorption [4] and pain sensation in higher animals [24]. In higher animals, the neuronal proton-gated acid-sensing ion channels (ASICs) form the largest DEG/ENaC subfamily, followed by the epithelial  $\text{Na}^+$  channel (ENaC) subfamily. BASIC represents the third subfamily [25]. DEG/ENaC channels form homo- or heterotrimeric channels, each subunit is characterized by two transmembrane segments which are linked on the extracellular side by a large glycosylated domain. The N- and C-terminus protrude into the cytosol [12, 30].

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BASIC is mainly found in the brain, liver, and intestinal tract but to a weaker extent also in the testes, lung, and kidney [17, 19]. In the liver, BASIC expression is restricted to cholangiocytes [25, 28], the epithelial cells, which line the bile duct and modify bile composition. In the brain, BASIC is expressed in a subset of unipolar brush cells in the cerebellum [3]. The function of BASIC neither in the liver nor in the brain is known [3, 28]. As the name bile acid-sensitive ion channel suggest, bile acids are natural activators of BASIC. Milimolar concentrations of various bile acids can robustly and reversibly activate BASIC [25, 28, 29]. This stimulatory effect of bile acids on BASIC is putatively not direct but rather an indirect bile acid-dependent alteration of the channel's membrane surrounding, which induces the opening of the channel [20, 21]. Consequently, specific membrane properties of different cell types, which express BASIC, may determine the physiological function of the channel in this particular cell type. Another study proposed that the degenerin region within the transmembrane domain of hBASIC is involved in bile acid sensitivity [11].

BASIC from rat and mouse share 97% sequence identity [17] but despite this similarity, they are characterized by different electrophysiological and pharmacological properties. When expressed in *Xenopus* oocytes, rBASIC and hBASIC show a weak constitutive activity. In this low activity resting state, the channels are not selective for Na<sup>+</sup> over K<sup>+</sup> [26]. mBASIC on the other hand is highly active at rest and selective for Na<sup>+</sup> over K<sup>+</sup>. This difference is due to one amino acid change between rBASIC and mBASIC. At position 387, rBASIC contains a serine, mBASIC an alanine. This amino acid difference leads to drastic changes in their affinity for extracellular Ca<sup>2+</sup> [26]. While rBASIC has a high affinity for Ca<sup>2+</sup>, which blocks the channel at physiological concentrations of extracellular Ca<sup>2+</sup>, mBASIC has a very low affinity for Ca<sup>2+</sup> and is not blocked by physiological concentrations of Ca<sup>2+</sup><sub>ex</sub>. Interestingly, hBASIC, just like mBASIC, contains an alanine at this crucial position; however, the channel shows the same biophysical properties like rBASIC: weak resting activity due to a high affinity block by Ca<sup>2+</sup>. Another difference between rBASIC, hBASIC, and mBASIC is their different affinity for the diuretic amiloride, a general blocker of DEG/ENaC channels. While rBASIC is only partly inhibited by amiloride with a low affinity, mBASIC is inhibited by amiloride with a dramatically higher apparent affinity [26]. Another inhibitor of DEG/ENaCs is the diarylamidine diminazene [27]. Interestingly, no difference in apparent affinity for diminazene is observed between rBASIC and mBASIC.

Because most studies on BASIC were performed using *Xenopus* oocytes as heterologous expression system [17, 19, 26–28], we aimed to verify these data using a different heterologous system. We expressed

rBASIC, mBASIC, and hBASIC in Hek293 cells and screened various typical electrophysiological and pharmacological features of BASICs. While some results for rBASIC and hBASIC were expected and similar to results from *Xenopus* oocyte experiments, the results we obtained for mBASIC were surprising and strikingly different than expected. mBASIC behaved very similar to rBASIC and hBASIC. We conclude that the expression system strongly affects key properties of these channels and suggest that the membrane composition is responsible for this difference.

## Materials and methods

### Molecular biology

cDNA of rBASIC (GenBank™ accession no. [NM\\_022227](#)), mBASIC (GenBank™ accession no. [NM\\_022227](#)), and hBASIC (GenBank™ accession no. [NM\\_017419](#)) were cloned into the vector pDNA3.1.

### Whole cell patch clamp recordings

Whole cell patch clamp measurements on Hek293 cells were conducted as described previously [21]. Patch pipettes of 4–6 MΩ resistance filled with (in mM) 10 NaCl, 121 KCl, 2 MgCl<sub>2</sub>, 5 EGTA, 2 Na<sub>2</sub>-ATP, 10 HEPES, and pH 7.25 were used. The bath solution contained (in mM) 128 NaCl, 5.4 KCl, 1 MgCl<sub>2</sub>, 2 CaCl<sub>2</sub>, 5.55 glucose, 10 HEPES, and pH 7.4 with NaOH. Osmolarity was adjusted with sucrose to 280 mosmol/l for the pipette solution and to 290 mosmol/l for the extracellular solution. Data were acquired using an Axon Digidata 1440A and an Axon Axopatch 200B and recorded, filtered at 2 kHz and digitized at 20 kHz and analyzed using the pClamp software (Axon Instruments, Molecular Devices, Sunnyvale, CA). All experiments were conducted at room temperature.

### Chemicals

Taurocholic acid (CA), taurodeoxycholic acid (DCA), taurochenodeoxycholic acid (CDCA), tauroolithocholic acid (LCA), and taurohyodeoxycholic (HDCA) acid were purchased from Sigma Aldrich (Germany). Tauroursodeoxycholic (UDCA) acid was purchased from Merck (Germany). Bile acids induce unspecific currents in *Xenopus* oocytes when applied at solubilizing concentrations. To avoid unspecific currents the maximal concentrations used in this study were 1 mM for DCA and CDCA; 2 mM for CA, HDCA, and LCA; and 7.5 mM for UDCA.

## Data analysis and statistics

Data were analyzed using the software IgorPro (WaveMetrics, Lake Oswego, OR) and are presented as means  $\pm$  S.E. Statistical significance was calculated using Student's unpaired *t* test.

Concentration-response curves for  $[Ca^{2+}]_{ex}$ , UDCA and diminazene were fitted with a Hill function:

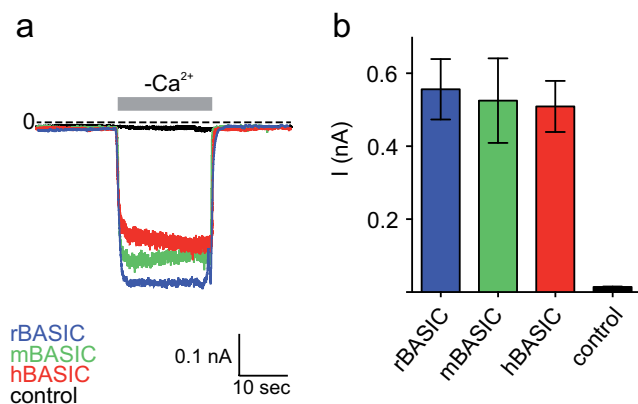
$$I = I_{max} - I_0 / [1 + (EC_{50}/[c])^n] \quad (1)$$

where  $I_{max}$  is the maximal current,  $I_0$  is the residual current,  $[c]$  is the concentration of the substance,  $n$  is the Hill-coefficient, and  $EC_{50}$  is the concentration at which half-maximal response occurs.

## Results

### BASIC from different species is inactive at rest and inhibited by physiological concentrations of extracellular $Ca^{2+}$

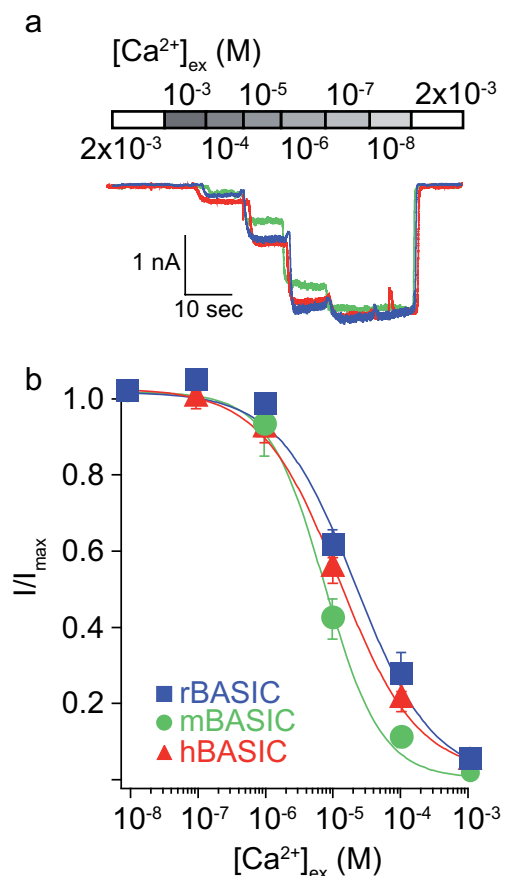
The heterologous expression of BASIC from rat, mouse, and human revealed complete inactivity at rest and activation upon removal of extracellular divalent cations ( $-Ca^{2+}$ ) (Fig. 1). The resulting currents did not desensitize and were reversed upon increase of the extracellular  $-Ca^{2+}$ -concentration. Current amplitudes were similar between rat, mouse, and human BASIC (rBASIC,  $556 \pm 83$  nA; mBASIC,  $525 \pm 116$  nA; and hBASIC  $509 \pm 70$  nA) (Fig. 1b). This result was somewhat unexpected since mBASIC is active at rest when expressed in *Xenopus* oocytes and suggested that the cellular surrounding



**Fig. 1** rBASIC, mBASIC, and hBASIC are activated by removal of extracellular  $Ca^{2+}$ . **a** Representative current traces of Hek293 cells expressing rBASIC (blue), mBASIC (green), or hBASIC (red) and untransfected Hek293 cells (black). Currents were recorded in 1.8 mM  $Ca^{2+}$  and 10 nM  $Ca^{2+}$  ( $-Ca^{2+}$ ), respectively; holding potential was  $-70$  mV. **b** Quantitative analysis of current amplitudes from **a**. Untransfected cells did not respond to 10 nM  $Ca^{2+}$  ( $-Ca^{2+}$ ) and served as control,  $n = 8$ . Error bars, S.E

of the channel is important for its electrophysiological properties.

Next, we aimed at defining the apparent affinity of all three channels for extracellular  $-Ca^{2+}$ . Decreasing concentrations of extracellular  $-Ca^{2+}$  were applied to Hek293 cells expressing the respective channel, increasing the BASIC conducted inward current (Fig. 2a). The apparent  $EC_{50}$  for  $[Ca^{2+}]_{ex}$  was  $38 \pm 2$   $\mu$ M ( $n = 8$ ) for rBASIC, which is approximately four-fold lower compared to expression in *Xenopus* oocytes (10  $\mu$ M) [26] (Fig. 2b). For hBASIC, the apparent  $EC_{50}$  for  $[Ca^{2+}]_{ex}$  was  $12 \pm 0.5$   $\mu$ M ( $n = 8$ ), which is also similar to results obtained with expression *Xenopus* oocytes (18  $\mu$ M) [15]. Different to expression in *Xenopus* oocytes, mBASIC in Hek293 cells showed the highest apparent affinity for  $[Ca^{2+}]_{ex}$  ( $EC_{50}$ ,  $6 \pm 0.01$   $\mu$ M,  $n = 8$ ) (Fig. 2b). Taken together physiological concentrations of extracellular divalent cations almost completely inhibit all three orthologs, suggesting that extracellular divalent cations stabilize their inactive resting state when heterologously expressed in Hek293 cells. Only



**Fig. 2** rBASIC, mBASIC, and hBASIC are inhibited by physiological concentrations of extracellular  $Ca^{2+}$ . **a** Representative current traces of Hek293 cells expressing rBASIC (blue), mBASIC (green), and hBASIC (red) activated by decreasing concentrations of extracellular  $Ca^{2+}$ . **b** Concentration-dependent inhibition of rBASIC, mBASIC, and hBASIC by  $[Ca^{2+}]_{ex}$ . Currents were normalized to the maximum current in the presence of 10 nM  $[Ca^{2+}]_{ex}$ ;  $n = 8$ . Error bars, S.E

under non-physiological concentrations of  $[Ca^{2+}]_{ex}$ , the three channels were active.

**Rat and mouse BASIC have similar pharmacological characteristics** Rat BASIC is strongly activated by millimolar concentrations of various bile acids. Among those bile acids, ursodeoxycholic acid (UDCA) is one of the most potent activators. In contrast, mBASIC is not affected by bile acids when expressed in *Xenopus* oocytes [28]. To analyze the sensitivity of rBASIC and mBASIC in Hek293 cells, we tested the effect of UDCA in the absence and presence of  $[Ca^{2+}]_{ex}$ .

We applied increasing concentrations of UDCA on Hek293, which resulted for both rBASIC and mBASIC in a reversible dose-dependent increase in current amplitude (Fig. 3a). The apparent affinity for UDCA of rBASIC and mBASIC was in a similar range; however, the  $EC_{50}$  of UDCA for rBASIC was slightly but significantly lower than for mBASIC (rBASIC  $2.7 \pm 0.1$  mM, mBASIC  $4.5 \pm 0.03$  mM,  $n = 8$ ). Surprisingly, the current amplitude of rBASIC at all concentrations of UDCA was dramatically higher compared to mBASIC suggesting that the efficacy of UDCA is higher for rBASIC than for mBASIC (Fig. 3a).

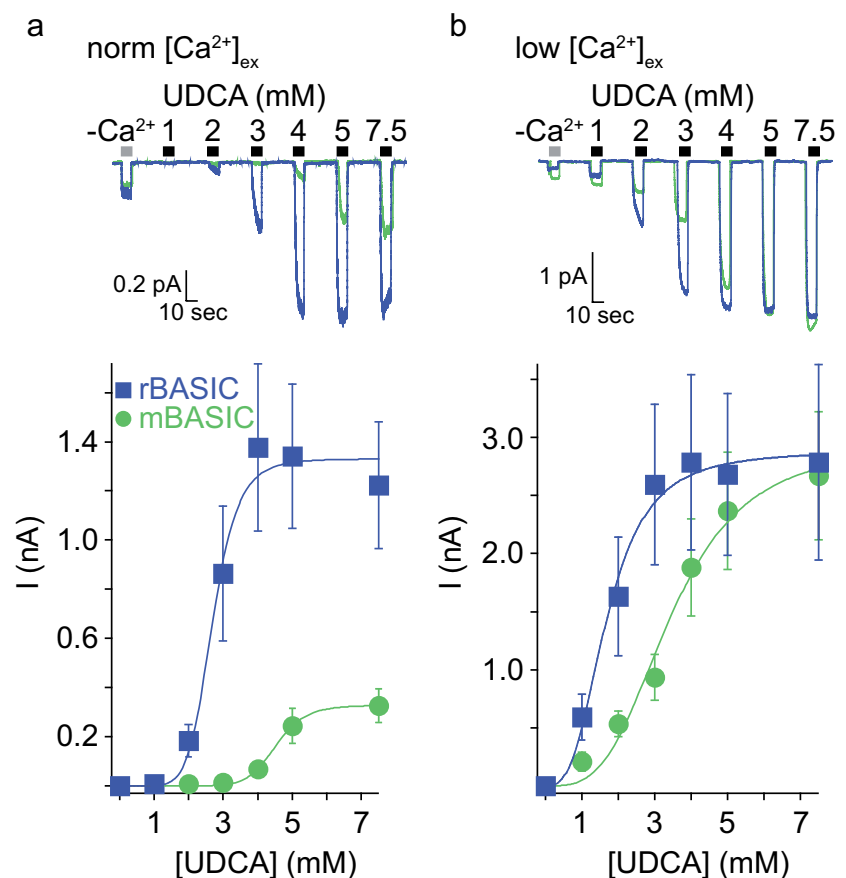
We repeated the experiment in the absence of divalent cations. The apparent affinity of UDCA for both rBASIC and mBASIC was not significantly affected by the absence of

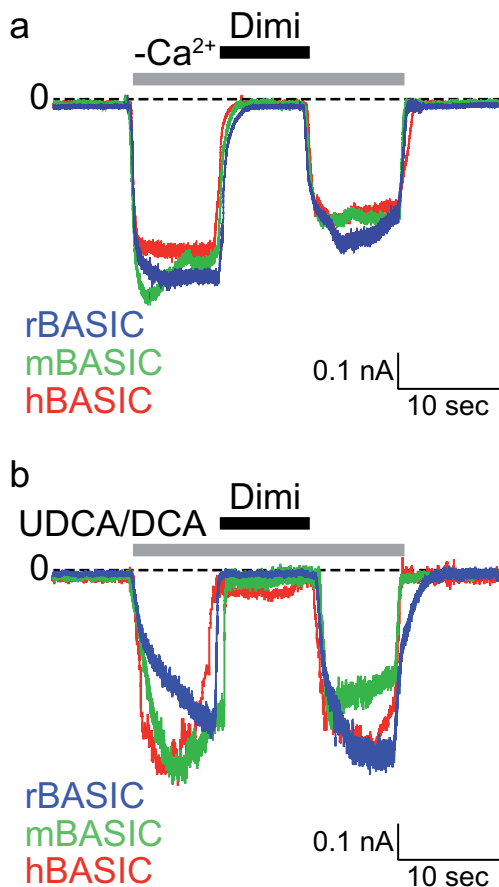
divalent cations (rBASIC  $1.7 \pm 0.1$  mM, mBASIC  $3.5 \pm 0.03$  mM,  $n = 9$ ) (Fig. 3b). However, we did not observe a difference in efficacy of UDCA for rBASIC and mBASIC. The maximum current amplitudes at 7.5 mM UDCA of both rBASIC and mBASIC were similar (rBASIC  $2.8 \text{ nA} \pm 0.8 \text{ nA}$ , mBASIC  $2.7 \pm 0.6 \text{ nA}$ ,  $n = 9$ ). Taken together, despite some variations in apparent affinity for UDCA and UDCA efficacy, rBASIC and mBASIC expressed in Hek293 cells behave more similar in response to UDCA than when expressed in *Xenopus* oocytes.

Diminazene is a potent inhibitor of ASICs [5, 22] and BASIC [27]. All three channels were almost completely inhibited by 100  $\mu\text{M}$  diminazene when activated either by extracellular  $Ca^{2+}$ -removal or 5 mM UDCA (rBASIC, mBASIC) or DCA (hBASIC) respectively (Fig. 4). Next, we determined the apparent affinity of rBASIC and mBASIC for diminazene (Fig. 5). Increasing concentrations of diminazene were applied inducing a dose-dependent decrease in current amplitude (Fig. 5a, c). Similar to *Xenopus* oocytes, the apparent affinities of rBASIC and mBASIC for diminazene were in a similar micromolar range ( $IC_{50}$   $[Ca^{2+}]_{ex}$  rBASIC,  $7.9 \pm 1.9 \mu\text{M}$ ; mBASIC,  $16.8 \pm 5.6 \mu\text{M}$ ;  $IC_{50}$  UDCA rBASIC,  $5 \pm 1.6 \mu\text{M}$ ; mBASIC,  $3.7 \pm 0.4 \mu\text{M}$ ;  $n = 9$ ) (Fig. 5b, d).

rBASIC expressed in *Xenopus* oocytes is characterized by an unselective ion pore in its low activity resting state [26] and

**Fig. 3** Effect of extracellular  $Ca^{2+}$  on the apparent affinity of rBASIC and mBASIC for the bile acid UDCA. **a** Upper, representative current traces from Hek293 cells transfected with rBASIC (blue) and mBASIC (green) activated by increasing concentrations of UDCA in the presence of physiological concentrations of extracellular  $Ca^{2+}$  (1.8 mM). Lower, concentration-response curves of UDCA for rBASIC (blue) and mBASIC (green) in the presence of 1.8 mM extracellular  $Ca^{2+}$ . **b** Upper, representative current traces from Hek293 cells transfected with rBASIC (blue) and mBASIC (green) activated by increasing concentrations of UDCA in the presence of low extracellular  $Ca^{2+}$  concentrations (10 nM). Lower, concentration-response curves of UDCA for rBASIC (blue) and mBASIC (green) in the presence of 10 nM extracellular  $Ca^{2+}$ . Error bars, S.E.,  $n = 8$ . Curves represent fits to the Hill-equation





**Fig. 4** Diminazene reversibly inhibits BASICs. **a, b** Representative current traces of Hek293 cells expressing rBASIC (blue), mBASIC (green), and hBASIC (red), activated by  $\text{Ca}^{2+}$ -removal (**a**) and UDCA (r/mBASIC) or DCA (hBASIC) (**b**) and inhibited by 100  $\mu\text{M}$  diminazene

when activated by bile acids [28]. However, upon relief of the  $\text{Ca}^{2+}$ -block, the channel becomes more selective for  $\text{Na}^+$  [26]. hBASIC shows similar characteristics [15]. However, mBASIC is more selective for  $\text{Na}^+$  over  $\text{K}^+$  [26]. We analyzed the selectivity of rBASIC and mBASIC by determining the current-voltage relation of rBASIC and mBASIC when activated by relief from the  $\text{Ca}^{2+}$ -block or 5 mM UDCA. Both, rBASIC and mBASIC, showed similar current-voltage relations either upon activation by removal of extracellular divalent cations (Fig. 6a) or by application of UDCA (Fig. 6b). The reversal potential was in the range of +25 to +30 mV (rev. pot.: rBASIC $_{[-\text{Ca}^{2+}]_{\text{ex}}}$ ,  $27.3 \pm 3.9$  mV; mBASIC $_{[-\text{Ca}^{2+}]_{\text{ex}}}$ ,  $24.6 \pm 3.5$  mV; rBASIC $_{\text{UDCA}}$ ,  $32.5 \pm 2.8$  mV; mBASIC $_{\text{UDCA}}$ ,  $28.3 \pm 3.2$  mV;  $n = 8$ ). This indicates that rBASIC and mBASIC are selective for  $\text{Na}^+$  over  $\text{K}^+$  and is in line with previous results for mBASIC and rBASIC, when activated by extracellular divalent removal.

Taken together, in Hek293 cells, rBASIC and mBASIC show similar electrophysiological and pharmacological features, which is in contrast to expression in *Xenopus* oocytes. This suggests that the expression system and possibly the

plasma membrane may affect biophysical properties of these highly similar ion channels differently.

### The bile acid sensitivity profile of hBASIC is different from rBASIC and mBASIC

The composition of bile acids varies between species. Rat and mouse bile is mainly composed of cholic acid (CA),  $\beta$ -muricholic acid ( $\beta\text{MCA}$ ), and hyodeoxycholic acid (HDCA) [18]. Human bile on the other hand contains mainly CA, deoxycholic acid (DCA), and chenodeoxycholic acid (CDCA) [8, 23]. In *Xenopus* oocytes, hBASIC was most potently activated by CDCA, one of the most abundant bile acids in humans. UDCA or HDCA only weakly activated hBASIC [28]. rBASIC on the other hand was most potently activated by HDCA and UDCA [28]. Here, we re-evaluate the differences in bile acid profiles for rBASIC, mBASIC, and hBASIC in Hek293 cells. All six bile acids tested resulted in robust responses. For rBASIC, the strongest response was induced by HDCA and UDCA. CA, LCA, DCA, and CDCA induced similar responses. For mBASIC, different bile acids induced similar responses (Fig. 7). hBASIC showed a distinctly different bile acid sensitivity profile. CA, UDCA, HDCA, and LCA induced only weak responses, while DCA and CDCA strongly activated hBASIC (Fig. 7). The effects of DCA and CDCA were of similar potency as UDCA and HDCA for rBASIC, although they were applied at 1 mM and not at 2 mM, thus the potency of DCA and CDCA for hBASIC activation is probably underestimated. However, the adverse effect of DCA and CDCA on Hek293 at concentrations above 1 mM renders a more precise analysis difficult.

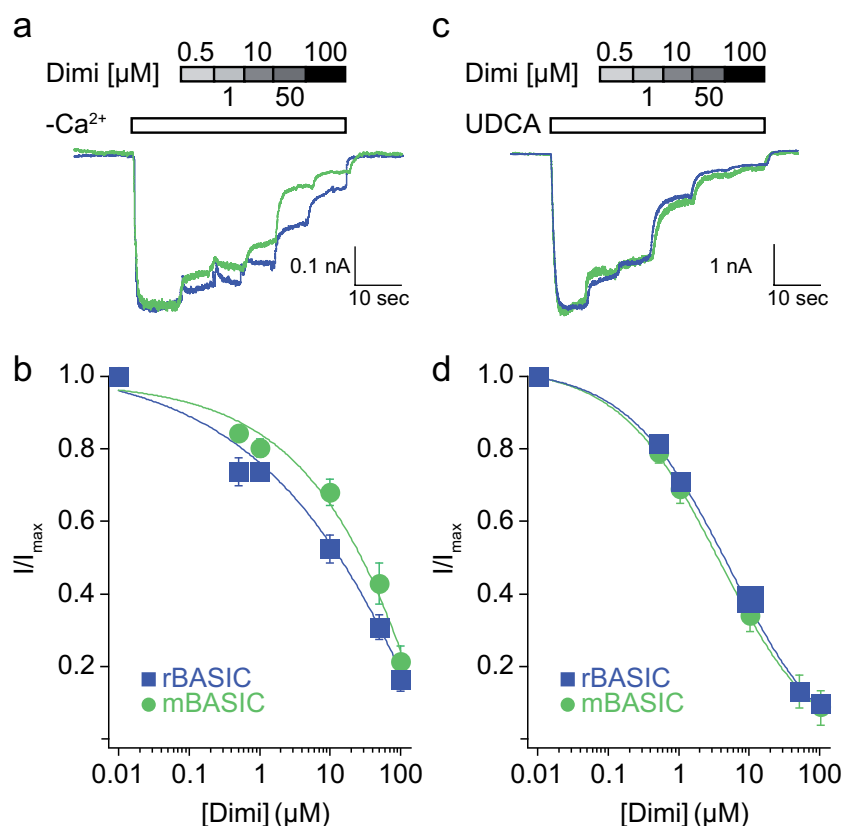
Taken together, rBASIC, mBASIC, and hBASIC responded differently to various bile acids, a finding which is similar to previous results obtained in *Xenopus* oocytes. The difference in bile acid sensitivity might represent an adaptation to the bile acid composition of different species.

## Discussion

Despite a high degree of sequence similarity, the electrophysiological and pharmacological properties of BASIC from mouse, rat, and human differ strongly when studied in *Xenopus laevis* oocytes [15, 17, 26]. The relevance of these differences for their possible physiological function remains a complete puzzle. In this study, we revisited the properties of BASICs but employed Hek293 cells as expression system.

Our study has two novel findings: (1) when expressed in Hek293 cells the previously described discrepancies between BASIC from mouse and rat diminish, rBASIC and mBASIC show almost identical properties. (2) Human BASIC also shows the same characteristics as rat and mouse BASIC;

**Fig. 5** Apparent affinity of rBASIC and mBASIC for diminazene. **a** Representative current traces of Hek293 cells expressing rBASIC (blue) and mBASIC (green) activated by removal of extracellular divalent cations ( $-\text{Ca}^{2+}$ ) and inhibited by increasing concentrations of diminazene. **b** Concentration-dependent inhibition of rBASIC and mBASIC by diminazene. Currents were normalized to the maximum current in the absence of diminazene;  $n = 8$ . Error bars, S.E. **c** Representative current traces of Hek293 cells expressing rBASIC (blue) and mBASIC (green) activated by 5 mM UDCA and inhibited by increasing concentrations of diminazene. **d** concentration-dependent inhibition of rBASIC and mBASIC by diminazene. Currents were normalized to the maximum current in the absence of diminazene;  $n = 8$ . Error bars, S.E.



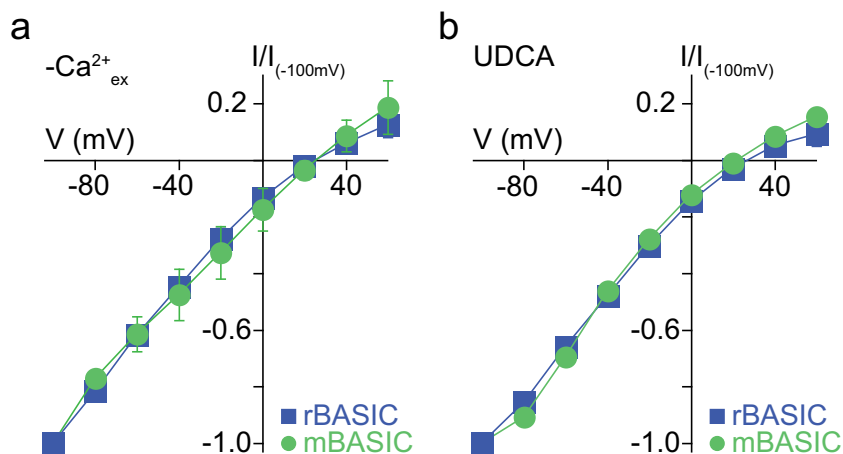
however, it shows a difference in responsiveness to different bile acids.

In line with our previous studies, rBASIC shows some typical electrophysiological and pharmacological characteristics when expressed in Hek293 cells: it is inactive at rest, which is due to a block by physiological concentrations of extracellular calcium. Consequently, removal of extracellular calcium induces an activation of the channel. Furthermore, it is activated by various bile acids and inhibited by diminazene. Contrary to previous results, mouse BASIC is also inactive at rest and not constitutively active as in oocytes. Just like

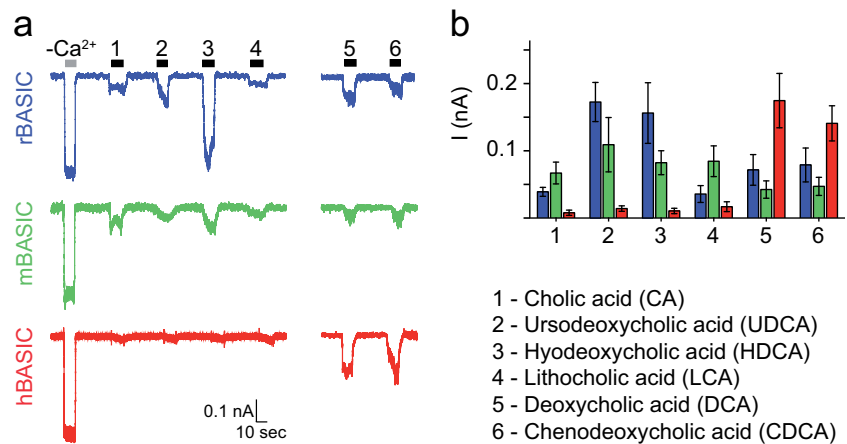
rBASIC, mBASIC is blocked by similar concentrations of extracellular calcium. Furthermore, it is inhibited by diminazene and activated by a range of different bile acids. This finding was somewhat surprising and suggests that the cellular context seems to be important for shaping the properties of the channel. Since BASIC was shown to be sensitive to its membrane surrounding, we speculate that different membrane properties of *Xenopus* oocytes and Hek293 cells may be important for the biophysical properties of the channels.

Is constitutive activity of mBASIC an oocyte artifact? It is puzzling how and why such drastic differences of mBASIC

**Fig. 6** BASICs are  $\text{Na}^+$ -selective channels when expressed in Hek293 cells. **a**, **b** Normalized mean current-voltage relationships of the maximum currents of rBASIC (blue) and mBASIC (green) induced by removal of extracellular divalent cations ( $-\text{Ca}^{2+}$ ) (**a**) or 5 mM UDCA (**b**). The holding potential was increased stepwise from  $-100$  to  $+60$  mV in 20-mV steps. Error bars, S.E.;  $n = 8$



**Fig. 7** The bile acid sensitivity profiles vary between BASICs from different species. **a** Representative current traces of rBASIC (blue), mBASIC (green), and hBASIC (red) by six different bile acids. CA, UDCA, HDCA, and LCA were applied at 2 mM; DCA and CDCA were applied at 1 mM only due to their non-specific solubilization properties above 1 mM. **b** Quantitative comparison of current amplitudes induced by the six bile acids. Error bars, S.E.;  $n = 8$



features occur depending on the cellular expression system. Since BASIC is expressed in different organs and tissues such as cholangiocytes in the liver [28] and unipolar brush cells (UBCs) in the cerebellum [3], it is tempting to speculate that the different cell types lead to different channel properties and thus physiological functions. It would therefore be interesting to study the channel in other heterologous expression systems as well as in cholangiocytes and UBCs of the cerebellum. Unfortunately, these approaches were not successful so far. Therefore, it remains unclear whether constitutive activity of mBASIC in *Xenopus* oocytes is a non-physiological artifact or whether it is of physiological relevance. Another explanation for the variation of the electrophysiological characteristics of mBASIC may be the regulation by additional proteins, which might be present in *Xenopus* oocytes and absent from Hek293 cells. However, since rBASIC and mBASIC are 97% identical, this conclusion is not very likely, as an interaction with other proteins would likely occur for both channels.

In our study we have tested six different bile acids. Interestingly, rBASIC and mBASIC showed a similar activation profile while hBASIC was insensitive to some bile acids, which are present in rodents and sensitive to bile acids predominantly found in humans. This supports the hypothesis that bile acid sensitivity might have adapted to the bile acid pool present in humans. Neither *Xenopus* oocytes nor Hek293 cells are under physiological conditions in contact with bile acids, which are natural detergents affecting the cell's membrane. Bile acid sensitivity of BASIC might therefore be an artifact of these expression systems. However, control experiments with oocytes or Hek293 cells not expressing BASIC rather suggest that the observed effects are specific to BASIC.

We have shown previously that bile acid activation of BASIC is mediated indirectly by a modification of membrane properties due to a bile acid adsorption to the plasma membrane [20]. These membrane modifications are sensed by BASIC and translated into activity. Since different bile acids are structurally very similar and therefore can be expected to

have similar effects on the plasma membrane, the fact that various bile acids affect hBASIC differently, may support the notion that bile acids modify BASIC by direct interaction. This is in line with studies describing the possible binding site for bile acids for ASIC, ENaC and hBASIC to be located within the degenerin site [9–11].

Taken together, our data show that BASIC from rat, mouse, and human show similar characteristics and suggest that they serve similar physiological roles in these species.

## References

1. Assmann M, Kuhn A, Dürmangel S, Holstein TW, Gründer S (2014) The comprehensive analysis of DEG/ENaC subunits in Hydra reveals a large variety of peptide-gated channels, potentially involved in neuromuscular transmission. *BMC Biol* 12:84. <https://doi.org/10.1186/s12915-014-0084-2>
2. Bianchi L (2007) Mechanotransduction: touch and feel at the molecular level as modeled in *Caenorhabditis elegans*. *Mol Neurobiol* 36:254–271. <https://doi.org/10.1007/s12035-007-8009-5>
3. Boiko N, Kucher V, Wang B, Stockand JD (2014) Restrictive expression of acid-sensing ion channel 5 (*asic5*) in unipolar brush cells of the vestibulocerebellum. *PLoS One* 9:e91326. <https://doi.org/10.1371/journal.pone.0091326>
4. Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC (1994) Amiloride-sensitive epithelial Na<sup>+</sup> channel is made of three homologous subunits. *Nature* 367:463–467. <https://doi.org/10.1038/367463a0>
5. Chen X, Qiu L, Li M, Dürmangel S, Orser BA, Xiong ZG, MacDonald JF (2010) Diarylamidines: high potency inhibitors of acid-sensing ion channels. *Neuropharmacology* 58:1045–1053. <https://doi.org/10.1016/j.neuropharm.2010.01.011>
6. Driscoll M, Chalfie M (1991) The *mec-4* gene is a member of a family of *Caenorhabditis elegans* genes that can mutate to induce neuronal degeneration. *Nature* 349:588–593. <https://doi.org/10.1038/349588a0>
7. Dürmangel S, Kuhn A, Tsiairis CD, Williamson M, Kalbacher H, Grimmlikhuijzen CJ, Holstein TW, Gründer S (2010) Three homologous subunits form a high affinity peptide-gated ion channel in Hydra. *J Biol Chem* 285:11958–11965. <https://doi.org/10.1074/jbc.M109.059998>

8. Fisher MM, Yousef IM (1973) Sex differences in the bile acid composition of human bile: studies in patients with and without gallstones. *Can Med Assoc J* 109:190–193
9. Ilyaskin AV, Diakov A, Korbmacher C, Haerteis S (2016) Activation of the human epithelial sodium channel (ENaC) by bile acids involves the degenerin site. *J Biol Chem* 291:19835–19847. <https://doi.org/10.1074/jbc.M116.726471>
10. Ilyaskin AV, Diakov A, Korbmacher C, Haerteis S (2017) Bile acids potentiate proton-activated currents in *Xenopus laevis* oocytes expressing human acid-sensing ion channel (ASIC1a). *Physiol Rep* 5. <https://doi.org/10.14814/phy2.13132>
11. Ilyaskin AV, Kirsch SA, Bockmann RA, Sticht H, Korbmacher C, Haerteis S, Diakov A (2018) The degenerin region of the human bile acid-sensitive ion channel (BASIC) is involved in channel inhibition by calcium and activation by bile acids. *Pflugers Arch* 470:1087–1102. <https://doi.org/10.1007/s00424-018-2142-z>
12. Jasti J, Furukawa H, Gonzales EB, Gouaux E (2007) Structure of acid-sensing ion channel 1 at 1.9 Å resolution and low pH. *Nature* 449:316–323. <https://doi.org/10.1038/nature06163>
13. Kellenberger S, Schild L (2002) Epithelial sodium channel/degenerin family of ion channels: a variety of functions for a shared structure. *Physiol Rev* 82:735–767. <https://doi.org/10.1152/physrev.00007.2002>
14. Kellenberger S, Schild L (2015) International Union of Basic and Clinical Pharmacology. XCI. Structure, function, and pharmacology of acid-sensing ion channels and the epithelial Na<sup>+</sup> channel. *Pharmacol Rev* 67:1–35. <https://doi.org/10.1124/pr.114.009225>
15. Lefevre CM, Diakov A, Haerteis S, Korbmacher C, Gründer S, Wiemuth D (2013) Pharmacological and electrophysiological characterization of the human bile acid-sensitive ion channel (hBASIC). *Pflugers Archiv* 466:253–263. <https://doi.org/10.1007/s00424-013-1310-4>
16. Lingueglia E, Champigny G, Lazdunski M, Barbry P (1995) Cloning of the amiloride-sensitive FMRFamide peptide-gated sodium channel. *Nature* 378:730–733. <https://doi.org/10.1038/378730a0>
17. Sakai H, Lingueglia E, Champigny G, Mattei MG, Lazdunski M (1999) Cloning and functional expression of a novel degenerin-like Na<sup>+</sup> channel gene in mammals. *J Physiol* 519(Pt 2):323–333
18. Sakakura H, Suzuki M, Kimura N, Takeda H, Nagata S, Maeda M (1993) Simultaneous determination of bile acids in rat bile and serum by high-performance liquid chromatography. *J Chromatogr* 621:123–131
19. Schaefer L, Sakai H, Mattei M, Lazdunski M, Lingueglia E (2000) Molecular cloning, functional expression and chromosomal localization of an amiloride-sensitive Na<sup>+</sup> channel from human small intestine. *FEBS Lett* 471:205–210. [https://doi.org/10.1016/S0014-5793\(00\)01403-4](https://doi.org/10.1016/S0014-5793(00)01403-4)
20. Schmidt A, Lenzig P, Oslender-Bujotzek A, Kusch J, Lucas SD, Gründer S, Wiemuth D (2014) The bile acid-sensitive ion channel (BASIC) is activated by alterations of its membrane environment. *PLoS One* 9:e111549. <https://doi.org/10.1371/journal.pone.0111549>
21. Schmidt A, Löhner D, Alsop RJ, Lenzig P, Oslender-Bujotzek A, Wirtz M, Rheinstadter MC, Gründer S, Wiemuth D (2016) A cytosolic amphiphilic alpha-helix controls the activity of the bile acid-sensitive ion channel (BASIC). *J Biol Chem* 291:24551–24565. <https://doi.org/10.1074/jbc.M116.756437>
22. Schmidt A, Rossetti G, Joussen S, Grunder S (2017) Diminazene is a slow pore blocker of acid-sensing ion channel 1a (ASIC1a). *Mol Pharmacol* 92:665–675. <https://doi.org/10.1124/mol.117.110064>
23. Setchell KD, Rodrigues CM, Clerici C, Solinas A, Morelli A, Gartung C, Boyer J (1997) Bile acid concentrations in human and rat liver tissue and in hepatocyte nuclei. *Gastroenterology* 112:226–235. [https://doi.org/10.1016/S0016-5085\(97\)70239-7](https://doi.org/10.1016/S0016-5085(97)70239-7)
24. Wemmie JA, Taugher RJ, Kreple CJ (2013) Acid-sensing ion channels in pain and disease. *Nat Rev Neurosci* 14:461–471. <https://doi.org/10.1038/nrn3529>
25. Wiemuth D, Assmann M, Grunder S (2014) The bile acid-sensitive ion channel (BASIC), the ignored cousin of ASICs and ENaC. *Channels* 8:29–34. <https://doi.org/10.4161/chan.27493>
26. Wiemuth D, Gründer S (2010) A single amino acid tunes Ca<sup>2+</sup> inhibition of brain liver intestine Na<sup>+</sup> channel (BLINaC). *J Biol Chem* 285:30404–30410. <https://doi.org/10.1074/jbc.M110.153064>
27. Wiemuth D, Gründer S (2011) The pharmacological profile of brain liver intestine Na<sup>+</sup> channel: inhibition by diarylamidines and activation by fenamates. *Mol Pharmacol* 80:911–919. <https://doi.org/10.1124/mol.111.073726>
28. Wiemuth D, Sahin H, Falkenburger BH, Lefevre CM, Wasmuth HE, Gründer S (2012) BASIC - a bile acid-sensitive ion channel highly expressed in bile ducts. *FASEB J* 26:4122–4130. <https://doi.org/10.1096/fj.12-207043>
29. Wiemuth D, Sahin H, Lefevre CM, Wasmuth HE, Grunder S (2013) Strong activation of bile acid-sensitive ion channel (BASIC) by ursodeoxycholic acid. *Channels* 7:38–42. <https://doi.org/10.4161/chan.22406>
30. Yoder N, Yoshioka C, Gouaux E (2018) Gating mechanisms of acid-sensing ion channels. *Nature* 555:397–401. <https://doi.org/10.1038/nature25782>