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Sensitivity of oocyte-expressed epithelial Na^+ channel to glibenclamide

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Abstract

The effect of glibenclamide on heterologously expressed amiloride-sensitive sodium channels (ENaCs) was investigated in *Xenopus* oocytes. The ENaC is a heteromer and consists of α-, β- and γ-subunits and the α- and β-subunits have previously been shown to confer sensitivity to glibenclamide. We coexpressed either colonic rat α- (rα) or guinea-pig α-subunit (gpα) with *Xenopus* βγ-subunits. The gpαxβγ was significantly stimulated by glibenclamide (100 μM) (184 ± 15%), whereas the rα-combination was slightly down-regulated by the sulfonylurea (79 ± 4%). The stimulating effect did not interfere with Na^+-self-inhibition resulting from intracellular accumulation of Na^+-ions. We exchanged cytosolic termini between both orthologs but the gpα-chimera with the termini from rat retained sensitivity to glibenclamide. The effect of glibenclamide on *Xenopus* ENaC (xENaC) was inhibited by ADP-β-S but not by ATP-γ-S, when applied intracellularly. Intracellular loading with Na^+-ions after inhibition of Na^+/K^+-ATPases with ouabain prevented an up-regulation of ENaC activity by glibenclamide. Pretreatment of oocytes expressing xENaC with edelfosine (ET-18-OCH_3) slightly reduced stimulation of I_{ami} (118 ± 12%; control: 132 ± 9%) while phosphatidylinositol-4,5-biphosphate (PIP_2) significantly reduced the effect of glibenclamide to 101 ± 3%.

Keywords: Glibenclamide; Epithelial Na^+ channel; *Xenopus* oocyte; ET-18-OCH_3; PIP_2

1. Introduction

Transcellular electrogenic Na^+ transport in tight epithelia as in the distal nephron, the distal colon or in the lung is limited by apical Na^+-selective channels (ENaCs). The functional channels comprise homologous α-, β- and γ-subunits which share a topology of two cytosolic termini, two transmembranous domains and a large extracellular loop. Regulation of ENaCs has been subject to intensive studies and in this regard proteins which associate with these channels recently attracted increasing attention [1,2].

Among such factors, ATP-binding cassette (ABC) proteins were proposed to play a possible role in regulation of ENaCs. An interaction between ENaC and the cystic fibrosis regulator (CFTR), a member of ABC protein family, was suggested to down-regulate apical sodium permeabilities [3–6]. Recently, glibenclamide, a high-affinity inhibitor of sulfonylurea receptors (SUR) but a low-affinity inhibitor of CFTR [7], was reported to stimulate heterologously expressed ENaCs in *Xenopus* oocytes [8]. While the ENaC from the *Xenopus* nephron (xαβγ) was strongly activated by this sulfonylurea, the colonic rat ENaC (rαβγ) was insensitive to glibenclamide. This remarkable sensitivity to this sulfonylurea was conferred by the xα- and xβ-subunits. Patch-clamp experiments in the outside-out configuration indicated that glibenclamide increased the NP_o product (N = number of channels; P_o = open probability) but single-channel conductance remained unaltered [8]. Equally, a superfusion of ENaC-expressing oocytes with glibenclamide did not affect single-channel behaviour in cell-attached patches. The authors concluded that glibenclamide may act directly on the ENaCs or on channel-associated proteins instead of triggering a diffusion of intracellular second messengers after binding to distant membrane receptors.

In the present study, we express ENaCs in oocytes of *Xenopus laevis* and investigate the effect of glibenclamide on further subunit combinations using two-electrode voltage-clamp technique. We expressed heteromeric channels composed of an α-subunit that we have cloned from guinea-pig colon (gpα) [9] and xβγ and compare those with the

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sulfonylurea-insensitive combination of rat α with xβγ. To investigate whether the stimulating effect of glibenclamide is triggered from within the cell, we took advantage of the difference of both orthologs in sensitivity to glibenclamide and switched cytosolic domains between rα- and gpα-subunits. Furthermore, we investigated the hypothesis of sulfonylurea receptors or related proteins with ABCs to bind glibenclamide and hence to modulate ENaCs.

Phospholipids have been shown to be important regulators of ion transporters and channels [10]. These lipids have to be inserted into the membranous bilayer where they interact directly with membrane proteins on the basis of electrostatics. The role of phospholipids in the glibenclamide-induced stimulation of ENaCs was investigated by inhibition of PIP-degrading phospholipase C as well as direct application of phosphatidylinositol-4,5-biphosphate (PIP_2).

Our study provides evidence that glibenclamide controls ENaC activity by a mechanism which is restricted to the oocyte membrane or associated elements. The effect is likely not to be transduced by a second-messenger-mediated modification of cytosolic parts of the α-subunits.

2. Materials and methods

2.1. Expression of ENaC in X. laevis oocytes

Capped cRNA (0.1 ng each) for α-, β- and γ-subunits of *Xenopus* ENaC, rat αENaC, guinea-pig αENaC or chimeric rat/guinea-pig α-subunits were injected into stage V/VI oocytes of *X. laevis* [9,11,12]. Injected oocytes were kept in storage solution (10 mM NaCl, 80 mM NMDG, 3 mM KCl, 2 mM $CaCl_2$, 2.5 mM pyruvate, 0.08 mM penicillin and 0.03 mM streptomycin, pH = 7.4 adjusted with HCl) for 24–48 h at 14 °C.

2.2. Electrophysiological measurements with two-electrode voltage-clamp technique

For two-microelectrode voltage-clamp experiments, two bathing solutions were used: high Na^+ Ringer: 90 mM NaCl, 1 mM KCl, 2 mM $CaCl_2$ and 5 mM HEPES (pH 7.4). Low Na^+ Ringer: 20 mM NaCl, 1 mM KCl, 2 mM $CaCl_2$, 70 mM NMDG and 5 mM HEPES (pH 7.4). Oocytes were continuously superfused at a flow rate of 3 ml/min.

The membrane potential was clamped to a holding potential of 0, −20 or −60 mV with a voltage-clamp amplifier (OC-725B Oocyte clamp, Warner Instrument Corp.) and controlled by a personal computer via CED 1401 (CED, Cambridge, UK). The current sensitive to 10 μM amiloride (I_{ami}) was determined. Glibenclamide was used at a concentration of 100 μM (from 0.2 M stock solution in dimethyl sulfoxide). The current difference between the respective decreases in response to amiloride were considered as effect of glibenclamide. Results are reported as means ± S.E. and represent the mean of *n* independent experiments with oocytes originating from *N* different donors. The results were analyzed with the Student's *t*-test or paired *t*-test and values which are significantly different are indicated.

2.3. Construction of chimeric rat/guinea-pig α-subunits

Using PCR, a BSPE1 restriction site was introduced into gpα cDNA sequence at amino acid position 585–589 (sense: *cggttccggagccgg*; antisense: *ccggctccggaaccg*). *Sac*II restriction sites were introduced into gpα cDNA (sequence position 224–228) and into rα cDNA (sequence position 285–290) (sense: *caaccgcggaagacg*; antisense: *cgtcttcccgcgttg*) [13]. All constructs were cloned into pSDEasy [14].

2.4. Chemicals and enzymes

Amiloride, ATP-γ-S; ADP-β-S, glibenclamide, ouabain and PIP_2 were obtained from Sigma. Edelfosine (ET-18-OCH_3) was obtained from Calbiochem Corp.

3. Results

3.1. Specific α-subunits mediate the effect of glibenclamide

The activity of heterologously expressed ENaCs in the membranes of oocytes is down-regulated by time-dependent autoregulative processes that are related to extra- and intracellular Na^+ concentrations [15]. In orientating experiments, superfusion of oocytes expressing *Xenopus* αβγENaC (xENaC) with 100 μM glibenclamide stimulated the amiloride-sensitive part of the current (I_{ami}) to 190 ± 15% ($n = 6$) even after the clamp current (I_m) had down-regulated

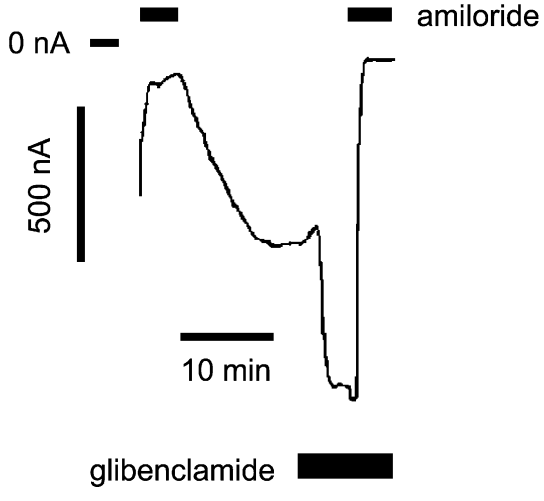

Fig. 1. Original current recording from gpαxβγENaC-expressing oocyte. The current sensitive to 10 μM amiloride was determined before and after glibenclamide (100 μM) was added. The holding potential was −60 mV.

to a steady-state value at a holding potential of −60 mV in response to accumulation of intracellular Na^+ (90 mM Na^+ extracellular). To investigate the role of the α-subunits for the sensitivity to glibenclamide, we coexpressed two further α-subunits, the colonic rat α (rα) and guinea-pig α (gpα) with xβγ-subunits. Glibenclamide 100 μM activated the gpαxβγ hybrid channel to 184 ± 13% (Fig. 1) and thus to a similar degree as the xENaC, whereas the rαxβγ combination was found to be slightly reduced by this sulfonylurea (79 ± 5%; $n=6$). To reduce accumulation of intracellular Na^+, we exposed oocytes to solutions with low Na^+ content. With 20 mM Na^+ in the bath, the oocytes expressing gpαxβγ or rαxβγ exhibited membrane potentials of −8.8 ± 1 mV ($n=28$) and −5.8 ± 1 mV ($n=23$), respectively. To avoid channel rundown in response to cytosolic accumulation of Na^+, the oocytes were clamped to a holding potential of 0 mV. After I_m had stabilized, membrane potentials were clamped to −20 mV for 5 min followed by the return to 0 mV (Fig. 2A). The clamp protocol was executed again in the presence of glibenclamide. Fig. 2B shows the immediate current values at the beginning of the −20-mV step and after 5 min. I_m in oocytes expressing rαxβγENaC was significantly down-regulated within 5 min ($P<0.01$) and this was not affected by glibenclamide ($n=10$; $N=4$). Activity of gpαxβγENaC was slightly down-regulated during the clamp step but the absolute current values were significantly increased by glibenclamide ($P<0.005$; $n=12$; $N=4$) (Fig. 2B).

To address the question of whether modifications of the intracellular domains transduct the stimulation, we took advantage of the regulatory differences of both hybrid channels and constructed r/gpα-chimeras by exchanging the cytosolic termini between these two orthologs. These constructs were coexpressed with the xβγ and tested for their sensitivity to glibenclamide. Fig. 3 shows the mean changes in I_{ami} induced by glibenclamide for the different

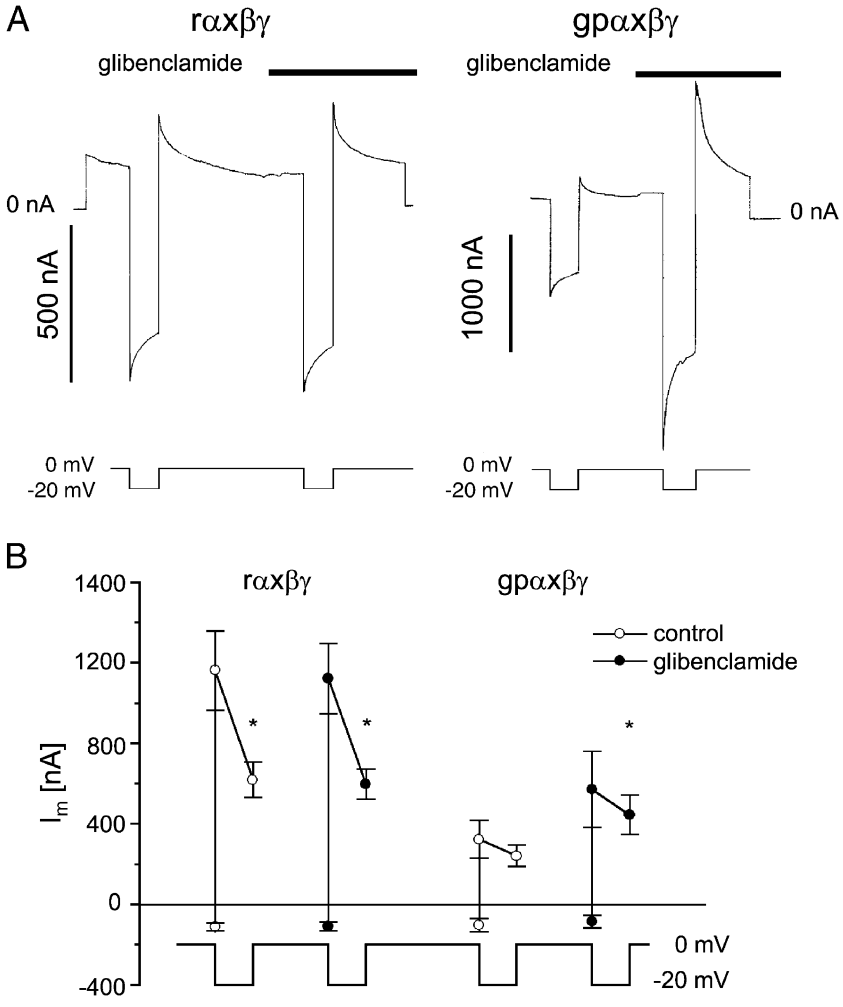

Fig. 2. Glibenclamide and channel rundown in response to intracellular Na^+ accumulation. Shown are current traces from oocytes expressing gpαxβγ- or rαxβγ-ENaC (A). To reduce intracellular accumulation of Na^+, bath solution contained 20 mM NaCl and oocytes were voltage-clamped to a holding potential of 0 mV. After membrane potential shortly (5 min) was clamped to −20 mV and I_m re-equilibrated after return to 0 mV glibenclamide (100 μM) was added. (B) Current values measured before and during superfusion with glibenclamide at start and after 5 min of the −20-mV clamp step ($n=12$; $N=4$). *: Significantly downregulated, $P<0.01$; **: significantly downregulated, $P<0.005$.

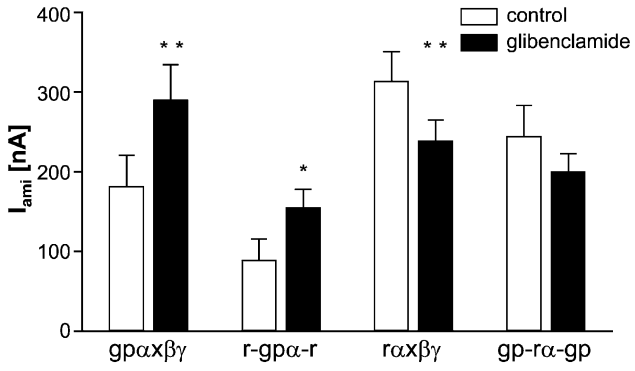

Fig. 3. Comparison of the effect of glibenclamide on different heteromeric channels. α-subunits from rat and guinea pig or chimeric constructs with exchanged cytosolic termini were coexpressed with *Xenopus* β- and γ-subunits. The current sensitive to 10 μM amiloride (I_{ami}) and the effect of glibenclamide (100 μM) were measured at a holding potential of −20 mV ($n=12-15$; $N=4$). *: Significantly different from control, $P<0.01$; **: significantly different from control, $P<0.005$.

channel combinations at a holding potential of −20 mV and 20 mM Na^+ in the bath. An increased amplitude of I_{ami} in response to glibenclamide was observed in oocytes that expressed channels with gpα-subunit (184 ± 15%; $n=14$; $N=4$) or the chimera which contained the transmembranous domains and the extracellular loop of the gpα-isoform (243 ± 15%; $n=12$; $N=4$). While glibenclamide significantly reduced I_{ami} in oocytes expressing rαxβγENaC (79 ± 4%; $n=13$; $N=4$), the activity of channels containing the rα-construct with both cytosolic termini originating from the guinea pig was not affected by the compound (98 ± 13%; $n=13$; $N=4$) (Fig. 4).

3.2. Are ABC-related proteins involved in mediating the glibenclamide effect?

SURs possess two ABCs in their cytosolic segments, the NBF1 and NBF2, which exhibit affinity to ATP and ADP [16]. We tested whether non-hydrolysable analogs of these nucleotides affect the activation of ENaCs by glibenclamide. Oocytes expressing the xENaC were injected with ATP-γ-S

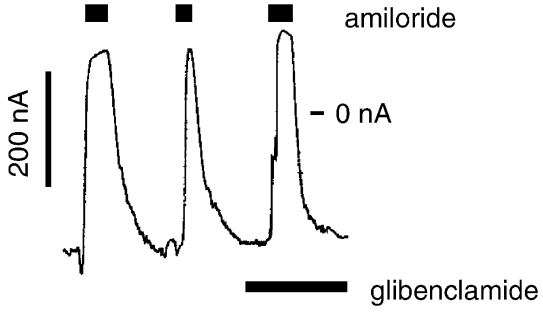

Fig. 4. Original current recording obtained in ENaC-expressing oocyte. Oocyte coexpressed chimeric rα (both cytosolic termini originating from guinea pig α-isoform) with *Xenopus* βγ. The holding potential was −20 mV. Note: In contrast to gpαxβγ, this channel is not stimulated by glibenclamide (100 μM).

or ADP-β-S, respectively. The increase in I_{ami} in response to 100 μM glibenclamide was determined and compared to data obtained from control oocytes. As shown in Fig. 5, ATP-γ-S at a cytosolic concentration of approximately 5 mM did not significantly affect the glibenclamide-induced I_{ami}. In contrast, after an injection of ADP-β-S (final ~5 mM intracellular), I_{ami} decreased significantly by approximately 24% in response to glibenclamide. In orientating experiments, we examined if the reduced response of I_{ami} to glibenclamide after ADP-β-S treatment was due to an interference with the action of Na^+/K^+-ATPases followed by Na^+ loading of the oocytes. An exposure of xENaC-expressing oocytes to 1 mM ouabain was sufficient to abolish stimulation of I_{ami} by glibenclamide (only 2 ± 2%; $N=2$; $n=6$), but the sulfonylurea did not induce a reduction of current as observed in the ADP-β-S experiments. An injection of ADP-β-S lowered V_m in all oocytes from +9.3 ± 1.3 mV in control to −2.7 ± 2.8 mV (with 90 mM Na^+ extracellular). This indicated that Na^+/K^+-pump activity was not shut down by ADP-β-S and thus no Na^+ loading of the oocytes. We tested for the effect of extracellular non-hydrolysable adenosine nucleotides and 30 min pretreatment of xENaC-expressing oocytes with 1 mM extracellular ATP-γ-S or ADP-β-S in the presence of 1 mM Mg^{2+}-ions [16] did not significantly alter the effect of glibenclamide on activity of xENaC (control: 143 ± 10%; ATP-γ-S: 133 ± 10% and ADP-β-S: 143 ± 10%; $n=6-10$; $N=3$).

3.3. Phospholipids reduced the effect of glibenclamide

There is evidence that phospholipids (PIPs) play a prominent role in regulation of diverse ion channels [10,17,18]. First, we increased the level of PIPs in the plasma membrane by application of a selective inhibitor of phosphatidyl-inositol-specific phospholipase C. A 30-min preincubation of oocytes expressing xENaC with 10

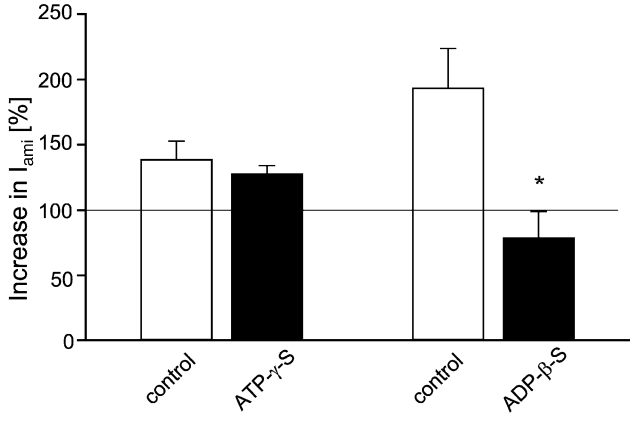

Fig. 5. Effect of intracellular non-hydrolysable adenosine nucleotides. xENaC-expressing oocytes were injected with either 50 nl of 50 mM ATP-γ-S or 50 mM ADP-β-S in H_2O. The holding potential was −20 mV. After clamp current reached the maximum in response to 100 μM glibenclamide, I_{ami} was determined ($n=6$; $N=3$). Control oocytes were injected with an equal amount of Li^+-ions. *: Significantly different from control, $P<0.005$.

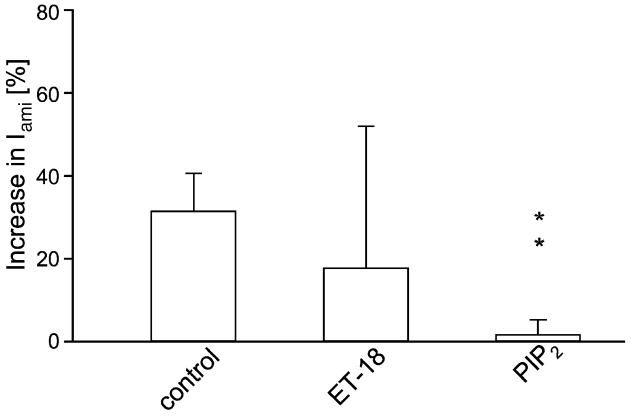

Fig. 6. Effect of ET-18-OCH_3 and PIP_2 on glibenclamide-induced increase in I_{ami}. Oocytes expressing xENaC were either incubated for 30 min in 10 μM ET-18-OCH_3 (ET-18) or additionally injected with 50 nl of PIP_2 (PIP_2) (25 mM in H_2O). Oocytes were clamped to a holding potential of −20 mV and then superfused with 100 μM glibenclamide. The current sensitive to 10 μM amiloride was determined. The number of measurements was $n=7–8$, $N=3$. **: Significantly different from control, $P<0.005$.

μM ET-18-OCH_3 decreased the average stimulatory effect of glibenclamide (Fig. 6). An additional injection of PIP_2 (50 nl PIP_2; 25 mM in H_2O) into ET-18-OCH_3-pretreated oocytes revealed a more pronounced and significant reduction of the glibenclamide effect (Fig. 6) [19].

4. Discussion

Glibenclamide, a high-affinity inhibitor of SUR was recently tested for its effect on the epithelial sodium channels. Besides, native amiloride-sensitive sodium conductances in the *Xenopus* kidney A6 cell line, the xENaC and the human ENaC heterologously expressed in *Xenopus* oocytes were stimulated by glibenclamide. Interestingly, the oocyte-expressed ratENaC could not be activated by glibenclamide and combinations with subunits from *Xenopus* revealed that the xα- and the xβ-subunit confer the sensitivity to glibenclamide [8]. We coexpressed *Xenopus* βγ-subunits with an α-subunit either from rat (rαxβγ) or with an α-subunit originating from the guinea-pig colon (gpαxβγ) [9,11]. While the rαxβγ remained insensitive to glibenclamide, as expected, the gpαxβγ was activated as the xENaC.

High cytosolic Na^+ concentrations decrease activity of ENaCs due to autoregulative processes. These mechanisms are possibly mediated via Na^+-sensing receptors and appear as well in oocytes [20–22]. We tested whether glibenclamide releases gpαxβγ from such Na^+-self-inhibition. We prevented accumulation of Na^+-ions by superfusion with low-Na^+ solution (20 mM), which is similar to physiological intracellular Na^+ concentrations in oocytes. The clamp potential was shifted from holding potential of 0 to −20 mV and held for 5 min. Although the initial amplitude of I_m in response to the −20-mV step was increased, the time-dependent down-regulation seemed to be not affected by glibenclamide. Thus, the stimulation by glibenclamide is possibly due to other mechanisms. We switched the intracellular termini between the gpα- and the ratα-subunit and tested channels containing these constructs for their sensitivity to glibenclamide. A substitution of both of the two cytosolic gpα-termini by the respective termini from the rat isoform did not prevent the stimulation of I_{ami} by glibenclamide. The effect on channels with the chimeric gpα was even more pronounced which may result from the weaker basal expression in our experiments (Fig. 3). The chimera with the extracellular loop and the transmembranous domains from the rat and intracellular parts from gpα was insensitive to glibenclamide. These findings coincide to some degree with conclusion of a previous study that extracellular glibenclamide mediates activation of ENaCs by either direct binding to the channel proteins or membrane-restricted associated factors [8]. The mechanisms of stimulation may only be exerted when certain conformational changes take place or with specific binding affinities that demand an integrity of the extracellular part of the protein. At least a modification of cytosolic parts of the α-ENaC-subunits as, for example, phosphorylation initiated by a distinct receptor which triggers the diffusion of second messengers seems not to be likely.

One putative regulatory protein that is thought to interact with ENaCs is the cystic fibrosis transmembrane conductance regulator (CFTR) [23]. In native epithelia, ENaC is inhibited when CFTR is activated [24–26]. The PKA-dependent stimulation of CFTR has been attributed to an interaction of the C-terminal PDZ-binding domain with scaffolding proteins and ezrin, a putative PKA anchoring protein [27–30]. Such an assemblage with PDZ- or similar domain factors could offer a novel way of how ABC proteins control ENaCs. SUR-type proteins could associate in scaffolding complexes that sequester, e.g. protein kinases or phosphatases in close proximity with ENaC and organize physically signal transduction pathways.

The glucose-induced insulin secretion in the pancreatic β-cells underlies a quite complex control of cytosolic ATP/ADP ratio [7,16]. SUR1 builds a subunit of the K_{ATP}-channel and binds ATP at NBF1 and ADP at the NBF2 in a cooperative manner. Glibenclamide possibly modulates the cooperative interaction of the NBFs of SUR1. SURs and ENaCs are coexpressed in renal epithelia where they are possibly colocalized in renal collecting duct principal cells [31]. Recently, heterologously expressed SUR was reported to control the processing and transport of ENaCs to the oocyte membrane in a chaperone-like manner [32]. This may suggest that glibenclamide binds to a SUR or to another as yet unidentified ABC protein and these interact with ENaCs in the *Xenopus* oocyte membrane.

To determine the involvement of any SUR-related ABC protein in the stimulation of ENaCs by glibenclamide, we

applied non-hydrolysable derivatives of ATP and ADP. An extracellular application of ATP-γ-S or ADP-β-S had no significant impact on the glibenclamide-induced stimulation of ENaC. However, an intracellular application of ADP-β-S but not ATP-γ-S abolished the stimulation of I_{ami} in response to glibenclamide. Meanwhile, K_{ATP} channels serve as a prototypic example for cytosolic ATP/ADP ratio [7,16]. SUR1 builds a subunit of the complex SUR-mediated regulation by intracellular adenosine nucleotides [17]. Because the inhibitory and stimulatory impacts of adenosine nucleotides on SUR-mediated regulation on ion channels are still under debate, it was difficult to predict whether ATP or ADP should positively or negatively influence the glibenclamide effect upon oocyte-expressed ENaCs. First of all, an interference with the activity of the Na^+/K^+-ATPases has to be taken into consideration. However, as we have shown, an inhibition of the Na^+/K^+-pumps by ouabain prevented an increase of I_{ami} in response to glibenclamide. In our experiments, an injection of ADP-β-S depolarized membrane potentials, an opposite effect from what one expects from an inhibition of Na^+/K^+-ATPases. In our opinion, this may indicate that the ADP-β-S effect was not due to the shutdown of pump activity. Further, both adenosine nucleotides were intracellularly offered as Li^+-salts. Li^+-ions are known to accumulate in the cells; nevertheless, only the ADP-β-S decreased significantly the response of I_{ami} to glibenclamide. However, our experiments provided no direct evidence that ADP-β-S did not affect activity of Na^+/K^+-ATPases.

Among the adenosine nucleotides, ATP plays a central role in polymerization and depolymerization processes of cytoskeletal elements as the actin filaments. An involvement of actinous elements had been postulated in regulation of ENaCs and other ion channels [33,34]. Thus, the inhibitory effect of ADP-β-S may also be due to an interference with such cytoskeletal elements. This would stress the importance of the transmembranous domains and the proximate sections of the ENaC proteins.

PI-kinases generate PIPs from phosphorylation of phosphatidylinositol by an ATP-consuming process [35,36]. The ABC proteins seem to link phospholipid metabolism to the residual cellular metabolism by regulating ATP sensitivity of ion channels as, e.g., the inward rectifying K_{ATP} [17,18]. The action of PIPs on ion channels requires their insertion into the plasma membrane where they interact with cytosolic parts of the channel proteins on the basis of electrostatics [37,38]. In these cases, membrane-inserted PIPs interact directly through their negatively charged head groups with the cytosolic portion of channel proteins or associated factors. In the present study, an increase of PIP_2 levels prevented the stimulation of I_{ami} by glibenclamide. In any case, inhibition of phosphatidyl-inositol-specific phospholipase C with ET-18-OCH_3 accumulates PIP_2 but reduces diacylglycerol (DAG) and 1,4,5-inositol triphosphate (IP_3). This may affect further signaling pathways and, for example, an inhibiting effect of a depletion in DAG has to be taken into consideration. Because PIP-mediated control is not a common feature of all kinds of membrane proteins, the regulatory impact on the oocyte-expressed ENaCs may be assessed as a further indicator for an involvement of SUR-related membrane receptors [10]. PIP_2 additionally inhibits a broad spectrum of F-actin-severing proteins and therefore acts as a stabilizer of filaments [34]. However, from our data, we cannot exclude the possibility that assembly and disassembly of the actin cytoskeletal network interfere with the stimulatory effect of glibenclamide on oocyte-expressed ENaCs.

In summary, these data indicate that the extracellular loop or transmembranous domains of α-ENaC confer sensitivity to glibenclamide. Glibenclamide does not interfere with Na^+-self-inhibition. The mechanism proved to be influenced by adenosine nucleotides and this sensitivity was possibly not due to down-regulation of Na^+/K^+-pumps. An increase of PIP_2 after exposure to ET-18-OCH_3 abolished the stimulation of ENaCs by glibenclamide. Whether ABC-related proteins and/or the actin cytoskeleton mediate the effect of glibenclamide cannot be decided at this point.

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References

[1] A.M. Hruska-Hageman, J.A. Wemmie, M.P. Price, M.J. Welsh, Biochem. J. 361 (2002) 443–450.
[2] N. Anzai, E. Deval, L. Schaefer, V. Friend, M. Lazdunski, E. Lingueglia, J. Biol. Chem. 277 (2002) 16655–16661.
[3] I.I. Ismailov, M.S. Awayda, B. Jovov, B.K. Berdiev, C.M. Fuller, J.R. Dedman, M. Kaetzel, D.J. Benos, J. Biol. Chem. 271 (1996) 4725–4732.
[4] M. Mall, A. Hipper, R. Greger, K. Kunzelmann, FEBS Lett. 381 (1996) 47–52.
[5] M.J. Stutts, C.M. Canessa, J.C. Olsen, M. Hamrick, J.A. Cohn, B.C. Rossier, R.C. Boucher, Science 269 (1995) 847–850.
[6] M.J. Stutts, B.C. Rossier, R.C. Boucher, J. Biol. Chem. 272 (1997) 14037–14040.
[7] J. Bryan, L. Aguilar-Bryan, Biochim. Biophys. Acta 1461 (1999) 285–303.
[8] A. Chrabi, J.D. Horisberger, J. Pharmacol. Exp. Ther. 290 (1999) 341–347.
[9] M. Schnizler, L. Mastroberardino, F. Reifarth, W.M. Weber, F. Verrey, W. Clauss, Pflugers Arch. 439 (2000) 579–587.
[10] D.W. Hilgemann, R. Ball, Science 273 (1996) 956–959.
[11] C.M. Canessa, L. Schild, G. Buell, B. Thorens, I. Gautschi, J.D. Horisberger, B.C. Rossier, Nature 367 (1994) 463–467.

[12] A. Puoti, A. May, C.M. Canessa, J.D. Horisberger, L. Schild, B.C. Rossier, Am. J. Physiol. 269 (1995) C188–C197.
[13] A. Chraibi, M. Schnizler, W. Clauss, J.D. Horisberger, J. Membr. Biol. 183 (2001) 15–23.
[14] A. Puoti, A. May, B.C. Rossier, J.D. Horisberger, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 5949–5954.
[15] H. Abriel, J.D. Horisberger, J. Physiol. 516 (1999) 31–43.
[16] K. Ueda, M. Matsuo, K. Tanabe, K. Morita, N. Kioka, T. Amachi, Biochim. Biophys. Acta 1461 (1999) 305–313.
[17] T. Baukrowitz, B. Fakler, Biochem. Pharmacol. 60 (2000) 735–740.
[18] Z. Fan, J.C. Makielski, J. Biol. Chem. 272 (1997) 5388–5395.
[19] G. Powis, D. Phil, Cancer Metastasis Rev. 13 (1994) 91–103.
[20] M. Hubner, R. Schreiber, A. Boucherot, A. Sanchez-Perez, P. Poronnik, D.I. Cook, K. Kunzelmann, FEBS Lett. 459 (1999) 443–447.
[21] H. Ishibashi, A. Dinudom, K.F. Harvey, S. Kumar, J.A. Young, D.I. Cook, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 9949–9953.
[22] P. Komwatana, A. Dinudom, J.A. Young, D.I. Cook, J. Membr. Biol. 162 (1998) 225–232.
[23] K. Kunzelmann, R. Schreiber, A. Boucherot, Kidney Int. 60 (2001) 455–461.
[24] M. Mall, M. Bleich, R. Greger, R. Schreiber, K. Kunzelmann, J. Clin. Invest. 102 (1998) 15–21.
[25] M. Mall, M. Bleich, J. Kuehr, M. Brandis, R. Greger, K. Kunzelmann, Am. J. Physiol. 277 (1999) G709–G716.
[26] B. Letz, C. Korbmacher, Am. J. Physiol. 272 (1997) C657–C666.
[27] B.D. Moyer, M. Duhaime, C. Shaw, J. Denton, D. Reynolds, K.H. Karlson, J. Pfeiffer, S. Wang, J.E. Mickle, M. Milewski, G.R. Cutting, W.B. Guggino, M. Li, B.A. Stanton, J. Biol. Chem. 275 (2000) 27069–27074.
[28] D.B. Short, K.W. Trotter, D. Reczek, S.M. Kreda, A. Bretscher, R.C. Boucher, M.J. Stutts, S.L. Milgram, J. Biol. Chem. 273 (1998) 19797–19801.
[29] F. Sun, M.J. Hug, C.M. Lewarchik, C.H. Yun, N.A. Bradbury, R.A. Frizzell, J. Biol. Chem. 275 (2000) 29539–29546.
[30] F. Sun, M.J. Hug, N.A. Bradbury, R.A. Frizzell, J. Biol. Chem. 275 (2000) 14360–14366.
[31] A.H. Beesley, I.Z. Qureshi, A.N. Giesberts, A.J. Parker, S.J. White, Pflugers Arch. 438 (1999) 1–7.
[32] A.A. Konstas, A. Bielfeld-Ackermann, C. Korbmacher, Pflugers Arch. 442 (2001) 752–761.
[33] H.F. Cantiello, J.L. Stow, A.G. Prat, D.A. Ausiello, Am. J. Physiol. 261 (1991) C882–C888.
[34] T. Furukawa, T. Yamane, T. Terai, Y. Katayama, M. Hiraoka, Pflugers Arch. 431 (1996) 504–512.
[35] A. Collins, A.V. Somlyo, D.W. Hilgemann, J. Physiol. 454 (1992) 27–57.
[36] D.W. Hilgemann, A. Collins, J. Physiol. 454 (1992) 59–82.
[37] T. Baukrowitz, U. Schulte, D. Oliver, S. Herlitze, T. Krauter, S.J. Tucker, J.P. Ruppersberg, B. Fakler, Science 282 (1998) 1141–1144.
[38] S.L. Shyng, C.G. Nichols, Science 282 (1998) 1138–1141.