Original Paper

Annexin 7 in the Regulation of Gastric Acid Secretion

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Key Words

Stomach • H⁺/K⁺ ATPase • K⁺ recycling • H⁺ secretion • glucocorticoids

Abstract

Background/Aims: Glucocorticoids enhance gastric acid secretion and inhibit gastric cyclooxygenase, thus downregulating formation of PGE₂, an inhibitor of gastric acid secretion. In erythrocytes, PGE₂ formation is inhibited by annexin 7. The present study thus explored whether annexin 7 participates in the regulation of gastric acid secretion. **Methods:** Annexin 7 protein expression was determined by Western blotting, cytosolic pH (pHi) of parietal cells utilizing BCECF-fluorescence, and gastric acid secretion by determination of Na⁺-independent pHi recovery from an ammonium pulse (ΔpHi/min). Experiments were performed in isolated glands from gene targeted mice lacking annexin 7 (*anx7*^{-/-}) and in respective wild type animals (*anx7*^{+/+}). **Results:** Prior to treatment pHi and ΔpHi/min were similar in isolated gastric glands from *anx7*^{-/-} and from *anx7*^{+/+} mice. Aspirin (100 μM added to the glands 1 hr prior to the experiment) significantly increased ΔpHi/min to similar values in both genotypes. The administration of dexamethasone (10 μg/g BW subcutaneously for 4 consecutive days prior to the experiments) significantly increased ΔpH/min in *anx7*^{+/+} mice but not in *anx7*^{-/-} mice. Following dexamethasone treatment, the luminal pH was significantly lower and the acid content significantly higher in *anx7*^{+/+} mice than in *anx7*^{-/-} mice. An increase of extracellular K⁺ concentration to 35 mM (replacing Na⁺/NMDG⁺) significantly increased ΔpHi/min in both genotypes. In neither genotype dexamethasone increased ΔpH/min further in the presence of 35 mM K⁺ or presence of aspirin. **Conclusions:** Annexin 7 is required for the stimulation of gastric acid secretion by glucocorticoids.

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Introduction

Annexins, Ca^{2+} - and phospholipid-binding intracellular proteins [1-5], contribute to the regulation of a wide variety of functions such as inhibition of phospholipase A_2 [6, 7], regulation of ion channels [8], aggregation of chromaffin granules [9], endo- and exocytosis [10, 11] or crosslinking of proteins within the cell cortex [12]. The isoform annexin 7 (or annexin VII, synexin) has been shown to interfere with prostaglandin E_2 (PGE_2) formation [13] and to participate in the regulation of secretion [14-16], hormone release [17], as well as cell survival and tumor growth [18-27]. Annexin 7 contributes to spherocytosis [28], cardiac remodelling [29] and inflammatory myopathies [30].

Initially generated annexin 7 knockout mice were lethal on embryonic day 10 [31]. In a second attempt viable annexin 7 knockout mice were generated [32], suffering from disorders of cardiac excitation [32, 33], enhanced glial cell proliferation [34] as well as accelerated suicidal erythrocyte death [35] associated with slightly increased osmotic erythrocyte resistance [36] and relative resistance to malaria [13]. The increased suicidal erythrocyte death and partial resistance to malaria resulted from excessive formation of PGE_2 [13].

PGE_2 is known to inhibit gastric acid secretion [37-39], which is accomplished by the H^+ / K^+ ATPase pumping H^+ into the gastric lumen in exchange of K^+ [40-43]. K^+ recycles into the lumen through KCNQ1/KCNE K^+ channels [44-46]. Genetic knockout of KCNQ1 thus abrogates gastric acid secretion [47]. The channels [48-51] and thus gastric acid secretion [41, 43, 52] are stimulated by cAMP. Gastric acid secretion is further stimulated by glucocorticoids [53], which decrease prostaglandin formation by downregulating cyclooxygenase 2 [38]. Nothing is known about the involvement of annexins in the glucocorticoid-dependent regulation of gastric acid secretion.

The present study thus explored, whether annexin 7 contributes to the regulation of gastric acid secretion. To this end, gastric acid secretion was determined in gene-targeted mice lacking functional annexin 7 and their wild type littermates.

Materials and Methods

Animals

Experiments were performed in gene-targeted mice lacking annexin 7 (*anx7^{-/-}*) and in corresponding wild-type mice (*anx7^{+/+}*). Generation, properties and genotyping of *anx7^{-/-}* mice were described earlier [32]. Sex and age-matched mice with age more than 3 months were used for the experiments. All animal experiments were conducted according to the German law for the care and use of laboratory animals and were approved by local authorities (Regierungspräsidium Tübingen).

Treatments

Mice had free access to a standard mouse diet (C1310, Altromin, Lage, Germany) and tap water. Dexamethasone was administered subcutaneously at a dose of 10 $\mu\text{g/g}$ BW for 4 consecutive days to the mice prior to the experiments. Aspirin (100 μM) was added to the glands 1 hr prior to the experiment. The substances were obtained from Sigma (Taufkirchen, Germany). The stock solutions were prepared in DMSO. Sham experiments were performed by adding DMSO or plain distilled water to the perfusing solution at a final dilution of 0.1 %.

Western blot analysis

After sacrificing the animals, tissues were immediately snap frozen in liquid nitrogen. Samples were lysed with ice-cold lysis buffer (Thermo Fisher Scientific) supplemented with complete protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). After centrifugation at 10000 rpm for 5 min, 30 μg protein were boiled in Roti-Load1 Buffer (Carl Roth GmbH) at 100°C for 10 min. Proteins were separated on SDS-polyacrylamide gels and transferred to PVDF membranes. The membranes were incubated overnight at 4°C with primary goat anti-Annexin 7 antibody (diluted 1:200, R&D Systems) or rabbit anti-GAPDH antibody (diluted 1:1000, Cell Signaling) and then with secondary anti-goat HRP-conjugated antibody

(diluted 1:2000, Santa Cruz Biotechnology) or with secondary anti-rabbit HRP-conjugated antibody (diluted 1:1000, Cell Signaling) for 1 hour at room temperature. For loading controls, the membranes were stripped in stripping buffer (Carl Roth GmbH) at 56°C for 5 min. Antibody binding was detected with the ECL detection reagent (Amersham). Bands were quantified with Quantity One Software (Bio-Rad Laboratories).

Gastric H⁺ secretion

For isolation of gastric glands animals were fasted for 16 hours prior to experiments on wire grids with free access to tap water. After sacrificing the animals the stomach was removed and cut longitudinally. After washing with standard HEPES solution the fundic and pyloric regions were discarded and the gastric corpus was sliced into 0.3 cm² sections. The tissues were transferred onto the cooled stage of a dissecting microscope and individual glands were carefully detached from the gastric wall by snapping off the tissue using sharpened microdissection tweezers. Care was taken not to touch the apical part of the glands. The glands were attached to a glass coverslip precoated with Cell-Tak adhesive (BD Biosciences) [54].

The solutions, flow lines and perfusion chamber were maintained at 37°C by a thermostatically controlled heating system. The volume of the perfusion chamber was 600 µl and the flow rate was 4 ml/min for all solutions. For digital imaging of cytosolic pH (pH_i) isolated individual glands were incubated in a HEPES-buffered Ringer solution containing 10 µM BCECF-AM (Molecular Probes, Leiden, The Netherlands) for 15 min at 37°C. After loading, the chamber was flushed for 5 min with Ringer solution to remove any deesterified dye sticking to the outside of the glands. The perfusion chamber was mounted on the stage of an inverted microscope (Zeiss Axiovert 135), which was used in the epifluorescence mode with a 40 x oil immersion objective (Zeiss Neoplan, Germany). BCECF was successively excited at 490/10 and 440/10 nm and the resultant fluorescent signal was monitored at 535/10 nm using an intensified charge-coupled device camera (Proxitronic, Germany) and specialized computer software (Metafluor, USA) [55]. Between 8 and 20 parietal cells were outlined and monitored during the course of the measurements. The results from each cell were averaged and taken for final analysis. Intensity ratio (490/440) data were converted into pH values using the high-K⁺/nigericin calibration technique [56, 57]. To this end the glands were perfused at the end of each experiment for 5 minutes with standard high-K⁺/nigericin (10 µg/ml) solution (pH 7.0). The intensity ratio data thus obtained were converted into pH values using the $r_{\max}$, $r_{\min}$, pK_a values previously generated from calibration experiments performed in isolated gastric glands to generate a standard nonlinear curve (pH range 5 to 8.5). The intensity ratio data were converted into pH values using the following equation:

$$pH = pK_a [\log((ratio_{\text{test}}/ratio_{pH\ 7.0}) - r_{\min}) / (r_{\max} - (ratio_{\text{test}}/ratio_{pH\ 7.0}))].$$

For acid loading, cells were transiently exposed to a solution containing 20 mM NH₄Cl leading to marked initial alkalization of cytosolic pH (pH_i) due to entry of NH₃ and binding of H⁺ to form NH₄⁺ [58, 59]. The acidification of cytosolic pH upon removal of ammonia allowed calculating the mean intrinsic buffering power (β) of the cells [58]. Assuming that NH₄⁺ and NH₃ are in equilibrium in cytosolic and extracellular fluid and that ammonia leaves the cells as NH₃:

$$\beta = \Delta[NH_4^+]_i / \Delta pH_i$$

where ΔpH_i is the decrease of cytosolic pH (pH_i) following ammonia removal and $\Delta[NH_4^+]_i$ is the decrease of cytosolic NH₄⁺ concentration, which is identical to the concentration of [NH₄⁺]_i immediately before the removal of ammonia. The pK for NH₄⁺/NH₃ is 8.9 [60] and at an extracellular pH (pH_o) of 7.4 the NH₄⁺ concentration in extracellular fluid ([NH₄⁺]_o) is 19.37 [20/(1+10^{pH_o-pK})] mM. The intracellular NH₄⁺ concentration ([NH₄⁺]_i) was calculated from:

$$[NH_4^+]_i = 19.37 \cdot 10^{pH_o - pH_i} \text{ mM.}$$

The calculation of the buffer capacity required that NH₄⁺ exits completely. After the initial decline, pH_i indeed showed little further change in the absence of Na⁺ and presence of omeprazole, indicating that there was no relevant further exit of NH₄⁺. The pH recovery during and following the NH₄⁺ pulse was determined in the absence of Na⁺ to prevent cellular realkalinization by the Na⁺/H⁺ exchanger.

To calculate the $\Delta pH/\text{min}$ during re-alkalinization, a manual linear fit was placed over a narrow pH range (pH 6.7 to 6.9) which could be applied to all measured cells [61]. The solutions were composed of (in mM): standard HEPES: 115 NaCl, 5 KCl, 1 CaCl₂, 1.2 MgSO₄, 2 NaH₂PO₄, 10 glucose, 32.2 HEPES; sodium free HEPES: 132.8 NMDG⁺Cl⁻, 3 KCl, 1 CaCl₂, 1.2 MgSO₄, 2 KH₂PO₄, 32.2 HEPES, 10 mannitol, 10 glucose (for sodium free ammonium chloride 10 mM NMDG⁺Cl⁻ and mannitol were replaced with 20 mM NH₄Cl); high K⁺ for calibration: 105 KCl, 1 CaCl₂, 1.2 MgSO₄, 32.2 HEPES, 10 mannitol, 10 µg/ml nigericin. The pH of the

solutions was titrated to 7.4 or 7.0 with HCl/NaOH, HCl/NMDG and HCl/KOH, respectively, at 37°C. Where indicated, the K⁺ concentration was increased at the expense of Na⁺/NMDG⁺ to 35 mM.

Determination of pH and acid content of the gastric lumen

The pH of the luminal stomach contents depends on the amount of acid secreted. Activation of gastric gland leads to decrease in luminal pH and conversely inhibition leads to increase in pH. The luminal pH of the overnight fasted mice serves therefore as a useful indicator of basal gastric acid secretion. To determine the basal intraluminal pH, a previously described method was slightly modified and used [62-64]. Briefly, the mice were fasted overnight on wire grids with free access to tap water and sacrificed under slight ether anaesthesia. The whole stomach was then carefully removed and cut open and the luminal contents were collected by washing the stomach with 10 ml unbuffered isotonic sodium chloride solution (containing additionally 5 mM KCl and 10 mM glucose). The washings were then centrifuged at 150 g for 5 minutes to remove the particulate matter and the pH of the supernatant solution was measured by a standard pH meter (Knick, Germany). The acid content in the solution was measured by titration of the above obtained supernatant solution with 0.01 N NaOH to an endpoint of pH 7.0 by monitoring the pH with a pH meter and additionally adding a universal pH indicator (Sigma, Taufkirchen, Germany) to the solution. The acid content thus obtained was then corrected by the acid content of the blank isotonic sodium chloride solution and used for further statistical analysis.

Statistics

Intracellular pH, pH recovery and buffer capacity of all parietal cells from one gland were averaged and the respective mean values were used for further statistical analysis. Data are provided as arithmetic means ± SEM, *n* represents the number of mice used. All data were tested for significance using Student's *t*-test with Welch's correction or ANOVA (Dunnets test), where applicable, and only results with *p* < 0.05 were considered statistically significant.

Results

The present paper pursued the hypothesis that annexin 7 participates in the regulation of gastric acid secretion by glucocorticoids, such as dexamethasone. To possibly disclose a role of annexin 7 in the regulation of gastric acid secretion, annexin 7 knockout (*anx7^{-/-}*) mice and corresponding wild-type animals (*anx7^{+/+}*) were analysed. Following an overnight fasting period the body weight and the weight of empty stomach were determined. The body weight was similar in *anx7^{-/-}* mice (20.5 ± 1.8 g, *n* = 5) and *anx7^{+/+}* mice (23.6 ± 1.6 g, *n* = 5). The stomach weight was again similar in *anx7^{-/-}* mice (0.27 ± 0.02 g, *n* = 5) and *anx7^{+/+}* mice (0.24 ± 0.01 g, *n* = 5). Western blot analysis was performed in gastric tissue from *anx7^{-/-}* and *anx7^{+/+}* mice without treatment or following prior treatment with dexamethasone (10 µg/g BW for 4 consecutive days prior to the experiments) to elucidate the expression of annexin 7. As illustrated in Fig. 1, annexin 7 is expressed in parietal cells from *anx7^{+/+}* mice but not from *anx7^{-/-}* mice. Dexamethasone treatment did not appreciably alter annexin 7 expression in neither *anx7^{-/-}* nor *anx7^{+/+}* mice. Thus, annexin 7 is expressed in gastric glands but its expression is not modified by glucocorticoids.

The impact of annexin 7 on gastric acid secretion was explored by utilizing BCECF fluorescence for the determination of cytosolic pH. Without treatment cytosolic pH was similar in isolated gastric glands from *anx7^{-/-}* and from *anx7^{+/+}* mice. Cytosolic pH was not significantly modified by treatment with dexamethasone (10 µg/g BW for 4 consecutive days to the mice prior to the experiments) or aspirin (100 µM added to the glands 1 hr prior to the experiment) or by increasing extracellular K⁺ concentration from 5 mM to 35 mM (Table 1). The buffer capacity was also not significantly different between gastric gland cells from *anx7^{-/-}* and *anx7^{+/+}* mice and was again not significantly different between presence or absence of dexamethasone, aspirin or enhanced extracellular K⁺ concentration (Table 1). Accordingly, the effect of H⁺ movement across the cell membrane is reflected by the respective changes of cytosolic pH in gastric glands from *anx7^{-/-}* and *anx7^{+/+}* mice. Gastric H⁺/K⁺ ATPase activity

Fig. 1. Expression of annexin 7 in gastric tissue.

A. Original Western blot of the expression of annexin 7 protein in the gastric tissue from *anx7^{-/-}* mice and *anx7^{+/+}* mice without treatment or following treatment with dexamethasone (10 µg/g BW for 4 consecutive days to the experiments). B. Arithmetic means ± SEM (n = 4 mice each group, a.u.) of the relative expression of annexin 7 protein in the gastric tissue from *anx7^{-/-}* mice (black bars) and *anx7^{+/+}* mice (white bars) without treatment or following prior treatment with dexamethasone (10 µg/g BW for 4 consecutive days prior to the experiments).

Table 1. Gastric acid secretion in *anx7^{-/-}* mice and *anx7^{+/+}* mice without treatment (Control) or following treatment with dexamethasone (10 µg/g BW for 4 consecutive days prior to the experiments) or aspirin (100 µM added to the glands 1 hr prior to the experiment) or both. Effect of an increase of bath K⁺ concentration from 5 mM to 35 mM. * - indicates significant difference to respective wild type mice. # - indicates significant difference to respective control (untreated or 0.1 % DMSO treated mice). § - indicates significant difference to presence and absence of aspirin during dexamethasone treatment

Experimental condition	Mouse genotype	Rate of pH recovery (delta pH units/minute)	Intracellular pH (units)	Buffer capacity (mM / pH unit)	Number of mice	Number of cells
Control	<i>anx7^{+/+}</i>	0.044 ± 0.011	7.20 ± 0.04	34.8 ± 5.0	10	100
	<i>anx7^{-/-}</i>	0.045 ± 0.010	7.21 ± 0.07	28.4 ± 4.1	12	138
Aspirin	<i>anx7^{+/+}</i>	0.123 ± 0.015 [#]	7.25 ± 0.05	31.3 ± 7.9	6	60
	<i>anx7^{-/-}</i>	0.128 ± 0.023 [#]	7.29 ± 0.03	23.0 ± 2.4	6	61
Omeprazole	<i>anx7^{+/+}</i>	0.003 ± 0.014	7.31 ± 0.08	17.9 ± 3.3	4	65
	<i>anx7^{-/-}</i>	0.002 ± 0.011	7.26 ± 0.07	17.8 ± 4.2	4	68
Dexamethasone	<i>anx7^{+/+}</i>	0.130 ± 0.032	7.22 ± 0.05	35.8 ± 7.4	6	82
	<i>anx7^{-/-}</i>	0.049 ± 0.008 [*]	7.20 ± 0.03	30.9 ± 4.0	6	70
Dexamethasone + Aspirin	<i>anx7^{+/+}</i>	0.123 ± 0.020 [#]	7.14 ± 0.02	41.2 ± 5.6	6	53
	<i>anx7^{-/-}</i>	0.114 ± 0.016 ^{#§}	7.20 ± 0.03	27.8 ± 5.2	6	44
35 mM K ⁺	<i>anx7^{+/+}</i>	0.255 ± 0.041 [#]	7.32 ± 0.06 [#]	31.9 ± 6.2	6	76
	<i>anx7^{-/-}</i>	0.233 ± 0.016 [#]	7.30 ± 0.05 [#]	22.7 ± 2.9	6	83
Dexamethasone + 35 mM K ⁺	<i>anx7^{+/+}</i>	0.284 ± 0.059 [#]	7.28 ± 0.02	32.6 ± 3.3	6	92
	<i>anx7^{-/-}</i>	0.275 ± 0.032 [#]	7.30 ± 0.03 ^{*#}	23.6 ± 4.1	6	99

was estimated from the Na⁺-independent pH recovery following an ammonium pulse ($\Delta\text{pH}_i/\text{min}$). As shown in Fig. 2, $\Delta\text{pH}_i/\text{min}$ in untreated gastric glands was not significantly different between *anx7^{-/-}* and *anx7^{+/+}* mice. Aspirin increased $\Delta\text{pH}_i/\text{min}$ in both *anx7^{-/-}* and *anx7^{+/+}* mice. The administration of omeprazole (100 µM) virtually abolished $\Delta\text{pH}_i/\text{min}$ in both genotypes (Fig. 2) indicating that the observed alkalization was due to H⁺/K⁺ ATPase activity. The observations suggest that in the absence of glucocorticoid treatment H⁺/K⁺ ATPase activity is downregulated by cyclooxygenase irrespective of the presence of annexin 7.

As shown in Fig. 3, treatment with dexamethasone (10 µg/g BW for 4 consecutive days prior to the experiments) increased $\Delta\text{pH}_i/\text{min}$ significantly in *anx7^{+/+}* mice but not in

Fig. 2. pH recovery in parietal cells in the absence and presence of aspirin. Alterations of cytosolic pH (ΔpH_i) in parietal cells following an ammonium pulse. To load the cells with H^+ , 20 mM NH_4Cl was added and Na^+ removed (replaced by NMDG $^+$, '0' Na^+) in a first step (see bars below each original tracing), NH_4Cl removed in a second step, Na^+ added in a third step and nigericin (pH_0 '7') applied in a fourth step to calibrate each individual experiment. A. Time dependent alterations of pH_i (arithmetic means $\pm$ SEM of 6-10 cells each) in representative single experiments in *anx7 $^{+/+}$* mice (left panels) and *anx7 $^{-/-}$* mice

(right panels) without (upper panels) or with (middle panels) aspirin treatment (100 μM added to the glands 1 hr prior to the experiment) or in the presence of 100 μM omeprazole (lower panels). B. Arithmetic means $\pm$ SEM (n = 4-6 mice each group) of Na^+ -independent alterations of cytosolic pH ($\Delta\text{pH}_i/\text{min}$) in parietal cells from *anx7 $^{-/-}$* mice (black bars) and *anx7 $^{+/+}$* mice (white bars) without (left bars) and with (middle bars) aspirin treatment (100 μM added to the glands 1 hr prior to the experiment) or in the presence of 100 μM omeprazole (right bars). # indicates significant difference from absence of aspirin. § indicates significant difference from absence of omeprazole.

anx7 $^{-/-}$ mice. Additional experiments were performed following exposure of gastric glands to increased extracellular K^+ concentration. An increase of the bath K^+ concentration to 35 mM (replacing $\text{Na}^+/\text{NMDG}^+$) increased $\Delta\text{pH}_i/\text{min}$ to similarly high levels in *anx7 $^{+/+}$* mice and in *anx7 $^{-/-}$* mice (Fig. 3). In neither genotype dexamethasone treatment increased $\Delta\text{pH}_i/\text{min}$ further in the presence of 35 mM K^+ . Accordingly, an increase of extracellular K^+ concentration abolished the stimulation of gastric acid secretion by dexamethasone treatment. The observations indicate that in the absence of annexin 7, the stimulating effect of glucocorticoids on gastric acid secretion is virtually abrogated, an effect apparently involving K^+ channels.

Further experiments were performed under inhibition of cyclooxygenase by aspirin. The additional treatment of dexamethasone gastric glands with aspirin did not significantly

Fig. 3. Effects of dexamethasone on pH recovery in parietal cells at normal and enhanced extracellular K^+ concentration. **A.** Time dependent alterations of pH_i (arithmetic means $\pm$ SEM of 6-10 cells each) in representative single experiments in *anx7^{+/+}* mice (left panels) and *anx7^{-/-}* mice (right panels) without (upper panels) and with stimulation with dexamethasone (10 μ g/g BW for 4 consecutive days prior to the experiments) at 5 mM K^+ (middle panels) and at 35 mM K^+ (lower panels). **B.** Arithmetic means $\pm$ SEM (n = 5-6 mice each group) of Na^+ -independent alterations of cytosolic pH (ΔpH_i /min) in parietal cells from *anx7^{-/-}* mice (black bars) and *anx7^{+/+}* mice (white bars) at 5 mM K^+ (left and middle left bars) and at 35 mM K^+ (right and middle right bars) without (left and middle left bars) or with dexamethasone treatment (right and middle right bars).

* indicates significant difference between *anx7^{-/-}* mice and *anx7^{+/+}* mice, # indicates significant difference to absence of dexamethasone, § indicates significant difference to 5 mM K^+ .

modify ΔpH_i /min in *anx7^{+/+}* mice but significantly increased ΔpH_i /min in *anx7^{-/-}* mice (Fig. 4). Accordingly, following treatment with both, dexamethasone and aspirin, ΔpH_i /min was similarly high in *anx7^{-/-}* and *anx7^{+/+}* mice. Those experiments suggest that annexin 7 is effective by inhibiting cyclooxygenase.

The difference in gastric acid secretion between *anx7^{-/-}* mice and *anx7^{+/+}* mice was further studied by determination of pH and acid content of the gastric lumen. As shown in Fig. 5, without treatment the pH of the solution containing the luminal aspirates was similar in both genotypes. Dexamethasone treatment decreased the pH in *anx7^{+/+}* mice but not in *anx7^{-/-}* mice. Accordingly, luminal pH was, following dexamethasone treatment, significantly lower in *anx7^{+/+}* mice than in *anx7^{-/-}* mice. Similarly, the acid content was significantly higher in the gastric lumen of *anx7^{+/+}* mice than of *anx7^{-/-}* mice. Thus, the effects of dexamethasone and annexin 7 on H^+ secretion are reflected by respective alterations of luminal pH and acid content.

Fig. 4. Effects of dexamethasone on pH recovery in parietal cells in the absence and presence of aspirin. **A.** Time dependent alterations of pH_i (arithmetic means $\pm$ SEM of 6-10 cells each) in representative single experiments in *anx7^{+/+}* mice (left panels) and *anx7^{-/-}* mice (right panels) without (upper panels) and with stimulation with dexamethasone (10 μ g/g BW for 4 consecutive days prior to the experiments) without (middle panels) and with (lower panels) additional treatment with aspirin (100 μ M added to the glands 1 hr prior to the experiment). **B.** Arithmetic means $\pm$ SEM (n = 6 mice each group) of Na^+ -independent alterations of cytosolic pH ($\Delta pH_i/min$) in parietal cells from *anx7^{-/-}* mice (black bars) and *anx7^{+/+}* mice (white

bars) in the absence of dexamethasone (left bars) or presence dexamethasone in the absence (middle bars) and presence (right bars) of aspirin. * indicates significant difference between *anx7^{-/-}* mice and *anx7^{+/+}* mice, # indicates significant difference to absence of dexamethasone, § indicates significant difference to absence of aspirin.

Fig. 5. Intraluminal pH and acid content of stomach from overnight fasted mice. Arithmetic means $\pm$ SEM of (n = 5-7 mice each group) of (A) the pH of the luminal contents and (B) acid equivalents in the luminal contents of stomach from *anx7^{-/-}* mice (black bars) and *anx7^{+/+}* mice (white bars) without (left bars) and with (right bars) dexamethasone treatment (10 μ g/g BW for 4 consecutive days to the mice prior to the experiments). * indicates significant difference between *anx7^{-/-}* mice and *anx7^{+/+}* mice. # indicates significant difference to absence of dexamethasone.

Discussion

The present study discloses a novel signaling molecule participating in the regulation of gastric acid secretion. Lack of annexin 7 virtually abolishes the effect of the glucocorticoid dexamethasone on gastric acid secretion, an effect reversed in the presence of cyclooxygenase inhibitor aspirin. The observations suggest that the stimulation of gastric acid secretion by glucocorticoids requires suppression of cyclooxygenase by annexin 7. Previously, we have shown that following *Plasmodium berghei* infection, PGE₂ plasma levels were larger in annexin 7-deficient mice than in their wild-type littermates [13]. Suppression of PGE₂ formation would indeed be expected to enhance gastric acid secretion. Surprisingly, the stimulatory effect of dexamethasone on gastric acid secretion fully depends on annexin 7. Moreover, it is fully mimicked by aspirin. In the presence of aspirin, dexamethasone does not stimulate gastric acid secretion further. Thus, annexin 7-dependent inhibition of cyclooxygenase appears to be critically important for the stimulation of gastric acid secretion by glucocorticoids.

Aspirin enhances gastric acid secretion in both genotypes, i.e. mice lacking annexin 7 (*anx7*^{-/-}) and wild type mice (*anx7*^{+/+}). It is tempting to speculate that basal gastric acid secretion is suppressed by a cyclooxygenase product, such as PGE₂, and that dexamethasone stimulates gastric acid secretion by annexin 7-dependent inhibition of cyclooxygenase.

An increase of extracellular K⁺ concentration to 35 mM enhanced gastric acid secretion, which could not be increased further by application of dexamethasone. An increase of extracellular K⁺ concentration should depolarize the cell membranes thus augmenting the K⁺ exit across the apical membrane. Gastric acid secretion critically depends on K⁺ recycling through KCNQ1 channels [47]. Possibly, annexin 7-sensitive cyclooxygenase activity leads to inhibition of KCNQ1 channels.

In theory annexin 7 may influence the regulation of gastric acid secretion by altered release of secretagogues. Moreover, annexin 7 could interfere with signaling stimulating gastric acid secretion. Gastric acid secretion is stimulated by increase of cytosolic Ca²⁺ activity and cAMP [43, 65, 66] as well as by phosphoinositide 3 kinase (PI3K)-dependent signaling [67-70].

According to an earlier study [53], the stimulation of gastric acid secretion depends on the serum- and glucocorticoid-inducible kinase (SGK1), which is known to stimulate a variety of epithelial channels and transporters [71]. The difference between SGK1-deficient mice and their littermates was similarly abrogated in the presence of 35 mM K⁺ [53]. Future studies may disclose interactions of SGK1 and annexin 7-dependent signaling in the regulation of gastric acid secretion.

In conclusion, the gastric acid secretion is similar in *anx7*^{-/-} mice and in *anx7*^{+/+} mice under basal conditions but cannot be upregulated by the glucocorticoid dexamethasone in *anx7*^{-/-} mice. Aspirin upregulates gastric acid secretion in both, *anx7*^{-/-} mice and *anx7*^{+/+} mice. Thus, Annexin 7-sensitive cyclooxygenase activity contributes to the stimulation of gastric acid secretion by glucocorticoids.

Abbreviations

BCECF (2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl este); DMSO (Dimethylsulfoxide); NMDG (N-methyl D- glucamine); HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); ATP4β (β-subunit of H⁺/K⁺ ATPase); Tbp (TATA box binding protein); PDK (phosphoinositide-dependent kinase); PI3K (phosphoinositide 3 kinase).

Conflict of Interests

All authors of this manuscript declare that they have no conflicts of interests.

Acknowledgements

The authors gratefully acknowledge the meticulous preparation of the manuscript by Lejla Subasic and Sari Rübe. This work was supported by grants from the DFG (SFB 773) and Open Access Publishing Fund of Tuebingen University.

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