PI3 Kinase Dependent Stimulation of Gastric Acid Secretion by Dexamethasone

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Key Words

Stomach • pH • H⁺/K⁺ ATPase • Dexamethasone

Abstract

Excessive gastric acid secretion plays an important role in the pathogenesis of peptic ulcers. Dexamethasone, a widely used drug, is known to stimulate gastric acid secretion and increase the incidence of peptic ulcers. However little is known about the mechanism of the dexamethasone's effect on parietal cells. The present study was performed to investigate the contribution of the phosphatidylinositol-3-kinase (PI3 kinase) to dexamethasone induced stimulation of gastric acid secretion. *In vivo* pretreatment with dexamethasone injections (150 µg/100g for 3 days) or *in vitro* exposure to (10 µM for > 20 minutes) significantly increased acid secretion in isolated gastric glands ~ 2-3 fold. The dexamethasone induced stimulation of gastric acid secretion was concentration dependent and significantly blunted by the H⁺/K⁺ ATPase inhibitor omeprazole (200 µM), the PI3 kinase inhibitor Wortmannin (500 nM), the protein kinase inhibitor staurosporine (2.5 µM) and the Cl⁻ channel blocker NPPB (100 µM); but not by the H₂ antagonist

cimetidine (100 µM). In conclusion, it was observed that dexamethasone's effect on proton extrusion requires the activity of a PI3 kinase pathway, an apical Cl⁻ channel and the H⁺/K⁺ ATPase.

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Introduction

Dexamethasone is a glucocorticoid widely used for the treatment of inflammatory diseases [1]. Untoward side effects of glucocorticoids include the generation of peptic ulcers with the risk of bleeding and perforation [2]. Glucocorticoids inhibit the inherent gastroprotective mechanisms of the stomach by inactivating the biosynthesis of prostaglandins [3]. Simultaneously, glucocorticoids stimulate gastric acid secretion [4, 5]. The mechanisms mediating the stimulatory effects on gastric acid secretion remained incompletely understood. On the one hand, glucocorticoids have been shown to enhance the degranulation of intramural mast cells with subsequently increased release of the secretagogue histamine [6]. On the other hand, glucocorticoids have been shown to inhibit the gastric peroxidase [7], which in turn inhibits gastric acid secretion [8].

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Fig. 1. Enhanced gastric acid secretion of dexamethasone treated rats. A: Original photographs of gastric glands after 10 minutes incubation of BCECF either from a saline injected rat (left panel) or from a dexamethasone injected rat (right panel). B: Original pH measurements in gastric glands from a control rat treated for three days with 140 mM saline (upper graph) or from a rat treated with 150 µg/ 100g dexamethasone (lower graph). C: Arithmetic means ± SEM of intracellular pH recovery (ΔpHi) in gastric glands from control rats (open column) or dexamethasone treated rats (closed column). * indicates significant difference from saline injected rats, $p < 0.05$ (unpaired t-test).

Gastric acid secretion is accomplished by H^+/K^+ -ATPase [9], KCNQ1 [10, 11] and Kir2.1 [12] K^+ channels, as well as chloride channels [13, 14] in the apical membrane of parietal cells.

Glucocorticoids have been shown to regulate the phosphatidylinositol (PI) 3 kinase [15], which has in turn been shown to regulate a variety of transport regulating kinases including protein Kinase C (PKC) [16] and the serum and glucocorticoid inducible Kinase (SGK) [17].

The present paper explores whether the stimulation of gastric proton extrusion via the H^+/K^+ -ATPase by dexamethasone involves a PI3 kinase pathway. Additional experiments have been performed following inhibition of H^+/K^+ -ATPase and chloride channels.

Materials and Methods

Animals and pre-treatment

Experiments were performed in gastric glands isolated from male Sprague-Dawley rats weighing ~200-300g, housed in climate- and humidity-controlled, light-cycled rooms and fed standard chow with free access to water. Where indicated, the rats were injected for three days with 150µg/100g bodyweight each day subcutaneously prior to the experiments.

	HEPES	0 Na ⁺ HEPES	0 Na ⁺ HEPES + NH ₄ Cl	High K ⁺
NaCl	115mM	-	-	-
NMDG	-	132.8mM	132.8mM	32.8mM
NH ₄ Cl	-	-	20mM	-
KCl	5mM	5mM	5mM	105mM
MgSO ₄	1.2mM	1.2mM	1.2mM	1.2mM
CaCl ₂	1mM	1mM	1mM	1mM
Mannitol	-	-	-	5mM
Glucose	10mM	10mM	10mM	-
HEPES	32.2mM	32.2mM	32.2mM	32.2mM
pH (37°C)	7.4	7.4	7.4	7

Table 1. Solutions for in vitro pH measurements

In vitro pH measurements in parietal cells of freshly isolated gastric glands

Before experimentation, animals were fasted for 18–24h to reduce basal acid secretion. Animals were killed with an overdose of isoflurane, and an abdominal incision was made. The stomach was ligated at the esophageal and duodenal junctures and excised. Stomachs were immediately rinsed with ice-cold HEPES to remove any residual food particles and kept on ice until use. Small segments of the gastric mucosa were prepared and later transferred to the stage of a dissecting microscope where individual gastric glands were hand-dissected using a

Fig. 2. Enhanced acid secretion of rat gastric glands treated with dexamethasone *in vitro*. A: Original pH measurements of a rat gastric gland incubated with BCECF in absence (upper graph) or presence (lower graph) of dexamethasone (10 μ M). B: Arithmetic means $\pm$ SEM of intracellular pH recovery (Δ pHi) in gastric glands without (open column) or with (closed column) 10 μ M dexamethasone. * indicates significant difference from control, $p < 0.05$ (unpaired *t*-test). C: Intracellular pH recovery (Δ pHi) in gastric glands (Arithmetic means $\pm$ SEM) as a function of dexamethasone concentration.

previously described technique [18]. Individual glands were subsequently placed on coverslips coated with Cell-Tak (cell adhesive, BD Biosciences; Bedford, MA) before the experiments were performed. Isolated gastric glands were loaded with 10 μ M 2',7-bis(2-carboxyethyl)-5-(and 6)-carboxyfluorescein acetomethyl ester (BCECF; Molecular Probes), a pH-sensitive dye [18]. After a 20-min dye-loading period and dexamethasone incubation (10 μ M), gland preparations were placed in a perfusion chamber maintained at 37°C on the stage of an inverted microscope (Olympus IX50) attached to a digital imaging system (Universal Imaging; Downingtown, PA) and perfused with HEPES to remove any deesterified dye. BCECF was excited at 490 ± 10 and 440 ± 10 nm, and the emitted fluorescence light was measured at 535 ± 10 nm using an intensified charge-coupled device camera [18]. Data points were collected every 15 s, and the ratio at 490/440 nm was initially recorded as arbitrary pH units, which were then converted to absolute pH using a high-K⁺/nigericin calibration technique [18, 19].

Proton extrusion by individual parietal cells was monitored by observing the recovery of intracellular pH (pH_i) after cells were acid loaded with a Na⁺-free HEPES solution containing 20 mM NH₄Cl. Parietal cells were subsequently superfused with Na⁺-free HEPES, which abolished all Na⁺/H⁺ exchanger activity, trapping H⁺ within the cytosol and initiating an immediate drop in pH_i. Under these conditions, the H⁺ extrusion pathway is exclusively via H⁺/K⁺-ATPase activation. pH_i recovery rates were measured in Na⁺-free HEPES solutions (see Table 1). Dexamethasone was added to all four solutions at a concentration of 10 μ M, H⁺/K⁺-ATPase inhibitor omeprazole

(200 μ M), PI3 kinase inhibitor Wortmannin (500 nM), protein kinase inhibitor staurosporine (2.5 μ M) or H₂ antagonist cimetidine (100 μ M) [all purchased from Sigma, BCH] were added as indicated.

Recovery rates (Δ pH_i) were calculated from the same initial starting pH to eliminate potential variations in the individual intracellular buffering power of cells under the different experimental conditions. The recovery rates are expressed as the change in pH (Δ pH) units per minute and were calculated over the pH range of 6.4–7.2.

Statistical analysis

An unpaired Student's *t*-test was used to test for differences in the pH of *in vitro* parietal cell pH_i experiments. ANOVA test was used to test differences of more than two groups.

Results

A subcutaneous injection with dexamethasone (150 μ g/100 g) for three days was followed by a significant increase of parietal acid secretion. As illustrated in Fig. 1, the H⁺ recovery averaged 0.038 ± 0.010 ($n = 5$) in the control rats (injected with 140mMol NaCl), and 0.096 ± 0.011 ($n = 6$) in the dexamethasone injected rats.

As illustrated in Fig. 2A and 2B, *in vitro* incubation with 10 μ M dexamethasone for 20 minutes in the ab-

Fig. 3. Lack of immediate dexamethasone effects on gastric acid secretion, effect of cimetidine. A: Original pH measurement of direct application (black arrow) of dexamethasone (10 μ M). B: Arithmetic means $\pm$ SEM of intracellular pH recovery (Δ pHi) in gastric glands before application of dexamethasone (open column) and after application of dexamethasone (closed column). Rectangles indicate arithmetic means $\pm$ SEM of intracellular pH recovery (Δ pHi) in gastric glands after 20 minutes incubation with dexamethasone (10 μ M) in absence (closed column) or presence (open column) of cimetidine (100 μ M). * indicates significant difference from control cells $p < 0.05$ (ANOVA using Dunnett's test).

sence of secretagogues increased the pH_i recovery following an acid load from 0.029 ± 0.005 ($n = 8$, vehicle treated) to 0.085 ± 0.006 ($n = 36$). The effect was concentration dependent (Fig. 2C). However, dexamethasone (10 μ M) had no effect within the first 3 minutes (Fig. 3A and 3B). The effect was not significantly modified by 5 μ M brefeldin A (data not shown).

A further series of experiments explored the possible role of histamine release from remaining ECL cells, which could lead to acid secretion in the absence of exogenous application of secretagogues. Glands were exposed to cimetidine (100 μ M) to block an endogenous histamine response. As shown in Figure 3B Δ pHi following dexamethasone treatment was not significantly different in the absence (Δ pHi = 0.069 ± 0.009) and presence (Δ pHi: 0.072 ± 0.011) of the H₂ receptor blocker

Fig. 4. Inhibition of dexamethasone stimulated acid secretion by H⁺/K⁺-ATPase blocker omeprazole. A: Original pH measurement of a 20 minutes incubation with dexamethasone (10 μ M) in absence (upper graph) or presence (lower graph) of omeprazole (200 μ M). B: Arithmetic means $\pm$ SEM of intracellular pH recovery (Δ pHi) in gastric glands after 20 minutes incubation with Ringer (-) and Ringer containing dexamethasone (+; 10 μ M) without (closed column) or with (open column) omeprazole (200 μ M). * indicates significant difference from control, $p < 0.05$, # indicates significant difference from dexamethasone (ANOVA using Tukey's test).

Cimetidine (100 μ M). Thus, the dexamethasone effect was not due to release of histamine and the concurrent stimulation of the H⁺/K⁺-ATPase.

Fig. 5. Blunting of dexamethasone stimulated acid secretion by the PI3 – kinase inhibitor Wortmannin. A: Original pH measurement of a 20 minutes incubation with dexamethasone (10 μM) in absence (upper graph) or presence (lower graph) of Wortmannin (500 nM). B: Arithmetic means ± SEM of intracellular pH recovery (ΔpHi) in gastric glands after 20 minutes incubation with Ringer (-) or Ringer containing dexamethasone (+; 10 μM) without (closed column) or with (open column) Wortmannin (500 nM) * indicates significant difference from controls, $p < 0.05$, # indicates significant difference from dexamethasone (ANOVA using Tukeys test).

To confirm that the effect of dexamethasone was mediated by K^+ dependent proton extrusion through the gastric H^+/K^+ -ATPase isoform, a series of studies was conducted with the potent H^+/K^+ -ATPase inhibitor

Fig. 6. Blunting of dexamethasone stimulated acid secretion by staurosporine. A: Original pH measurement of a 20 minutes incubation with dexamethasone (10 μM) in absence (upper graph) or presence (lower graph) of staurosporine 2.5 microMol. B: Arithmetic means ± SEM of intracellular pH recovery (ΔpHi) in gastric glands after 20 minutes incubation of dexamethasone (10 μM) without (closed column) or with (open column) staurosporine (2.5 μM). # indicates significant difference from dexamethasone, $p < 0.05$ (unpaired t-test).

omeprazole (200 μM). ΔpHi following dexamethasone treatment was significantly lower in the presence (0.053 ± 0.004 , $n = 6$) than in the absence (0.092 ± 0.010 , $n = 6$) of omeprazole (Fig. 4).

Fig. 7. Inhibition of dexamethasone stimulated acid secretion by Cl^- channel blocker NPPB. Arithmetic means $\pm$ SEM of intracellular pH recovery (ΔpH_i) in gastric glands after 20 minutes incubation of dexamethasone (10 μM) without (closed column) or with (open column) NPPB (100 μM). # indicates significant difference from dexamethasone, $p < 0.05$ (unpaired t-test).

To explore, whether the stimulating effect of dexamethasone required phosphatidylinositol (PI) 3 kinase, experiments have been performed in the absence and presence of PI3 kinase inhibitor Wortmannin (500 nM). Following dexamethasone treatment intracellular ΔpH_i was significantly lower in the presence (0.037 ± 0.011 , $n = 7$) than in the absence (0.117 ± 0.015 , $n = 7$) of Wortmannin (Fig. 5), demonstrating that active PI3 kinase is required for the effect of dexamethasone on gastric acid secretion.

To investigate the possible role of protein kinases, the potent but unspecific kinase inhibitor staurosporine (2.5 μM) was applied with dexamethasone (10 μM) 20 minutes prior to the measurements. ΔpH_i following dexamethasone treatment was significantly lower in the presence (0.036 ± 0.010 , $n = 3$) than in the absence (0.076 ± 0.008 , $n = 3$) of staurosporine (Fig. 6).

Further experiments explored whether the effect of dexamethasone could be blocked by inhibition of Cl^- channels. As shown in Fig. 7, the Cl^- channel blocker 5-Nitro-2-3-phenylpropylaminobenzoic acid (NPPB) significantly blunted H^+ secretion in dexamethasone treated glands. ΔpH_i following dexamethasone treatment was significantly lower in the presence (0.023 ± 0.018 , $n = 5$) than in the absence (0.117 ± 0.039 , $n = 3$) of 100 μM NPPB (100 μM).

Discussion

Dexamethasone is a well known potent stimulator of gastric acid secretion [4, 5]. The mechanisms underlying the stimulation of gastric acid secretion by dexamethasone are, however, a matter of controversy. Some studies provided evidence for an inhibitory effect of dexamethasone on the gastric peroxidase, which is in turn an inhibitor of gastric acid secretion [8, 20]. Other studies have shown that dexamethasone triggers the release of histamine from intestinal mast cells [6]. This mechanism suggests an indirect stimulation by dexamethasone through histamine via the H_2 -receptor. The results of our present study indicate that dexamethasone has in addition a direct secretagogue independent effect on the acid secretion in parietal cells. Specifically, we observed that subcutaneous injection of dexamethasone in rats for three days leads to elevated proton extrusion. A similar H^+/K^+ -ATPase dependent stimulation was observed in vitro when gastric glands were incubated for 20 minutes with dexamethasone. These studies were conducted on animals that were fasted prior to the study to assure that secretagogue induced acid secretion was minimal. However, there was still a variation in spontaneous acid secretion, which may have been due to differences in fasting periods. We could rule out that the stimulatory effect was influenced by coincubation with cimetidine, a competitive H_2 -receptor blocker suggesting that histamine does not play a major role in the in vitro stimulation of gastric acid secretion by dexamethasone. The preparation of the glands was performed to expose only parietal cells to dexamethasone. As they have been isolated from the corpus, it is not likely, that the in vitro stimulation of dexamethasone is due to histamine or acetylcholine release.

In the unstimulated state, the parietal cell contains abundant intracellular tubulovesicles that sequester H^+/K^+ -ATPase and channel proteins [21]. Upon stimulation, the tubulovesicles fuse with the apical membrane leading to enhanced cell surface expression of the H^+/K^+ -ATPase protein and ion channel proteins. This trafficking process is a major regulating process of gastric acid secretion. To investigate the process of anterograde transports of proteins, we treated glands with Brefeldin A and dexamethasone. Brefeldin A inhibits vesicle formation at the Golgi apparatus [22] and thus stops anterograde transport of proteins to the membrane. Our study demonstrates that Brefeldin A does not significantly blunt the effect of dexamethasone. Thus, stimulation of acid secretion by dexamethasone is presumably not related to enhanced anterograde transport of channel or H^+/K^+ -ATPase pro-

teins to the membrane. Possibly, dexamethasone activates transport proteins already expressed at the apical cell surface. Direct application of dexamethasone at the beginning of acid secretion, however, has no stimulating influence on acid secretion. Thus, dexamethasone does not directly activate the H^+/K^+ -ATPase.

Our observations further point to an involvement of PI3 kinase in the glucocorticoid stimulation of acid secretion of rat gastric glands. The PI3 kinase is blocked by Wortmannin, which interacts with the catalytic center of PI3 kinase [23, 24]. As Wortmannin blunts the dexamethasone stimulated gastric acid secretion, we propose that the PI3 kinase plays an important role in the regulation of gastric acid secretion. Several PI3 kinase dependent kinases [16] are inhibited by staurosporine, a potent but rather unspecific inhibitor of several protein kinases including protein kinase C [25], and the Serum and Glucocorticoid inducible Kinase (SGK) [26]. The SGK kinase was established as a strong regulator of various membrane transport proteins [27-29] and ion channels [30, 31]. The SGK gene contains a Glucocorticoid Response Element (GRE) [32]. Thus SGK is under transcriptional control of glucocorticoids and thus dexamethasone increases the transcription of the SGK1 [15].

One of the possible targets of glucocorticoid regulation is the K^+ -channel KCNQ1, which is required for gastric acid secretion. KCNQ1 is colocalized with the

β -subunit KCNE2 in the apical membrane of parietal cells [33]. Since gastric acid secretion is mediated primarily by luminal H^+/K^+ -ATPase, it was postulated that KCNQ1/KCNE2 channel complexes recycle K^+ from the cell to the lumen to provide K^+ for H^+ , K^+ exchange mechanism [33]. In a mouse lacking functional KCNQ1, or the β -subunit of the H^+/K^+ -ATPase [9], a phenotypic change of gastric hyperplasia and achlorhydria [10, 11] were observed. Nevertheless, experiments with the chloride channel blocker NPPB point to the requirement of Cl^- channel activity in the stimulation of acid secretion by dexamethasone.

In conclusion, the present study reports a novel mechanism by which dexamethasone stimulates gastric acid secretion. The PI3 kinase pathway appears to play the major role in this stimulation. Blocking the PI3 kinase with Wortmannin or PKC/SGK with staurosporine inhibits the Dexamethasone induced secretagogue-independent acid secretion. Furthermore, the effect of dexamethasone also appears to require the activity of chloride channels within the parietal cells.

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