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## Bile Acids Accompanied by Acid and Pepsin Stimulate Salivary Secretion, Mediated by the Esophagosally Reflex, Both in Controls and Patients with Gastroesophageal Reflux Disease

Sole kwasów żółciowych oraz kwas solny i pepsyna – stymulacja wydzielania śliny przez odruch przełykowo-śliniankowy u ludzi zdrowych i chorych na chorobę refluksową

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### Abstract

**Objectives.** The aim of the study was to define the role of bile acids (BA), accompanied by HCl-Pepsin (HCl/P/BA), in stimulation of salivary secretory response mediated by the esophagosally reflex.

**Material and Methods.** The rate of secretion of salivary inorganic (bicarbonate, non-bicarbonate) and organic (protein, glycoconjugate, prostaglandin E<sub>2</sub> – PGE<sub>2</sub>, epidermal growth factor – EGF) protective components was investigated in 19 control subjects and 18 patients with gastroesophageal reflux disease (GERD) under basal conditions, mastication, and during intraesophageal mechanical (tubing, balloons) and chemical stimulation (HCl/Pepsin/BA) mimicking the duodenogastroesophageal reflux (DGER).

**Results.** The significantly increased rates of salivary bicarbonate ( $p < 0.05$ ) and nonbicarbonate ( $p < 0.05$ ) secretion, accompanied by a significant increase in pH and protein ( $p < 0.05$ ) in controls during HCl/P/BA stimulation were demonstrated. In patients with GERD, nonbicarbonate and protein outputs significantly increased ( $p < 0.05$ ) during stimulation with HCl/P/BA. Although, the rates of secretion of bicarbonate and protein in patients with GERD were significantly lower ( $p < 0.05$ ), the volume of saliva, EGF, and PGE<sub>2</sub> were significantly higher ( $p < 0.05$ ) than in controls during exposure to HCl/P/BA.

**Conclusions.** 1. Acid, pepsin and bile acids, mimicking the duodenogastroesophageal refluxate, elicit a significant salivary secretory response, therefore, are potent stimulants of the esophagosally reflex, both in controls and patients with GERD. 2. Significant differences in the content of salivary protective factors during intraesophageal stimulation with HCl/P/BA, mediated by the esophagosally reflex, between controls and patients with GERD indicates that protective mechanisms are heterogeneous and may contribute to the development of mucosal changes (Adv Clin Exp Med 2005, 14, 2, 237–245).

**Key words:** salivary esophagoprotection induced by bile acids, HCl, pepsin.

### Streszczenie

**Cel pracy.** Określenie roli soli kwasów żółciowych (BA) oraz HCl i pepsyny i BA (HCl/P/BA) w stymulacji wydzielania ślinowego przez odruch przełykowo-śliniankowy.

**Materiał i metody.** Oznaczano wydzielanie ślinowe nieorganicznych (dwuwęglany i niedwuwęglany) oraz organicznych (białko, glikokoniugaty, prostaglandyny E<sub>2</sub>, czynnik wzrostu naskórka – EGF) czynników ochronnych u 19 zdrowych ochotników i 18 pacjentów z chorobą refluksową (GERD) w warunkach podstawowych, podczas żucia i w czasie wewnątrzprzełykowej stymulacji mechanicznej i chemicznej naśladującej refluks dwunastniczo-żółdkowo-przełykowy (DGER).

**Wyniki.** Zanotowano znamieny wzrost wydzielania ślinowego dwuwęglanów ( $p < 0,05$ ) i niedwuwęglanów ( $p < 0,05$ ) z towarzyszącym wzrostem pH i wydzielaniem białka ( $p < 0,05$ ) w grupie zdrowych ochotników podczas stymulacji chemicznej. Wyrzut niedwuwęglanów i białka ( $p < 0,05$ ) wzrastał w czasie stymulacji chemicznej. Wyrzut dwuwęglanów, białka u pacjentów z GERD był niższy ( $p < 0,05$ ) niż u zdrowych ochotników. Wyrzut EGF, PGE2 był natomiast wyższy niż w grupie zdrowych ochotników w czasie stymulacji chemicznej ( $p < 0,05$ ).

**Wnioski.** 1. Kwas solny, pepsyna i sole kwasów żółciowych wywołują wydzielanie ślinowych czynników ochronnych przez stymulację odruchu przełykowo-ślinowego u chorych oraz u pacjentów chorych na GERD. 2. Różnice w zawartości ślinowych czynników ochronnych podczas wewnątrzprzełykowej stymulacji chemicznej HCl/P/BA między zdrowymi ochotnikami a pacjentami chorymi na GERD wskazuje na heterogeniczność tego procesu i może odpowiadać za uszkodzenia w błonie śluzowej (*Adv Clin Exp Med* 2005, 14, 2, 237–245).

**Słowa kluczowe:** ślinowe czynniki ochronne w refluksie żółciowym, kwas solny i pepsyna.

The integrity of the esophageal mucosa depends upon an equilibrium between aggressive factors and protective mechanisms [1–4]. The esophageal mucosal defense comprises three complementary barriers: a) pre-epithelial, b) epithelial and c) post-epithelial. The quantity and the quality of the pre-epithelial barrier is based on its two major components: saliva and the secretion elaborated by the esophageal submucosal mucus glands [5].

The salivary secretion in controls and patients with reflux esophagitis (RE) is augmented by intraesophageal chemical stimulation with HCl and pepsin, and is mediated by the esophagosali-vary reflex [6–11].

Evidence is accumulating that among aggressive factors components of bile play an important role in the development of the esophageal mucosal pathology [12–14].

However, the rate of salivary secretion mediated by the esophagosali-vary reflex in response to HCl and pepsin accompanied by bile acids, mimicking the duodenogastroesophageal reflux, remains to be explored.

The authors have studied, therefore, the rate of secretion of salivary inorganic and organic protective components during esophageal mechanical and chemical stimulation by acid, pepsin and bile acids, in controls and patients with gastroesophageal reflux disease (GERD).

## Material and Methods

### Collection of Saliva

The study was conducted in 19 asymptomatic volunteers (mean age – 33 years; 11 M, 8 F; 15 Caucasians, 2 African-Americans, 2 Asians) and 18 GERD patients (mean age – 29 years; 6 M, 10 F; 14 Caucasians, 2 Asians) with a long (over 1 year) history of heartburn. All investigated subjects in both groups were nonsmokers before enrolment into the study protocol. All subjects

were instructed not to swallow the saliva during all experimental periods. The saliva was expectorated every 10 sec during the experimental procedure. The following salivary samples were collected on ice for each subject:

- 1) basal salivary – during the first 10 min,
- 2) saliva stimulated by mastication – during chewing the parafilm for 5 min.

### Mechanical Stimulation of the Esophagus

1. Stimulation of saliva by tubing – after placement the intraesophageal catheter – three consecutive 1.5-min intervals.

2. Stimulation of saliva by balloons – after inflation of intraesophageal balloons – three consecutive 1.5-min intervals.

### Chemical Stimulation of the Esophagus

1. Production of saliva during the initial perfusion with saline – three consecutive 1.5-min intervals.

2. Production of saliva during the initial perfusion with hydrochloric acid, pepsin, and bile acids – three consecutive 1.5-min intervals.

3. Production of saliva during final hydrochloric acid, pepsin, and bile acids perfusion – the next three consecutive 1.5 min intervals

4. Production of saliva during the final perfusion with saline – the last three consecutive 1.5 min intervals.

Collection of saliva in all patients was done at the same time of the day. All subjects had not any mastication dysfunction and did not receive any medication before the experimental procedure.

### Esophageal Perfusion Catheter

Esophageal perfusion was performed with a specially designed six-channel catheter. The four larger diameter channels were used for infusion and aspiration of the perfusate, aspiration of gas-

tric juice and aspiration of incidentally swallowed saliva, which is retained above the upper balloon. The two small-diameter channels were used for inflation of the upper and lower balloons to compartmentalize a 3.75-cm segment of the lower esophagus.

### Esophagus Perfusion Solutions

For each 1.5-min intervals of perfusion fresh 10-ml solutions was prepared.

1. The initial saline perfusion – NaCl (0.15 M).
2. The initial hydrochloric acid, pepsin and bile acids perfusion – HCl (0.01 M; pH 2.1), pepsin 0.5 mg/ml and 10 mM mixture of bile acid salts. The bile acid salts mixture was prepared as follows:

- A. Glycocholic acid – 28% of the total solution (2.8 mM),

- B. Glycodeoxycholic acid – 10.7% of the total solution (1.07 mM),

- C. Glycochenodeoxycholic acid – 28% of the total solution (2.8 mM),

- D. Taurocholic acid – 14% of the total solution (1.4 mM),

- E. Taurodeoxycholic acid – 5.3% of the total solution (0.53 mM),

- F. Taurochenodeoxycholic acid – 14% of the total solution (1.4 mM).

3. The final hydrochloric acid, pepsin and bile acid perfusion – HCl (0.01 M; pH 2.1), pepsin 0.5 mg/ml and 10 mM mixture of bile acid salts (similar to described above).

4. The final perfusion with saline – NaCl (0.15 M).

HCl concentration corresponds to the most commonly seen pH of the gastroesophageal refluxate [15], pepsin concentration corresponds to the average proteolytic activity of human gastric juice [16, 17]. Concentration of bile acids corresponds to their most frequently found values in gastric juice in patients with duodenogastric reflux.

### Esophageal Perfusion Procedure

Subjects were placed in semirecumbent position. The nasopharynx was anesthetized with Xylocaine gel and esophageal catheter was inserted into the esophagus through the nares. Then catheter's balloons were insufflated. This procedure allows the compartmentalization of 3.75-cm segment of the lower esophagus with the distal balloon above the lower esophageal sphincter. During each of the chemical stimulation periods 10-ml solution of perfusate was circulated within the isolated 3.75-cm segment of esophagus.

## Biochemical Methods for the Measurement of the Salivary Secretory Components

Salivary volume of samples collected on ice was measured using a sialometer (Proflow Incorporated, Amityville, New York), as it was described in previous papers by the authors. Salivary pH was monitored using the Expandable Ion Analyzer EA 940 (Orion Res., Boston, Massachusetts). Salivary bicarbonate content was analyzed by titration and back titration using TitraLab 90 (Radiometer America Inc., Chicago Illinois) according to Izutsu [18]. Salivary glycoconjugate was measured using the periodic acid Schiff (PAS) methodology [19]. Salivary EGF was measured using the RIA kit (Amersham, Arlington Heights, Illinois). The details of methodology were described in previous publication. The salivary PGE<sub>2</sub> was measured using RIA kit (Amersham, Arlington Heights, Illinois), as described previously. The protein concentration was measured according to Lowry method [20].

Statistical analysis was performed using  $\Sigma$ -Stat software (Jandel Scientific, San Rafael, California). Data in each period of mechanical and chemical stimulation are presented as the mean  $\pm$  SEM value of three consecutive 1.5-min intervals.

## Results

### Salivary Inorganic Protective Components in Asymptomatic Volunteers (Tab. 1)

Salivary bicarbonate outputs in the control group during the chemical stimulation with initial and final HCl/P/BA, significantly increased when compared to the period of initial saline ( $84.05 \pm 16.1$  vs.  $20.57 \pm 4.04$ , and  $31.39 \pm 7.65$  vs.  $20.57 \pm 4.04$   $\mu\text{mol/min}$ ,  $p < 0.05$ , respectively). Nonbicarbonate output during perfusion with HCl/P/BA also significantly increased ( $62.5 \pm 9.64$  vs.  $21.36 \pm 5.27$   $\mu\text{mol/min}$ ,  $p < 0.05$ ) resulting in significantly higher salivary pH during the period of exposure to HCl/P/BA ( $7.56 \pm 0.01$  vs.  $7.48 \pm 0.02$ ,  $p < 0.05$ ).

### Salivary Organic Protective Components in Asymptomatic Volunteers (Tab. 2)

Protein output in saliva during esophageal exposure to HCl/P/BA significantly increased over its corresponding value during perfusion with ini-

**Table 1.** Salivary inorganic protective components in controls under basal condition and during intraesophageal mechanical and chemical stimulation mediated by esophagosally reflex (mean  $\pm$  SEM)**Tabela 1.** Nieorganiczne ślinowe czynniki ochronne w grupie kontrolnej w warunkach podstawowych i podczas stymulacji wewnątrzprzełykowej (wartości średnie  $\pm$  SEM)

Parameter	Basal	Mastication	Tubing	Balloon	NaCl	HCl/P/BA	HCl/P/BA	NaCl
Volume (ml/min)	0.76 $\pm$ 0.09	1.50 $\pm$ 0.14	3.05 $\pm$ 0.31	3.82 $\pm$ 0.48	3.69 $\pm$ 0.42	3.95 $\pm$ 0.45	4.18 $\pm$ 0.48	3.66 $\pm$ 0.34
pH	7.19 $\pm$ 0.03	7.20 $\pm$ 0.08	7.25 $\pm$ 0.05	7.24 $\pm$ 0.04	7.48 $\pm$ 0.02	7.56 $\pm$ 0.01 <sup>a</sup>	7.50 $\pm$ 0.02	7.48 $\pm$ 0.04
Bicarbonate ( $\mu$ mol/min)	6.94 $\pm$ 1.17	14.7 $\pm$ 2.46	34.66 $\pm$ 6.93	36.09 $\pm$ 7.16	20.51 $\pm$ 4.04	84.05 $\pm$ 16.14 <sup>a</sup>	31.39 $\pm$ 7.65 <sup>a</sup>	24.97 $\pm$ 4.33
Nonbicarbonate ( $\mu$ mol/min)	5.49 $\pm$ 1.19	12.53 $\pm$ 2.13	38.25 $\pm$ 4.79	34.20 $\pm$ 6.43	21.36 $\pm$ 5.27	62.48 $\pm$ 9.64 <sup>a</sup>	36.10 $\pm$ 8.61	21.52 $\pm$ 2.91

<sup>a</sup>  $p < 0.05$  vs. NaCl.

HCl/P/BA – hydrochloric acid + pepsin + bile acids solution.

**Table 2.** Salivary organic protective components in controls under basal condition and during intraesophageal mechanical and chemical stimulation mediated by esophagosally reflex (mean  $\pm$  SEM)**Tabela 2.** Organiczne ślinowe czynniki ochronne w grupie kontrolnej w warunkach podstawowych i podczas stymulacji wewnątrzprzełykowej (wartości średnie  $\pm$  SEM)

Parameter	Basal	Mastication	Tubing	Balloon	NaCl	HCl/P/BA	HCl/P/BA	NaCl
Glyco-conjugate (mg/min)	1.95 $\pm$ 0.30	3.11 $\pm$ 0.47	17.98 $\pm$ 2.43	22.20 $\pm$ 3.42	23.07 $\pm$ 4.12	22.56 $\pm$ 3.89	22.57 $\pm$ 3.43	17.74 $\pm$ 2.49
Protein (mg/min)	2.60 $\pm$ 0.4	3.70 $\pm$ 0.5	5.5 $\pm$ 0.7	6.8 $\pm$ 0.8	5.7 $\pm$ 0.7	6.6 $\pm$ 0.6 <sup>a</sup>	6.2 $\pm$ 0.6	6.0 $\pm$ 0.5
EGF (ng/min)	1.41 $\pm$ 0.28	2.59 $\pm$ 0.55	4.40 $\pm$ 0.79	4.24 $\pm$ 0.74	4.56 $\pm$ 1.02	5.11 $\pm$ 1.22	3.85 $\pm$ 1.07	6.92 $\pm$ 1.87 <sup>b</sup>
PGE <sub>2</sub> (pg/min)	112 $\pm$ 29.4	106 $\pm$ 28.2	205 $\pm$ 58.1	282 $\pm$ 132	286 $\pm$ 105	373 $\pm$ 117	251 $\pm$ 95.1	373 $\pm$ 69.2

<sup>a</sup>  $p < 0.05$  vs. NaCl.<sup>b</sup>  $p < 0.1$  vs. NaCl.

HCl/P/BA – hydrochloric acid + pepsin + bile acids solution.

tial saline (6.6  $\pm$  0.6 vs. 5.7  $\pm$  0.7 mg/min,  $p < 0.05$ ). The EGF output during the final saline, after discontinuation of HCl/P/BA, also increased (52%) when compared with initial NaCl, although this trend was not statistically significant ( $p < 0.1$ ).

### Salivary Inorganic Protective Components in GERD Patients (Tab. 3)

The nonbicarbonate output significantly increased during perfusion with HCl/P/BA over its corresponding values during the mucosal exposure to initial saline (54.22  $\pm$  10.04 vs. 20.46  $\pm$  4.33  $\mu$ mol/min,  $p < 0.05$ ).

### Salivary Organic Protective Components in GERD Patients (Tab. 4)

Both periods of mucosal exposure to HCl/P/BA significantly augmented protein outputs when compared to initial saline (5.17  $\pm$  0.21 vs. 3.93  $\pm$  0.14 mg/min,  $p < 0.05$  and 6.10  $\pm$  0.70 vs. 3.93  $\pm$  0.14 mg/min,  $p < 0.05$ , respectively). An increase (78%) in EGF output during final saline was also demonstrated, although this trend was not statistically significant ( $p < 0.1$ ).

**Table 3.** Salivary inorganic protective components in GERD group under basal condition and during intraesophageal mechanical and chemical stimulation mediated by esophagosalivary reflex (mean  $\pm$  SEM)**Tabela 3.** Nieorganiczne ślinowe czynniki ochronne w grupie GERD w warunkach podstawowych i podczas stymulacji wewnątrzprzełykowej (wartości średnie  $\pm$  SEM)

Parameter	Basal	Mastication	Tubing	Balloon	NaCl	HCl/P/BA	HCl/P/BA	NaCl
Volume (ml/min)	0.59 $\pm$ 0.07	1.57 $\pm$ 0.19	2.81 $\pm$ 0.23	4.05 $\pm$ 0.40	3.44 $\pm$ 0.34	4.11 $\pm$ 0.43	4.36 $\pm$ 0.44	3.87 $\pm$ 0.34
pH	7.08 $\pm$ 0.01	7.12 $\pm$ 0.01	7.16 $\pm$ 0.02	7.17 $\pm$ 0.03	7.38 $\pm$ 0.03	7.41 $\pm$ 0.03	7.40 $\pm$ 0.04	7.36 $\pm$ 0.06
Bicarbonate ( $\mu$ mol/min)	5.18 $\pm$ 1.06	10.01 $\pm$ 1.76	21.14 $\pm$ 2.93	32.79 $\pm$ 6.53	20.72 $\pm$ 3.29	35.48 $\pm$ 6.79	31.16 $\pm$ 7.23	20.94 $\pm$ 3.94
Nonbicarbonate ( $\mu$ mol/min)	2.64 $\pm$ 0.75	5.81 $\pm$ 1.11	18.54 $\pm$ 3.92	36.69 $\pm$ 6.32	20.46 $\pm$ 4.33	54.22 $\pm$ 10.04 <sup>a</sup>	24.22 $\pm$ 4.78	11.39 $\pm$ 2.89

<sup>a</sup>  $p < 0.05$  vs. NaCl.

HCl/P/BA – hydrochloric acid + pepsin + bile acids solution.

**Table 4.** Salivary organic protective components in GERD group under basal condition and during intraesophageal mechanical and chemical stimulation mediated by esophagosalivary reflex (mean  $\pm$  SEM)**Tabela 4.** Organiczne ślinowe czynniki ochronne w grupie GERD w warunkach podstawowych i podczas stymulacji wewnątrzprzełykowej (wartości średnie  $\pm$  SEM)

Parameter	Basal	Mastication	Tubing	Balloon	NaCl	HCl/P/BA	HCl/P/BA	NaCl
Glyco-conjugate (mg/min)	1.99 $\pm$ 0.19	4.69 $\pm$ 0.64	14.88 $\pm$ 1.59	22.04 $\pm$ 3.65	20.21 $\pm$ 2.46	21.57 $\pm$ 2.65	21.34 $\pm$ 2.92	17.54 $\pm$ 2.27
Protein (mg/min)	0.97 $\pm$ 0.07	1.60 $\pm$ 0.11	3.96 $\pm$ 0.11	7.89 $\pm$ 0.23	3.93 $\pm$ 0.14	5.17 $\pm$ 0.21 <sup>a</sup>	6.10 $\pm$ 0.70 <sup>a</sup>	6.24 $\pm$ 0.63
EGF (ng/min)	2.29 $\pm$ 0.62	4.09 $\pm$ 0.56	8.31 $\pm$ 1.77	9.80 $\pm$ 1.21	9.70 $\pm$ 1.31	12.29 $\pm$ 1.99	14.74 $\pm$ 3.04	17.28 $\pm$ 7.38 <sup>b</sup>
PGE <sub>2</sub> (pg/min)	171 $\pm$ 62.2	439 $\pm$ 166	318 $\pm$ 143	609 $\pm$ 221	393 $\pm$ 103	483 $\pm$ 132	472 $\pm$ 128	410 $\pm$ 85.2

<sup>a</sup>  $p < 0.05$  vs. NaCl.<sup>b</sup>  $p < 0.1$  vs. NaCl.

HCl/P/BA – hydrochloric acid + pepsin + bile acids solution.

### The Comparison of Salivary Inorganic Protective Components between Healthy Volunteers and Patients with GERD (Tab. 5)

In patients with GERD, the pH during initial saline, the initial and final HCl/P/BA, and during the final saline was significantly lower than in controls (7.38  $\pm$  0.03 vs. 7.48  $\pm$  0.03,  $p < 0.05$ ; 7.41  $\pm$  0.03 vs. 7.56  $\pm$  0.01,  $p < 0.05$ ; 7.40  $\pm$  0.04 vs. 7.50  $\pm$  0.02,  $p < 0.05$  and 7.36  $\pm$  0.06 vs. 7.48  $\pm$  0.04,  $p < 0.05$ , respectively).

The bicarbonate output in the GERD group was significantly lower than in control during perfusion with initial HCl/P/BA (35.5  $\pm$  6.79 vs. 84.0  $\pm$  16.1  $\mu$ mol/min,  $p < 0.05$ ).

The authors found the statistically lower output of the nonbicarbonate in GERD group during the

final HCl/P/BA perfusion when compared to controls (24.2  $\pm$  4.78 vs. 36.10  $\pm$  8.61  $\mu$ mol/min,  $p < 0.05$ ).

### The Comparison of Salivary Organic Protective Components between Healthy Volunteers and Patients with GERD (Tab. 6)

The protein output in the GERD group was significantly lower during the initial saline and initial HCl/P/BA than in controls (3.93  $\pm$  0.14 vs. 6.1  $\pm$  0.7 mg/min,  $p < 0.05$  and 5.17  $\pm$  0.21 vs. 6.1  $\pm$  0.6 mg/min,  $p < 0.05$ , respectively).

The salivary EGF output in GERD was significantly higher than in control group during initial saline, initial and final HCl/P/BA and final saline (9.70  $\pm$  1.31 vs. 4.56  $\pm$  1.02 ng/min,  $p < 0.05$ ; 12.29  $\pm$  1.99 vs. 5.11  $\pm$  1.22 ng/min,  $p < 0.05$ , 14.74  $\pm$  3.04 vs. 3.85  $\pm$  1.07 ng/min,  $p < 0.05$  and



**Table 5.** Salivary inorganic protective components in control (CTRL) and gastroesophageal reflux disease (GERD) groups under basal condition and during intraesophageal mechanical and chemical stimulation mediated by esophagosally reflex (mean  $\pm$  SEM)**Tabela 5.** Nieorganiczne ślinowe czynniki ochronne w grupie kontrolnej i GERD w warunkach podstawowych i podczas stymulacji wewnątrzprzełykowej (wartości średnie  $\pm$  SEM)

Parameter	Basal	Mastication	Tubing	Balloon	NaCl	HCl/P/BA	HCl/P/BA	NaCl
Volume CTRL (ml/min)	0.76 $\pm$ 0.09	1.50 $\pm$ 0.14	3.05 $\pm$ 0.31	3.82 $\pm$ 0.48	3.69 $\pm$ 0.42	3.95 $\pm$ 0.45	4.18 $\pm$ 0.48	3.66 $\pm$ 0.34
Volume GERD (ml/min)	0.59 $\pm$ 0.07 <sup>x</sup>	1.57 $\pm$ 0.19	2.81 $\pm$ 0.23	4.05 $\pm$ 0.40	3.78 $\pm$ 0.34	3.95 $\pm$ 0.37	4.36 $\pm$ 0.44	3.87 $\pm$ 0.34
pH CTRL	7.19 $\pm$ 0.03	7.20 $\pm$ 0.08	7.25 $\pm$ 0.05	7.24 $\pm$ 0.04	7.48 $\pm$ 0.03	7.56 $\pm$ 0.01	7.50 $\pm$ 0.02	7.48 $\pm$ 0.04
pH GERD	7.08 $\pm$ 0.01 <sup>x</sup>	7.12 $\pm$ 0.01	7.16 $\pm$ 0.02 <sup>x</sup>	7.17 $\pm$ 0.03 <sup>x</sup>	7.38 $\pm$ 0.03 <sup>x</sup>	7.41 $\pm$ 0.03 <sup>x</sup>	7.40 $\pm$ 0.04 <sup>x</sup>	7.36 $\pm$ 0.06 <sup>x</sup>
Bicarbonate CTRL ( $\mu$ mol/min)	6.94 $\pm$ 1.17	14.7 $\pm$ 2.46	34.66 $\pm$ 6.93	36.09 $\pm$ 7.16	20.51 $\pm$ 4.04	84.05 $\pm$ 16.14	31.39 $\pm$ 7.65	24.97 $\pm$ 4.33
Bicarbonate GERD ( $\mu$ mol/min)	5.18 $\pm$ 1.06	10.01 $\pm$ 1.76	21.14 $\pm$ 2.93 <sup>x</sup>	32.79 $\pm$ 6.53	20.72 $\pm$ 3.29	35.48 $\pm$ 6.79 <sup>x</sup>	31.16 $\pm$ 7.23	20.94 $\pm$ 3.94
Nonbicarbonate CTRL ( $\mu$ mol/min)	5.49 $\pm$ 1.19	12.53 $\pm$ 2.13	38.25 $\pm$ 4.79	34.20 $\pm$ 6.43	21.36 $\pm$ 5.27	62.48 $\pm$ 9.64	36.10 $\pm$ 8.61	21.52 $\pm$ 2.91
Nonbicarbonate GERD ( $\mu$ mol/min)	2.64 $\pm$ 0.75 <sup>x</sup>	5.81 $\pm$ 1.11 <sup>x</sup>	18.54 $\pm$ 3.92 <sup>x</sup>	36.69 $\pm$ 6.32	20.46 $\pm$ 4.33	54.22 $\pm$ 10.04	24.22 $\pm$ 4.78	11.39 $\pm$ 2.89 <sup>x</sup>

<sup>x</sup> p < 0.05 vs. CTRL.

HCl/P/BA – hydrochloric acid + pepsin + bile acids solution.

**Table 6.** Salivary organic protective components in control (CTRL) and gastroesophageal reflux disease (GERD) groups under basal condition and during intraesophageal mechanical and chemical stimulation mediated by esophagosally reflex (Mean  $\pm$  SEM)**Tabela 6.** Organiczne ślinowe czynniki ochronne w grupie kontrolnej i GERD w warunkach podstawowych i podczas stymulacji wewnątrzprzełykowej (wartości średnie  $\pm$  SEM)

Parameter	Basal	Mastication	Tubing	Balloon	NaCl	HCl/P/BA	HCl/P/BA	NaCl
Glyco-conjugate CTRL (mg/min)	1.95 $\pm$ 0.30	3.11 $\pm$ 0.47	17.98 $\pm$ 2.43	22.20 $\pm$ 3.42	23.07 $\pm$ 4.12	22.56 $\pm$ 3.89	22.57 $\pm$ 3.43	17.74 $\pm$ 2.49
Glyco-conjugate GERD (mg/min)	1.99 $\pm$ 0.19	4.69 $\pm$ 0.64	14.88 $\pm$ 1.59	22.04 $\pm$ 3.65	20.21 $\pm$ 2.46	21.57 $\pm$ 2.65	21.34 $\pm$ 2.92	17.54 $\pm$ 2.27
Protein CTRL (mg/min)	2.60 $\pm$ 0.4	3.70 $\pm$ 0.5	5.5 $\pm$ 0.7	6.8 $\pm$ 0.8	6.1 $\pm$ 0.7	6.1 $\pm$ 0.6	6.2 $\pm$ 0.6	6.0 $\pm$ 0.5
Protein GERD (mg/min)	0.97 $\pm$ 0.07 <sup>x</sup>	1.60 $\pm$ 0.11 <sup>x</sup>	3.96 $\pm$ 0.11 <sup>x</sup>	7.89 $\pm$ 0.23 <sup>x</sup>	3.93 $\pm$ 0.14 <sup>x</sup>	5.17 $\pm$ 0.21 <sup>x</sup>	6.10 $\pm$ 0.70	6.24 $\pm$ 0.63
EGF CTRL (ng/min)	1.41 $\pm$ 0.28	2.59 $\pm$ 0.55	4.40 $\pm$ 0.79	4.24 $\pm$ 0.74	4.56 $\pm$ 1.02	5.11 $\pm$ 1.22	3.85 $\pm$ 1.07	6.92 $\pm$ 1.87
EGF GERD (ng/min)	2.29 $\pm$ 0.62 <sup>x</sup>	4.09 $\pm$ 0.56 <sup>x</sup>	8.31 $\pm$ 1.77 <sup>x</sup>	9.80 $\pm$ 1.21 <sup>x</sup>	9.70 $\pm$ 1.31 <sup>x</sup>	12.29 $\pm$ 1.99 <sup>x</sup>	14.74 $\pm$ 3.04 <sup>x</sup>	17.28 $\pm$ 7.38 <sup>x</sup>
PGE <sub>2</sub> CTRL (pg/min)	112 $\pm$ 29.4	106 $\pm$ 28.2	205 $\pm$ 58.1	282 $\pm$ 132	286 $\pm$ 105	373 $\pm$ 117	251 $\pm$ 95.1	373 $\pm$ 69.2
PGE <sub>2</sub> GERD (pg/min)	171 $\pm$ 62.2	439 $\pm$ 166 <sup>x</sup>	318 $\pm$ 143	609 $\pm$ 221 <sup>x</sup>	393 $\pm$ 103	483 $\pm$ 132	472 $\pm$ 128 <sup>x</sup>	410 $\pm$ 85.2

<sup>x</sup> p < 0.05 vs. CTRL.

HCl/P/BA – hydrochloric acid + pepsin + bile acids solution.

17.28  $\pm$  7.38 vs. 6.92  $\pm$  1.87 ng/min,  $p < 0.05$ , respectively).

The salivary PGE<sub>2</sub> output in GERD group was significantly higher than in control group during perfusion with the final HCl/P/BA (472  $\pm$  128 vs. 251  $\pm$  95.1 pg/min,  $p < 0.05$ ).

## Discussion

The presented study demonstrated in controls a significant increase (300%) of salivary bicarbonate output during the chemical stimulation of the esophageal mucosa with hydrochloric acid, pepsin and bile acids (HCl/P/BA), and significant increase (200%) in production of nonbicarbonate, accompanied by significantly increased salivary pH. The output of protein during perfusion with the HCl/P/BA increased also significantly. The increased production of salivary protective components indicates, that esophageal exposure to HCl/P/BA in asymptomatic volunteers may stimulate salivary protective potential through the esophagosaliary reflex. This stimulation affects the quantity and the quality of the salivary secretory components without any significant increase in the volume of saliva.

In addition, in the study the authors observed an increased output of salivary inorganic and organic protective components in GERD patients during the chemical stimulation of the esophageal mucosa with HCl/P/BA. A significantly augmented nonbicarbonate output (165%) was accompanied by increase in salivary protein output (55%) during perfusion with HCl/P/BA. However, the authors did not observe the increase of bicarbonate in GERD group after HCl/P/BA as it was the case in controls.

The authors also observed some differences in the rate of secretion of salivary inorganic and organic protective components between population of controls and patients with GERD.

The authors demonstrated in patients with GERD significantly lower pH during the perfusion with initial saline, the initial and final HCl/P/BA and during the final saline than in controls. The bicarbonate output in the GERD group was significantly lower (57%) than in controls during perfusion with initial HCl/P/BA and this was accompanied by significantly lower (49%) output of nonbicarbonate during the final perfusion with HCl/P/BA.

In the GERD group, the protein output was also significantly lower than in controls during stimulation with HCl/P/BA. The authors found, however, in patients with GERD significantly higher salivary output of EGF during initial

HCl/P/BA (140%) and final HCl/P/BA (280%) and statistically significantly higher output of PGE<sub>2</sub> (88%) during the final perfusion with HCl/P/BA than in control group.

In previous studies the stimulatory impact of acid and pepsin on production of salivary protective components, mediated by the esophagosaliary reflex, in healthy volunteers has already been demonstrated. Intraesophageal perfusion with HCl and pepsin (HCl/P) increased significantly the salivary volume and viscosity [21], EGF and PGE<sub>2</sub> outputs in healthy volunteers [22, 23].

In patients with endoscopically negative GERD (E (-) GERD) the authors observed significantly increased salivary nonbicarbonate output during intraesophageal stimulation with HCl/P. In patients with E (-) GERD during stimulation of the lower esophagus with HCl/P the authors have found a trend – an increase in salivary volume, bicarbonate, protein, and EGF outputs (Poplawski C, Zbroch T, Goldin G et al.: Augmented salivary esophagoprotection in patients with gastroesophageal reflux disease: Its potential pathogenetic implications. Submitted).

In addition, the authors demonstrated the significantly higher salivary volume in E (-) GERD patients during intraesophageal chemical stimulation with HCl/P than in controls. The glycoconjugate output was also significantly higher in E (-) GERD than in controls during chemical stimulation with HCl/P and this was accompanied by significantly higher protein and TGF- $\alpha$  outputs. The authors also observed in E (-) GERD significantly higher salivary pH, and bicarbonate, glycoconjugate, protein, EGF output than in reflux esophagitis (RE) group during intraesophageal perfusion with HCl/P (Poplawski C, Zbroch T, Goldin G et al.: Augmented salivary esophagoprotection in patients with gastroesophageal reflux disease: Its potential pathogenetic implications. Submitted).

The increase in salivary bicarbonate and nonbicarbonate outputs in controls during chemical stimulation of esophageal mucosa with HCl/P/BA, observed in the present study, may play the major role in normalization of the esophageal pH during refluxate episodes. The significantly augmented salivary bicarbonate production the component of salivary buffering capacity may especially be helpful in protection of the esophageal mucosa during reflux episodes with duodenogastric contents. Based on the present results and previous study focused on salivary secretion response at the intraesophageal chemical stimulation with HCl/P in healthy subjects, one may assume that stimulation with HCl/P/BA of the afferent fibers within the esophagosaliary reflex is less efficient in

enhancement of salivary protection than stimulation with HCl/P only.

In the current study, GERD patients exposed to HCl/P/BA refluxate a significantly increased production of salivary nonbicarbonate but not bicarbonate was demonstrated. Therefore, the authors postulate that the response of salivary glands to stimulation of afferent fibers in esophagosalivary reflex in response to esophageal exposure to HCl/P/BA is less potent in GERD patients than in control group. This is important considering the fact that bile components play a significant role in the development of the esophageal mucosal injury.

Recently, Gotley et al. suggest that conjugated bile acids are detected in the oesophagus of most patients with oesophagitis, therefore bile acid salts seem to play an important role in the pathogenesis of oesophagitis especially in some patients with nocturnal gastroesophageal reflux [24]. Stein in his study has demonstrated that contamination of the refluxed gastric juice with bile acids predis-

poses the patient to development of strictures and Barrett's esophagus [25]. Patients with Barrett's oesophagus have significantly more duodenogastroesophageal reflux incidents than oesophagitis patient [26].

In the study, the authors demonstrated that control individuals and patients with GERD secrete significantly higher amount of salivary protective components during chemical stimulation of esophagus with bile acids than during esophageal mucosal exposure to saline solution. GERD group, however, has lower salivary protective capacity than healthy volunteers during stimulation with these esophageal aggressive factors. Inadequate protective capacity may lead to damage of the lower esophageal mucosa by bile acids accompanied by hydrochloric acid and pepsin. Compensatory overproduction of EGF and PGs may accelerate healing but potentially could also increase the risk of developing the Barrett's esophagus and further complications.

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