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Changes of Gastric Lipase Activity after Ethanol and Indomethacin Administration: Influence of Pretreatment with Allopurinol, Pentoxifylline and L-DOPA

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Summary

Gastric lipase (GL) plays an important role in emulsification and digestion of food fat. Lipids are components of the hydrophobic mucus and mucosa barrier. Damage of the gastric mucosa may therefore be related to changes in the lipid content and GL activity. In the present paper, we studied the effect of administration of a single dose of 96 % ethanol (E) and indomethacin 20 $mg.kg^{-1}$ (IND) on the activity of GL and on the concentrations of nonesterified fatty acids (NEFA) and triacylglycerols (TG) in the gastric mucosa of rats. Furthermore, we studied how these changes are affected by allopurinol (ALO), pentoxifylline (PX) and L-DOPA pretreatment 30 min before administration of E or IND. The effect of sialoadenectomy (SA) on these parameters was also evaluated. We found: 1) significant ($p<0.01$) inhibition of GL activity after administration of E and IND and also ALO, as well as after pretreatment with ALO before E and PX before IND. L-DOPA administered alone stimulated GL activity, but its administration before IND significantly ($p<0.01$) inhibited this enzymatic activity. GL activity was decreased to the threshold values in SA rats and after administration of E to SA animals. 2) NEFA concentrations were decreased after E and increased significantly ($p<0.01$) after IND administration. A marked significant ($p<0.01$) decrease in NEFA was found after PX and L-DOPA administration. The administration of ALO also lowered the concentration of NEFA. Pretreatment by drugs before E and IND resulted in a significant increase of NEFA in comparison with the drugs given alone ($p<0.05$ for ALO + E; $p<0.01$ for PX + IND). 3) TG were also decreased in all experimental groups in comparison with the control group, i.e. after E and IND, after ALO and SA and also after pretreatment by ALO before E. The concentration of TG decreased after PX, significantly ($p<0.05$) after L-DOPA and after pretreatment by PX before IND. Pretreatment by ALO before E and L-DOPA before IND resulted in the increase of TG in comparison with drugs alone. Thus, these results suggest certain protective effect of pretreatment with ALO, PX and L-DOPA against the E- and IND-induced decrease in NEFA and TG during injury of the gastric mucosa. On the other hand, inhibition of GL activity was also apparent after administration of these drugs before E and IND, which suggest presence of a persisting impairment of lipid digestion in the stomach.

Key words

Gastric lipase • Lipids • Ethanol • Indomethacin • Allopurinol • Pentoxifylline • L-DOPA

Introduction

Hydrolysis of food fat in the stomach by gastric lipase (GL) is the first step of their digestion in the gastrointestinal tract (GIT). GL is released from the gastric mucosa after different stimuli into the lumen of stomach, where it cleaves the sn-1 and sn-3 ester bonds of short-, mild-, and long-chain triacylglycerols (TG) to give rise to nonesterified fatty acids (NEFA) and diacylglycerols, principal products of gastric lipolysis, which play an important role in the functioning of GIT. Long-chain NEFA are activators of pancreatic lipase (Hamosh 1987, Gargouri *et al.* 1989, 1997, Borel *et al.* 1994, Embleton and Pouton 1997), short-chain NEFA stimulate the growth, differentiation and apoptosis of intestinal epithelial cells and other cells such as lymphocytes (Bernhard *et al.* 1999). The products of stomach lipolysis participate in the emulsification of food fat before it is metabolized in the intestine (Armand *et al.* 1994). Besides, NEFA are components of mucous glycoproteins (Slomiany *et al.* 1984) as well as lipids of the hydrophobic barrier of mucus and gastric mucosa (Slomiany *et al.* 1987, Natomi *et al.* 1993, Lichtenberger 1995, Bernhardt *et al.* 1996). The physiological role of GL is manifested mainly in pancreatic insufficiency (i.e. chronic pancreatitis), cystic fibrosis, and also in newborns (Hamosh 1987), where GL hydrolyses a large amount of food TG. In different animal species, GL has a different localization in the upper GIT. In man, rabbits, and dogs, it is localized in the secretory glands of chief cells of stomach fundus, in the rat and mice much activity is localized in the area around circumvallate papillae as so-called lingual lipase (Hamosh and Scow 1973, Moreau *et al.* 1988, Gargouri *et al.* 1989). In the rat stomach only a very weak GL activity was identified, which is about 40-times lower than in humans (Aoubala *et al.* 1997). It was found that human and rabbit GL as well as rat lingual lipase are similar in some characteristics: molecular mass approximately 43-45 kDa, high stability under acid conditions, optimum pH about 5.4, and 75-80 % homology of amino acids sequence (Aoubala *et al.* 1994, 1997, Gargouri *et al.* 1989, 1997, Verger *et al.* 1996). All enzymes contain three cystein residues and one disulfide bridge. The main stimulus for GL secretion and activity is fat in the food. Other stimuli include pentagastrin/gastrin, cholinergic and parasympathetic activation (Perret *et al.* 1993, Armand *et al.* 1994, Borovička *et al.* 1997, Wojdemann *et al.* 1997) and some food components such as NaCl, phosphatidylcholine, ovalbumin (Gargouri *et al.* 1989, Borel *et al.* 1994). High HCl secretion and elevated NEFA concentration, both endogenous and exogenous, inhibit secretion and activity of GL (Bernback *et al.* 1989, Borel *et al.* 1994, Gargouri *et al.* 1989). Epidermal growth factor (EGF) and transforming growth factor (TGF) from salivary glands and gastric mucosa are specific inhibitors of GL activity (Tremblay *et al.* 1999). Inhibition of GL activity is associated with decreased fat absorption and disturbances of the stomach and GIT functions (Embleton and Pouton 1997, Gargouri *et al.* 1997).

It was demonstrated that chemical substances such as ethanol, thiols and some drugs, due to changes of chemical and physical characteristics of the enzyme and/or its substrate and other undetermined mechanisms, may inhibit GL activity (Gargouri *et al.* 1989, 1997, Zhi *et al.* 1999).

Changes in HCl secretion, lesion formation, and some disturbances of the gastric mucosa after ethanol (E) and indomethacin (IND) administration, and also after sialoadenectomy (SA) have been described earlier (Mirossay *et al.* 1988, 1999, Kohút *et al.* 1992a,b, 1995, 1997, 1999, Mirossay and Kohút 1991, Kohút and Mojžiš 1993, Mojžiš and Kohút 1993, Šallingová and Kohút 1994)

The aim of the present paper was to:

1) evaluate changes in GL activity and NEFA and TG concentrations in the gastric mucosa of rats after intragastric administration of a single dose of 96 % E and/or after intraperitoneal administration of a single dose of IND (20 mg. kg^{-1}),

2) assess changes in the above-mentioned parameters on the day 10 after sialoadenectomy (SA),

3) investigate the effect of pretreatment with selected drugs on the E- and IND-induced changes: allopurinol (xantine oxidase inhibitor that possesses an antioxidant effect), pentoxifylline, and L-DOPA.

Methods

Male Wistar rats with average body weight 252.7±10.5 g received ethanol (E) (1 ml, intragastrically) or indomethacin (IND) (Sigma, 20 $mg.kg^{-1}$, intraperitoneally) after 24 h fasting. Allopurinol (ALO) (Sigma, 50 $mg.kg^{-1}$), pentoxifylline (PX) (Sigma, 45 $mg.kg^{-1}$) and L-DOPA (Spofa, 25 $mg.kg^{-1}$) were administered 30 min before E or IND, or separately. In

one group of rats, the bilateral excision of submandibulary glands (sialoadenectomy, SA) was performed. The stomach was removed under pentobarbital anesthesia (50 mg/kg, i.p.) always 1 h after E and 4 h after IND administration. SA animals were analyzed on the day 10 after the surgery and 24 h fasting and 50 % of the SA rats also 1 h after E administration. Individual groups consisted of 5-24 animals. The activity of gastric lipase (GL) was determined in gastric mucosa homogenates (about 100 mg/1 ml of 0.154 M NaCl) after centrifugation at 2500 rpm for 10 min by modified methods (Sedláková *et al.* 1979, Gargouri *et al.* 1989, Perret *et al.* 1993) as described earlier (Sedláková *et al.* 1997). 100 µl of homogenate containing the enzyme was added into 1 ml of the substrate mixture: Intralipid, 20 %, 2 parts, Tris-HCl buffer, 2 parts, albumin (human, lyofilized), 15 %, normal rat serum, 1 part, adjusted to pH 6.0. We suppose that lipolytic activity in gastric mucosa of rats is due to GL because no lipoprotein lipase activity was detected (Hamosh and Scow 1973). GL activity was quantified in µmol of nonesterified fatty acids (NEFA) released from TG substrate during 60 min incubation at 37 °C. NEFA were determined by a spectrophotometric micromethod (Novák 1965).

The concentration of nonesterified fatty acids (NEFA) by the assay method in tissues (Novák 1962) and triacylglycerols (TG) by kit TG 450T (Bio Lachema, ČR) were also determined in the samples of gastric mucosa.

The results were statistically evaluated by multiple-way ANOVA and Student's t-test for non-paired values.

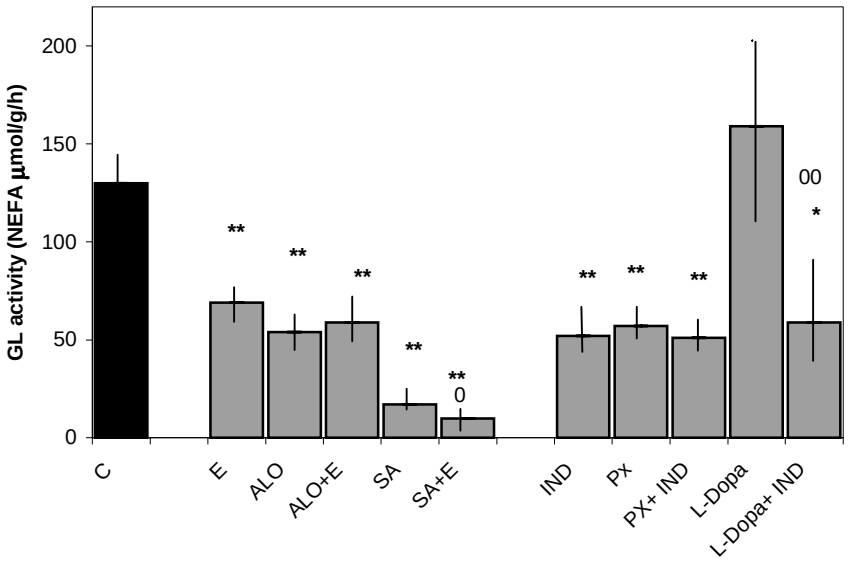

Fig. 1. *Gastric lipase activity in the gastric mucosa of rats after administration of ethanol (E), indomethacin (IND) and sialoadenectomy (SA); after administration of allopurinol (ALO), pentoxifylline (PX) and L-DOPA as well as after administration in combination: SA + E; ALO + E, PX + IND and L-DOPA + IND. Results are are expressed as mean ± S.E.M. Significant differences: ** for p<0.01 in comparison with control group (C), $^{+}$ for p<0.05 in comparison with ethanol (E).*

Results

The changes of GL activity in the gastric mucosa of rats under the conditions of our experiment, evaluated by ANOVA, were highly significant ($F=7.9278$, $p<0.01$). As seen in Figure 1, GL activity was decreased significantly ($p<0.01$) after administration of E and IND as well as after ALO and PX in comparison with the control group (C). On the contrary, administration of L-DOPA led to an increase in GL activity, which, however, was non-significant. In the gastric mucosa of SA rats, GL activity was drastically decreased ($p<0.01$, 13.9 % of C). The administration of E to SA rats caused even more profound decrease in GL activity (to about 8.0 % of C). Pretreatment with the selected drugs before E or IND did not correct the decrease in GL activity caused by E or IND. The activity of GL was decreased significantly ($p<0.01$), in comparison with C, after administration of ALO before E and also after administration of PX and L-DOPA before IND. PX administered before IND decreased GL activity non-significantly in comparison with PX alone, whereas the decrease after L-DOPA administered before IND was significant ($p<0.01$). Thus, our results show that E and IND, ALO and PX administered alone or in combination with E and IND, and also L-DOPA in combination with IND, inhibited GL activity.

Changes in NEFA concentration in the gastric mucosa of rats, statistically evaluated by ANOVA, were also significant ($F=6.7704$, $p<0.01$). Their levels (Fig. 2) were decreased non-significantly after administration of

E and ALO, and also after SA. E administered to SA rats caused an increase in NEFA to the control levels. Pretreatment by ALO before E resulted in a significant ($p<0.05$) increase of NEFA in comparison with the administration of ALO alone. The administration of IND caused a significant ($p<0.01$) increase in NEFA concentrations and attained almost two-fold level than in the C group. The NEFA concentration was decreased significantly ($p<0.01$) after administration of PX and L-DOPA alone and reached approximately only half values of C groups. Pretreatment with PX and L-DOPA before IND increased the NEFA values in comparison with administration of the drugs alone, after PX before IND significantly ($p<0.01$). However, their levels after pretreatment remained lower than the C values and were significantly ($p<0.01$) decreased in comparison with IND alone.

Changes in TG concentration in the gastric mucosa evaluated by ANOVA were significant at the 95 % limit ($F=1.8708$, $p<0.05$). TG concentrations were

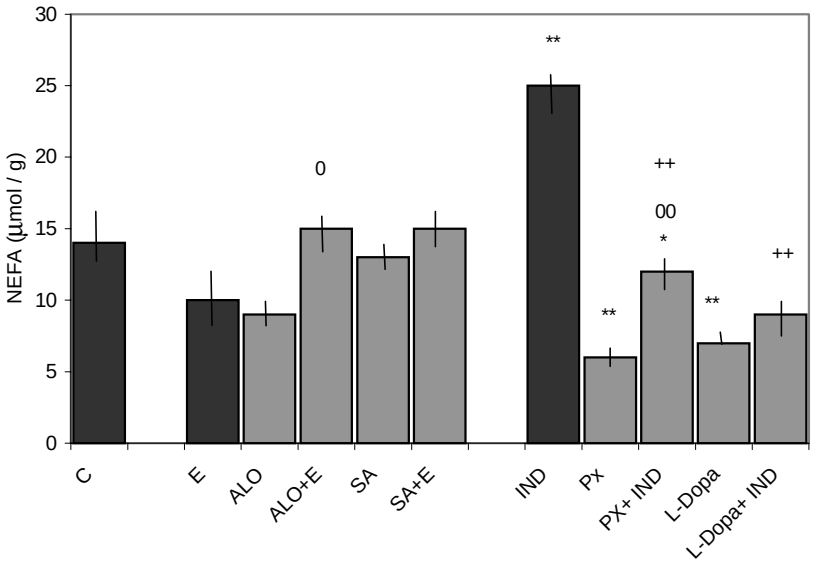

Fig. 2. *Concentration of nonesterified fatty acids (NEFA) in the gastric mucosa of rats after administration of ethanol (E), indomethacin (IND) and sialoadenectomy (SA); after administration of allopurinol (ALO), pentoxifylline (PX) and L-DOPA as well as after administration in combination: SA + E, ALO + E, PX + IND and L-DOPA + IND. Results are expressed as means ± S.E.M. Significant differences: ** for $p<0.01$ in comparison with control group (C), $^{++}$ for $p<0.01$ in comparison with IND. ^{0} for $p<0.05$ and ^{00} for $p<0.01$ in comparison with drug alone (ALO and PX, respectively).*

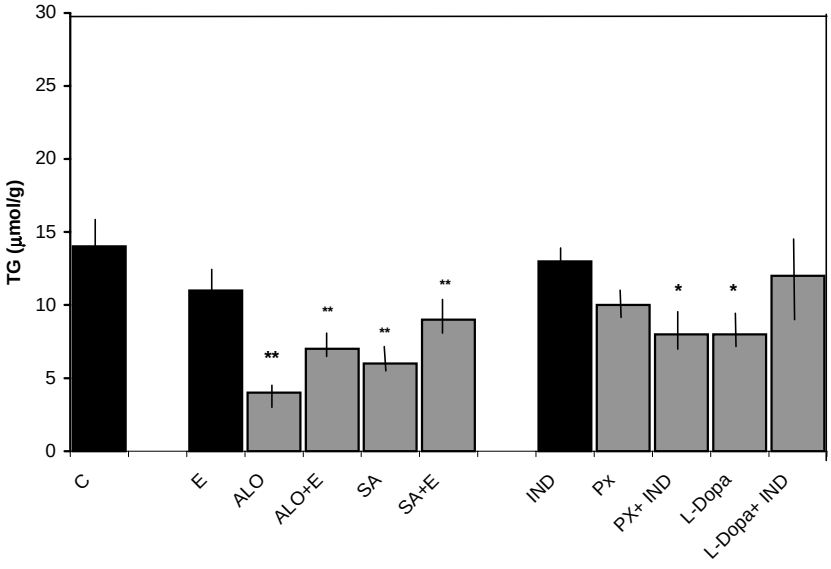

Fig. 3. *Concentration of triacylglycerols in the gastric mucosa of rats after administration of ethanol (E), indomethacin (IND) and sialoadenectomy (SA); after administration of allopurinol (ALO), pentoxifylline (PX) and L-DOPA as well as after administration in combination: SA + E, ALO + E, PX + IND and L-DOPA + IND Results are expressed as means ± S.E.M. Significant differences: * for $p<0.05$ and ** for $p<0.01$ in comparison with control group (C).*

decreased after administration of E and significantly ($p<0.01$) after administration of ALO and in SA rats (Fig. 3). TG also remained decreased after administration of E to SA animals and after pretreatment with ALO before E ($p<0.01$), although their levels increased in comparison with the SA alone and ALO alone groups. The TG concentration also decreased after administration of IND. Administration of PX and L-DOPA resulted in a more pronounced decrease in TG, in the case of L-DOPA for $p<0.05$. Pretreatment with PX before IND caused a further decrease in TG, which was significant in comparison with C ($p<0.05$). Pretreatment with L-DOPA before IND increased their concentration.

These results show that pretreatment with ALO, PX and L-DOPA before E or IND has a certain protective effect against E- a IND-induced decrease in NEFA and TG in the gastric mucosa.

Discussion

The first step in gastrointestinal lipolysis, i.e. hydrolysis of food fat by gastric lipase (GL) is considered to be a principal moment in the digestion of lipids, and inhibition of GL is associated with decreased emulsification and absorption of lipids, and with disturbances of the functions of stomach and GIT (Armand *et al.* 1994, Borel *et al.* 1994, Verger *et al.* 1996, Embleton and Pouton 1997, Gargouri *et al.* 1997). An increase in formation of lesions, vascular permeability and lipoperoxide levels were reported in the gastric mucosa after E and IND (Kohút *et al.* 1992b, 1994, Kohút and Mirossay 1993, Kohút and Mojžiš 1993), as well as thinning of the mucous layer and decrease in hexosamine and phospholipids (PL) content (Kohút *et al.* 1997, 1998). We may suggest a relation between changes in secretion/activity of GL and NEFA/TG concentrations in gastric mucosa and its damage. We have found a marked inhibition of GL activity after E and IND administration and a similar inhibitory effect was observed after ALO and PX treatment as well as after pretreatment by ALO before E and PX and L-DOPA before IND. All evaluated drugs and substances may have evoked the changes in GL on the biochemical level. The GL protein contains three cysteine residues and one disulfide bridge with one free thiol group which easily undergoes chemical modifications accompanied by a decrease or loss of enzymatic activity. Gargouri *et al.* (1989, 1997) showed that thiols, C12.0-NBS2 (dodecyldithio-5(2-nitrobenzoic acid), 4-PDS (4.4-dithiopyridine), natural disulfide ajoien and others, during *in vitro* incubation with rabbit and human GL, caused a fast and strong inhibition of GL activity with respect to the concentration of the inhibitor, time of incubation and pH. This was due to their competitive binding to the enzyme. Acetylation is also accompanied by inhibition of GL. Furthermore, tetrahydrolipstatine (THL), a lipstatine derivate, and probably general inhibitor of gastrointestinal lipases, orlistat, also inhibit GL activity by a similar mechanism (Gargouri *et al.* 1997, Zhi *et al.* 1999). Mutations, e.g. substitution of cysteine in the catalytic domain by other amino acid, lead to the loss of enzymatic activity (Canaan *et al.* 1999).

A decrease in GL activity was observed during incubation of native human GL and duodenal juice with E (Sternby *et al.* 1996). E is metabolized in the stomach in a similar way as in the liver (Lieber 1994), with formation of acetaldehyde, acetate, reactive oxygen radicals and lipoperoxides. Acetaldehyde generates adducts with proteins, hence it may be regarded as a modifier of GL. E is an organic solvent and as such it may be the cause of physical and chemical changes in a substrate, herein the content of lipids in the mucus and gastric mucosa. A decrease of PL in the stomach and gastric mucosa has been reported after E (Kohút *et al.* 1997, 1998). In the present work, we have found a decrease in NEFA and TG.

It is generally accepted that GL, in rats referred to as lingual lipase, has its origin in the salivary glands, however, only very low enzymatic activity was found in the stomach (Hamosh and Scow 1973, Hamosh 1987, Moreau *et al.* 1988). Therefore, a part of our experiments was peformed on rats 10 day after the removal of salivary glands. Salivary glands are a source of growth factors, EGF and TGF, which have trophic effects on the gastric mucosa and mucosa of GIT (Konturek *et al.* 1990). The removal of salivary glands is accompanied by morphological changes in GIT, inhibition of the mucosal epithelial cell proliferation and reduction of synthesis of DNA, RNA and proteins (Hiramatsu *et al.* 1991). SA potentiated E- and IND-induced injury of gastric mucosa which was ameliorated by administration of EGF (Kohút *et al.* 1992a). We observed a very low GL activity in SA rats which was even lower after administration of E. NEFA in the gastric mucosa after SA as well as after administration of E to SA rats did not change compared with the control group and reflected the low activity of GL. However, TG were markedly decreased after SA as a result of a general inhibition of synthetic processes. Administration of E to the SA rats caused a mild increase of TG.

Reactive oxygen radicals are regarded as the major cause of gastric mucosa injury. The major source of reactive oxygen species is the xantine-xantine oxidase system (X/XO) in the gastric mucosa as well as NADPH oxidase of activated polymorphonuclear leukocytes (PMNL) (Wada *et al.* 1996). The increase of activated PMNL in the circulation and enhanced lipoperoxide levels in the gastric mucosa of rats were found after administration of E and IND (Kohút *et al.* 1992b, Kohút and Mojžiš 1993, Šallingová and Kohút 1994). Inhibition of XO by ALO pretreatment before E and IND resulted in a decrease of lipoperoxide levels, lesion formation, and vascular permeability. In our experiments, ALO administered alone reduced GL activity to approximately half of the level of controls and this was accompanied by a decrease of NEFA. At the same time, we observed a drastic decrease of TG (30 % of controls), probably due to stimulation of other lipases and esterases caused by alkalization in the stomach after ALO. Pretreatment with ALO in combination with E leads to a further drop in GL activity, but increases the content of NEFA and TG. ALO is a purine derivate, which alone or *via* its metabolites may modify the GL protein, and this change is more marked after combination of ALO with E.

After administration of IND, GL activity was inhibited, NEFA were markedly increased, and there was a small decrease of TG. Inhibition of GL activity after IND is probably due to the fact that IND causes gastric injury mainly by inhibiting cyclooxygenase responsible for endogenous formation of gastroprotective prostaglandins (PG) with accumulation of activated neutrophils (Wallace *et al.* 1990, Anthony *et al.* 1996). The administration of IND to rats increases HCl secretion (Mirossay *et al.* 1988, Mirossay and Kohút 1991, Kohút *et al.* 1992a,b), which is the main inhibitor of GL activity. The increase of NEFA in the gastric mucosa after IND, which we report in this work, as well as their increase in the circulation (Sedláková *et al.* 1998) by a feedback mechanism, have an inhibitory effect on the GL activity. Besides, elevated NEFA in the gastric mucosa indicate disturbances in their metabolism, particularly inhibition of oxidation. Unsaturated fatty acids are a source of lipoperoxides, which are factors causing mucosal damage.

Apart from reactive oxygen radicals, activated leukocytes are also a source of various cytokines (TNF, IL, PAF) and adhesion molecules, which cause vascular wall/endothelium damage resulting in microcirculation disturbances in the stomach and small intestine (Santucci *et al.* 1994, Wada *et al.* 1996, Reuter and Wallace 1999). PX and L-DOPA, drugs with vasodilatory effects, may play a role in gastroprotection and by increasing cAMP and activating the signaling pathway of cAMP may affect the lipid content in the gastric mucosa and other functions of the stomach and GIT. It was found that PX specifically inhibits particular functions of activated leukocytes: adhesion, chemotaxis, formation of oxygen radicals and others (Wada *et al.* 1996). Pretreatment with PX before E and IND leads to a decrease in the number of activated PMNL in the circulation, and a reduction of lipoperoxidation and vascular permeability in the gastric mucosa of rats (Kohút *et al.* 1992b, Šallingová and Kohút 1994). Taking into account the parameters studied in our work, PX inhibited GL activity to the similar extent as IND and ALO, and this effect did not change substantially when PX was administered before IND. However, in comparison with IND, a marked decrease in NEFA and TG was found after PX. These changes indicate that the metabolic turnover of lipids, hydrolysis of TG and oxidation of NEFA are enhanced after PX administration. Pretreatment by PX in combination with IND increased the content of NEFA as a reflection of two contradictory effects: inhibition of NEFA oxidation after treatment with IND and stimulation of their oxidation by PX. TG were further lowered. The inhibition of GL activity may be a result of different effects of cAMP on cells in the gastric mucosa; stimulation of parietal cells to secrete HCl (Hersey and Sachs 1995) and concomitant inhibition of secretion in chief cells which probably leads to inhibition of GL activity.

L-DOPA, as a dopaminergic receptor DA_1 agonist, stimulates the adenylate cyclase and increases cAMP levels in the gastric mucosa. It was shown that under conditions of gastric injury induced by stress, IND or E in man or rats, dopamine is depleted in the stomach where it normally accounts for about 20 % of catecholamines and that these effects are accompanied by lesions (Glavin 1989). IND stimulates the basal and cholinergic-induced secretion of catecholamines from the adrenals (Warashina 1997). L-DOPA and activation of the dopaminergic system stimulate the bicarbonate secretion in the stomach and duodenum (Flemström 1994), which may be a good millieu for secretion and activity of GL. Pretreatment of rats with L-DOPA, dopamine and PGI_2 in combination with IND and E attenuated the damage to the gastric mucosa, i.e. decreased the lesion formation, vascular permeability and HCl secretion (Kohút and Mirossay 1993, Kohút *et al.*

1995), mucus formation and secretion as well as hexosamine and PL content (Kohút *et al.* 1997). We found out that of the drugs examined, only L-DOPA stimulated GL activity. However, after pretreatment with L-DOPA before IND, the enzymatic activity was repeatedly decreased to the levels achieved after IND alone. This may be due to the inhibition of dopaminergic activity of the stomach. Besides, the doses of L-DOPA and IND and timing of experimentation after dosage may influence the activity of GL. Changes in NEFA and TG after treatment with L-DOPA as well as after administration of L-DOPA before IND followed a similar course as after PX, i.e. stimulation of their metabolism with L-DOPA alone and tendency to normalization after combination of L-DOPA with IND.

Our results show that E and IND inhibited GL activity. ALO and PX inhibited, while L-DOPA, on the contrary, stimulated the activity of GL. Pretreatment by ALO before E and PX and L-DOPA before IND has no influence on E- and IND-induced inhibition of GL activity, but even enhances this inhibition in some circumstances. Furthermore, we found important changes in the levels of NEFA and TG in the gastric mucosa after E and IND and also after ALO, PX and L-Dopa. These changes are characterized by reduction of NEFA and TG with an exception of the increase of NEFA after IND. In summary, the pretreatment by ALO, PX and L-DOPA before E and IND resulted in amelioration, i.e. increase in NEFA and TG with small exceptions. Thus, our findings serve as further evidence of the gastroprotective effects of ALO, PX and L-Dopa, administered in combination with E and IND, on the hydrophobic lipid barrier of the gastric mucosa and mucus.

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