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EFFECTS OF TAUROCHOLIC ACID/HCl ALONE OR AFTER PRETREATMENT WITH GERANYLGERANYLACETONE ON PHOSPHOLIPID METABOLISM IN RAT GASTRIC MUCOSA

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Abstract—Changes in phospholipid metabolism in gastric mucosa caused by instillation of taurocholic acid (TCA)/HCl (80 mM/300 mM) into the stomach of rats and the effects of pretreatment with an antiulcer agent, geranylgeranylacetone (GGA), were studied after intravenous injection of radioisotope-labeled precursors. The instillation of TCA/HCl rapidly reduced the incorporation of labeled fatty acids and glycerol into phosphatidylcholine and phosphatidylethanolamine, indicating the inhibition of *de novo* synthesis of phospholipids. These changes were restored by 120–150 min after the TCA/HCl treatment. Pretreatment with GGA enhanced the incorporation of precursors into phosphatidylcholine immediately after the instillation of TCA/HCl. Experiments in which the mucosal lipids were labeled with fatty acids prior to the instillation of TCA/HCl showed that the degradation of cellular lipids and release of the products into the gastric lumen were induced by TCA/HCl and that these changes were not prevented by GGA. Since GGA almost completely inhibited the gastric lesions induced by TCA/HCl, the enhancement of synthesis of mucosal phosphatidylcholine induced by GGA may be involved in the prevention of gastric damage. The incorporation of labeled fatty acids into free fatty acid fraction and diacylglycerol was increased quickly by the TCA/HCl treatment, suggesting early damage to the blood vessels of the gastric mucosa; these changes were inhibited significantly by GGA.

Gastric mucosal cells excrete HCl and pepsin to digest foods, and these cells are covered with a layer of mucus, which they excrete, and which protects them against injury by noxious agents such as HCl, bile acids, and other chemicals. Although the mechanisms of excretion of HCl and mucus have been studied in many laboratories, little is known about lipid metabolism in gastric mucosa and its relation to the cellular functions.

Horowitz and his colleagues [1–3] detected in rat stomach several kinds of enzyme activities involved in the metabolism of phospholipids. Hills and his colleagues have recently found phospholipids in the stomach surface [4], and they have proposed that this phospholipid layer plays a defensive role, protecting the mucosal cells against injury [5–7]. Slomiany and his co-workers [8, 9] demonstrated that the lipids associated with mucus in the stomach play a role in maintaining the viscosity of the mucus and have a barrier function against hydrogen ions present in the gastric lumen. These studies were focused on the lipids presented in the surface layer of the stomach and on the role of the lipids as a protective barrier. We have found recently that absolute ethanol instilled into the stomach cavity inhibits the *de novo* synthesis of phospholipids in the mucosal cells [10].

The purpose of the present work was to investigate the changes in the metabolism of phospholipids and other lipids in rat gastric mucosa caused by a cell-damaging agent, taurocholic acid (TCA‡)/HCl. There are several lines of evidence that reflux of bile acids from the duodenum into the stomach is involved in the development of gastric ulcer, since bile reflux occurs with high incidence in certain patients [11–13]. Bile acids have been demonstrated in experimental animals and humans to break the mucosal barrier of the stomach, resulting in increases in the back-diffusion of H^+ and net flux of Na^+ into the gastric lumen and a rapid decrease in the transmucosal potential difference [14–16]. Although morphological and histological characterizations of bile acid-induced damage to the gastric mucosa have been carried out by many authors, the possibility that bile acids might alter the intracellular metabolism of lipids has not been examined previously.

In addition, the effect of pretreatment with an antiulcer agent, geranylgeranylacetone (GGA), on the changes in lipid metabolism induced by the instillation of TCA/HCl was also examined. GGA has been reported to have inhibitory effects on peptic ulcers induced not only by agents such as indomethacin, aspirin, prednisolone, reserpine [17] and ethanol [18], but also by TCA [19].

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‡ Abbreviations: TCA, taurocholic acid; GGA, geranylgeranylacetone; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TG, triacylglycerol; DG, diacylglycerol; and FFA, free fatty acid.

MATERIALS AND METHODS

Chemicals. [1-^{14}C]Palmitic acid (58 mCi/mmol) and [9,10-^{3}H]oleic acid (4.3 Ci/mmol) were purchased from Amersham Inc. (U.K.), and [2-^{3}H]glycerol (2 Ci/mmol) was from ICN Radiochemicals (U.S.A.). Bovine serum albumin and pancreatic phospholipase A_2 were obtained from the Sigma Chemical Co., and taurocholic acid sodium salt was from the Calibiochem-Behring Co. Other chemicals used were of reagent grade. GGA (6,10,14,18-tetramethyl-5,9,13,17-nonadecatetraen-2-one) was synthesized in our laboratory.

Administration of labeled precursors. Labeled precursor was dissolved in 5% (w/v) bovine serum albumin solution, and 0.5 ml of the solution (40 μCi for fatty acids, 100 μCi for glycerol) was injected into the femoral vein of rats. Male Sprague–Dawley rats aged 7 to 8 weeks were fasted for 24 hr before use. GGA (200 mg/kg, an emulsion with 1% Tween 80) or vehicle (1% Tween 80) was administered orally, and 120 min later TCA/HCl (80 mM/300 mM, 1.2 ml) was given intragastrically to the rats of the vehicle-pretreated or GGA-pretreated group. An additional group of rats (saline control) intragastrically received saline (1.2 ml) instead of TCA/HCl. The incorporation of labeled precursors was examined at several time points before or after TCA/HCl treatment. The rats of each group were injected with the label 20 sec before, 15 min after, or 120 min after the instillation of TCA/HCl, and then were killed 15 min or 30 min after the injection of the label. The rats treated with GGA alone, but without TCA/HCl instillation, were instilled with saline instead of TCA/HCl 120 min after administration of GGA, injected with the label simultaneously or 120 min after the instillation of saline, and killed 30 min after the injection of the label. In experiments to examine the degradation of mucosal lipids, the rats were injected with [^{3}H]oleic acid and [^{14}C]palmitic acid, instilled with TCA/HCl 30 min later, and then killed 60 min after the injection of the precursors.

Extraction of lipids. The stomach was excised, opened with scissors along the greater curvature, and washed in ice-cold saline. The gastric contents were collected in the experiments to examine the degradation of mucosal lipids into the gastric lumen. Gastric mucosa was then wiped with a filter paper and scraped with a metal spatula on an ice-cold glass plate. Mucosal cells were homogenized in 5 ml of ice-cold 0.5 mM EDTA with a Teflon homogenizer, and the volume of homogenate was adjusted to 7 ml. Total lipids were extracted from 5 ml of the homogenate by the method of Bligh and Dyer [20]. The chloroform layer obtained was evaporated to dryness, and the residue was dissolved in 5 ml of chloroform–methanol (2:1) and stored at −20° until use.

Determination of incorporated radioactivity. One milliliter of the extract was concentrated under nitrogen gas and applied to a precoated TLC plate (silica gel F_{254}, ER Merck, GR). Neutral lipids were separated by the method of Ando *et al.* [21], and phospholipids by the method of Billah *et al.* [22]. Lipids were located by exposure of the plate to iodine vapor, and the silica gel corresponding to each lipid fraction was scraped into counting vials. A scintillant (5 ml, ACS-II, Amersham Inc.) was added to each vial, and radioactivity was measured with an Aloka LSC-900 liquid scintillation spectrometer.

Analysis of the positional distribution of incorporated fatty acids. Lipid extract (1 ml) was separated into phospholipid fractions by TLC as described above, and the silica gel regions corresponding to phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were scraped into tubes. Methanol (2 ml) was added to each tube, and the mixture was sonicated for 10 min in a bath-type ultrasonicator (Branson Co.); then 4 ml of chloroform was added to the tube. Phospholipids were further extracted by shaking for 30 min, and the organic phase was transferred to another tube. The extract was dried under nitrogen gas, 0.5 ml of distilled water was added, and the mixture was sonicated for 5 min. Phospholipids dispersed in water were digested by incubation with phospholipase A_2 for 30 min at 37° by the method of Wassef *et al.* [1]. The reaction mixture was then extracted, and fatty acid fraction and lysophosphatide were separated by TLC as described above. The radioactivity of each fraction was measured. These conditions allowed complete hydrolysis of fatty acids incorporated into the *sn-2* position of phospholipids.

Examination of gastric ulcer. Ulcers induced by the instillation of TCA/HCl were examined by the method of Murakami *et al.* [17], and data are shown by the sum of lesions in length.

Statistical analysis. Results are expressed as the mean ± SE. The data were analyzed by using the unpaired Student's *t*-test.

RESULTS

Incorporation of fatty acids into phospholipids after TCA/HCl instillation. The instillation of TCA/HCl into the gastric cavity induced severe gastric lesions; this damage was inhibited almost completely in the rats pretreated with GGA at a dose of 200 mg/kg (Table 1). To examine the effects of TCA/HCl instilled into the stomach on the phospholipid metabolism in gastric mucosa, the incorporation of [^{14}C]palmitic acid into phospholipid fractions was measured. The effects of pretreatment with GGA on the TCA/HCl-induced changes in the incorporation of palmitic acid were also examined (Fig. 1).

First, the effects of GGA alone on the uptake of palmitic acid into phospholipids were examined. The incorporation into PC was decreased slightly in the GGA-treated rats as compared with the rats treated with vehicle, while the incorporations into PE and the phosphatidylinositol + phosphatidylserine (PI + PS) fraction were inhibited significantly during the observation period. The instillation of TCA/HCl into the stomach reduced the uptake of palmitic acid into PC within 15–45 min to 55% of the saline control, but the uptake into PC recovered by 120–150 min. The pretreatment with GGA enhanced the incorporation of palmitic acid into PC within 15 min, and the label in PC was 124% of the saline control. However, the incorporation into PC in the GGA-pretreated rats was restored by 15–45 min to the

Table 1. Effects of GGA on ulcer formation induced by the instillation of TCA/HCl

Treatment	Ulcer index	% Inhibition	Ulcer index	% Inhibition
	Time after TCA/HCl instillation 15 min		120 min	
Vehicle	67.8 ± 8.5		60.0 ± 4.5	
GGA (50 mg/kg)	20.2 ± 7.8*	70.2	31.6 ± 7.8†	47.3
GGA (200 mg/kg)	9.0 ± 6.0‡	86.7	9.4 ± 1.3‡	84.3

GGA or vehicle was administered orally 120 min before the instillation of TCA/HCl. Ulcers were examined 15 or 120 min after the TCA/HCl treatment and are expressed by the sum of lesions in length (mm). Data are the mean ± SE from five or six rats.
*‡ Significant differences: * $P < 0.01$, † $P < 0.05$, and ‡ $P < 0.001$ vs TCA/HCl instillation.

level in the rats treated with GGA alone and was unchanged at 120–150 min.

The incorporation of palmitic acid into PE was also decreased by the instillation of TCA/HCl within 15–45 min, but recovered by 120–150 min. Palmitic acid incorporation into PE was increased in the GGA-pretreated rats to the level of the saline control within 15 min, and then decreased by 15–45 min to the level in the rats with GGA alone. Practically no radioactivity was found in lysophosphatides, lysophosphatidylcholine or lysophosphatidylethanolamine, in the saline control rats, and there was no significant increase of labeling in the lysophosphatide fractions after the instillation of TCA/HCl. In contrast, the incorporation of palmitic acid into PI + PS was affected slightly by the instillation of TCA/HCl. The incorporation into PI + PS was increased slightly in the GGA-pretreated rats by the treatment with TCA/HCl, but did not reach the values of the saline control, and then decreased to the level in the rats with GGA alone.

The incorporation of [^{3}H]oleic acid into phospho-

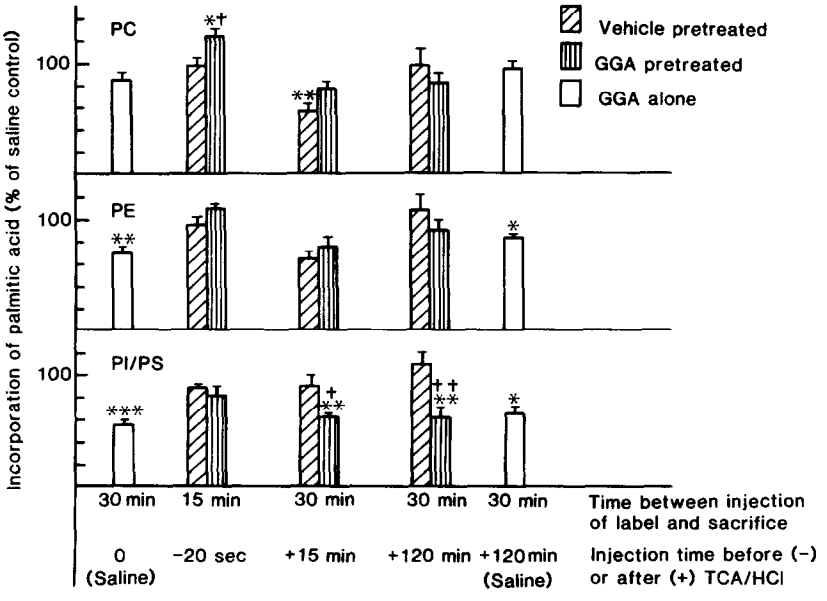

Fig. 1. Effects of TCA/HCl alone or after pretreatment with GGA on the incorporation of labeled palmitic acid into mucosal phospholipids. Rats, divided into three groups for each labeling period, were treated as follows: saline control, vehicle and saline; vehicle pretreated, vehicle and TCA/HCl; GGA pretreated, GGA and TCA/HCl. The rats of each group received GGA (200 mg/kg) or vehicle, were instilled with TCA/HCl or saline 120 min later, then injected with [^{14}C]palmitic acid before or after TCA/HCl treatment, and killed as indicated on the figure. An additional group of rats (GGA alone) was given GGA and instilled with saline instead of TCA/HCl. Radioactivity incorporated into PC, PE, and PI + PS is shown as percent of saline control. The variation of incorporated radioactivity among the saline control groups was 3383–4780, 796–1083, and 1076–1376 dpm/ml extract (mean value of five rats) for PC, PE, and PI + PS respectively. Each value is the mean ± SE of five rats. Significant differences: (*) $P < 0.05$, (**) $P < 0.01$ and (***) $P < 0.001$ vs saline control; (+) $P < 0.05$ and (++) $P < 0.01$ vs vehicle pretreatment.

lipids was also examined, and the results indicated that the intragastric instillation of TCA/HCl similarly reduced the incorporation of oleic acid into PC and PE, while the incorporation into PC and PE in the GGA-pretreated rats was enhanced more markedly as compared with that of palmitic acid within 15 min after TCA/HCl instillation. In these rats, oleic acid incorporation into PC and PE was 135 ± 6.5% (N = 5) and 125 ± 6.6% of the saline control respectively.

Positional distribution. The positional distribution of incorporated fatty acids in PC and PE was analyzed by TLC after digestion with phospholipase A_2 (Fig. 2). Palmitic acid was incorporated into the *sn*-1 position of PC and PE at a higher rate than *sn*-2 in the rats of the saline control. The instillation of TCA/HCl tended to slightly reduce the incorporation of palmitic acid into the *sn*-1 position of PC and PE. The pretreatment with GGA produced a significant increase in the incorporation of palmitic acid into the *sn-1* position of PC and PE. Similarly, oleic acid incorporation was slightly higher in the *sn-1* position of PC and PE than in the *sn-2* position in the saline control rats. The instillation of TCA/HCl slightly reduced the incorporation of oleic acid into the *sn-1* position of PC and PE, whereas the incorporation into the *sn-2* position tended to be enhanced as compared with the saline control. The pretreatment with GGA significantly enhanced the incorporation of oleic acid at both *sn-1* and *sn-2* of PC and at *sn-2* of PE.

Incorporation of fatty acids into neutral lipids after TCA/HCl instillation. The incorporation of labeled palmitic acid into neutral lipids, triacylglycerol (TG), diacylglycerol (DG), and free fatty acid (FFA) fraction after the instillation of TCA/HCl was also determined (Fig. 3). The treatment with GGA alone slightly enhanced the incorporation into TG during 120–150 min after the administration of GGA as compared with the rats treated with vehicle, whereas the incorporation into DG was inhibited. The label in FFA fraction was not affected by the GGA treatment.

The instillation of TCA/HCl slightly increased the incorporation of palmitic acid into TG within 15 min, and then the uptake into TG declined until 120–150 min. The pretreatment with GGA enhanced the incorporation into TG within 15 min as compared with that in the rats with GGA alone, and the labeling was 154% of the saline control. The uptake of palmitic acid into TG in the GGA-pretreated rats then declined until 120–150 min. The radioactivity present in the FFA fraction was increased markedly (about 7-fold over the saline control) within 15 min by the instillation of TCA/HCl, and the pretreatment with GGA reduced the labeling in the FFA fraction to the value of the saline control. The incorporation into DG was also increased rapidly by the instillation of TCA/HCl, and the pretreatment with GGA reduced the labeling in DG significantly.

These observations were also noted in the experiments with labeled oleic acid.

Incorporation of glycerol. Effects of TCA/HCl were examined further by using [^{3}H]glycerol which was injected into rats 20 sec before the instillation of TCA/HCl (Table 2). The incorporation of glycerol into PC and PE decreased immediately after the instillation of TCA/HCl. The pretreatment with GGA prevented the fall in uptake into PC induced TCA/HCl, but no statistical difference of the incorporation into PE was found between the vehicle-

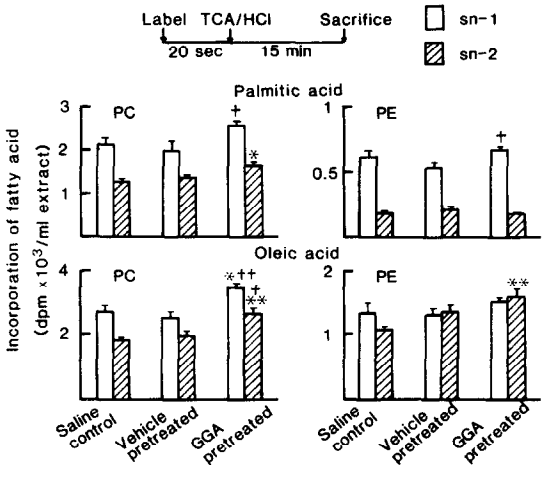

Fig. 2. Positional distribution of incorporated fatty acids. Rats were injected with [^{14}C]palmitic acid and [^{3}H]oleic acid intravenously 20 sec before the installation of TCA/HCl or saline (saline control) and killed 15 min after the injection of the label. GGA (200 mg/kg) or vehicle was administered orally 120 min before the instillation of TCA/HCl. The positional distribution of incorporated fatty acids into PC and PE was analyzed by TLC after digestion of phospholipids with phospholipase A_2. Each value is the mean ± SE of five rats. Significant differences: (*) $P < 0.05$ and (**) $P < 0.01$ vs saline control; (+) $P < 0.05$ and (++) $P < 0.01$ vs vehicle pretreatment.

Table 2. Effects of TCA/HCl alone or after pretreatment with GGA on the incorporation of [^{3}H]glycerol into mucosal lipids

	Incorporation of glycerol (% of saline control*)	
	Vehicle pretreated	GGA pretreated
PC	83.2 ± 2.7† (10)	113.4 ± 4.2‡, ‖ (8)
PE	77.9 ± 2.7§ (9)	86.8 ± 4.4‡ ()7)
PI + PS	87.2 ± 5.1‡ (10)	73.6 ± 4.3§ (8)
TG	99.2 ± 6.7 (6)	150.3 ± 17.2‡, ¶ (5)
DG	112.0 ± 12.3 (6)	91.2 ± 10.7 (5)

Rats were injected with [^{3}H]glycerol intravenously 20 sec before the instillation of TCA/HCl and killed 15 min after the injection of the label. GGA (200 mg/kg) or vehicle was administered orally 120 min before the instillation of TCA/HCl. The incorporation of radioactivity is shown as percent of saline control. Data are the mean ± SE of the number of experiments in parentheses.

* The incorporation of [^{3}H]glycerol into the lipid fractions in the saline control rats was 4052, 709, 720, 1168, and 182 dpm/ml extract (mean value of six to ten rats) for PC, PE, PE + PS, TG, and DG respectively.

†–¶ Significant differences: † $P < 0.01$, † $P < 0.05$, and § $P < 0.001$ vs saline control; and ‖ $P < 0.001$ and ¶ $P < 0.05$ vs TCA/HCl instillation.

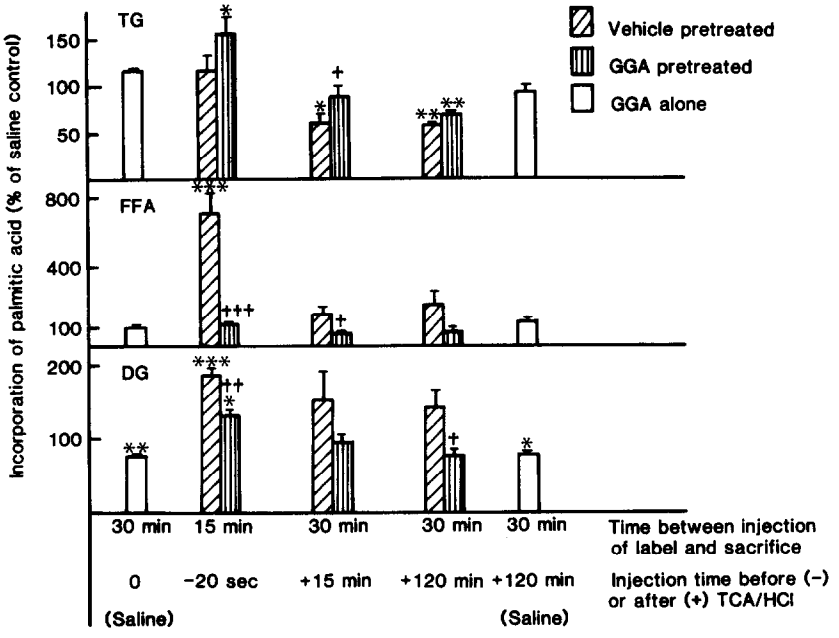

Fig. 3. Effects of TCA/HCl alone or after pretreatment with GGA on the incorporation of labeled palmitic acid into mucosal lipids. The details of treatments are described in the legend of Fig. 1. Radioactivity incorporated into TG, FFA fraction, and DG is shown as percent of saline control. The variation of incorporated radioactivity among the saline control groups was 6,607—10,230, 403–457, and 541–577 dpm/ml extract (mean value of five rats) for TG, FFA, and DG respectively. Each value is the mean ± SE of five rats. Significant differences: (*) $P < 0.05$, (**) $P < 0.01$ and (***) $P < 0.001$ vs saline control; (+) $P < 0.05$, (++) $P < 0.01$ and (+++) $P < 0.001$ vs vehicle pretreatment.

pretreated rats and the GGA-pretreated rats. The incorporation of glycerol into PI + PS was decreased slightly by the instillation of TCA/HCl, and the pretreatment with GGA significantly reduced the upake of glycerol into PI + PS. The incorporation of glycerol into TG was not affected by TCA/HCl, but an increase in the incorporation of glycerol into TG was observed in the rats pretreated with GGA. The incorporation of glycerol into DG was not affected by the instillation of TCA/HCl.

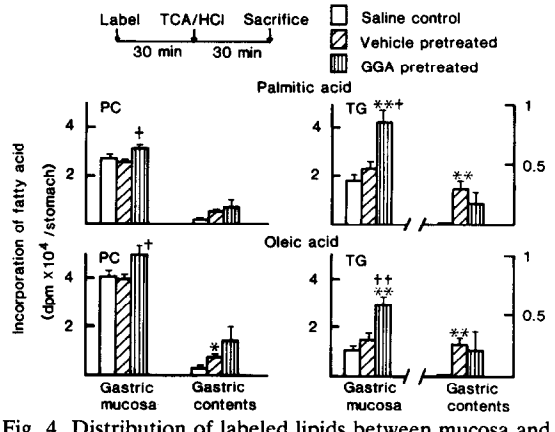

Fig. 4. Distribution of labeled lipids between mucosa and gastric contents after the instillation of TCA/HCl. Rats were injected with [^{14}C]palmitic acid and [^{3}H]oleic acid intravenously, instilled with TCA/HCl 30 min later, and killed 30 min after the instillation of TCA/HCl. GGA (200 mg/kg) or vehicle was administered orally 120 min before the instillation of TCA/HCl. Saline control rats received saline instead of TCA/HCl. The label in PC and TG in mucosa and gastric contents was measured. Each value is the mean ± SE of five rats. Significant differences: (*) $P < 0.05$ and (**) $P < 0.01$ vs saline control; (+) $P < 0.05$ and (++) $P < 0.01$ vs vehicle pretreatment.

Degradation of mucosal lipids. We investigated whether TCA/HCl induces the release of the mucosal lipids into the gastric lumen and examined the possibility that GGA may inhibit the degradation of lipids. The rats injected with [^{14}C]palmitic acid and [^{3}H]oleic acid 30 min prior to the instillation of TCA/HCl, and were killed 30 min after the instillation. Radioactivity in the mucosal lipids and present in the intragastric contents was determined (Fig. 4). The label in PC and TG present in the gastric mucosa was not changed by TCA/HCl instillation, but the labeling of PC and TG in the gastric contents was increased by TCA/HCl instillation as compared with the saline control. The pretreatment with GGA increased the labeling in mucosal PC and TG as compared with the vehicle-pretreated rats; however, GGA showed no effect on the TCA/HCl-induced degradation of lipids into the gastric lumen.

DISCUSSION

It was found in rat liver that the $C_{16:0}$–$C_{18:1}$ and $C_{16:0}$–$C_{18:2}$ molecular species of PC are mainly synthesized *de novo* [23]. Thus, we examined the effects of instillation TCA/HCl into the stomach on phospholipid metabolism in gastric mucosa using [^{14}C]palmitic acid and [^{3}H]oleic acid as precursors.

Fatty acid incorporation into PC and PE was decreased by the instillation of TCA/HCl; furthermore, there was not any significant increase of labeling in lysophosphatide fractions. The experiment using [^{3}H]glycerol, together with the above results, indicated that *de novo* synthesis of PC and PE was decreased by the TCA/HCl instillation. TCA as a protonated form can be absorbed by gastric mucosa [24], and may disrupt the intracellular integrity of phospholipid synthesis in the mucosal cells. Our previous studies demonstrated that intragastric instillation of absolute ethanol reduces the incorporation of fatty acids and glycerol into PC and PE [10]. Ethanol also can penetrate into mucosal cells when administered orally [25]. It is therefore likely that cell-damaging agents such as bile acid and ethanol directly affect the synthetic activity of phospholipids in the mucosal cells, and this effect may be involved in the development of gastric lesions induced by these agents. Our studies were focused on the mucosal metabolism of phospholipids, while lipids present in the mucous layer of the stomach have been demonstrated to be involved in the process of gastric mucosal defense through maintaining the viscosity of the mucous [8], H^+ retardation [9], and the inhibition of proteolytic digestion of mucous glycoprotein [26]. Hence, the inhibition of phospholipid synthesis in the mucosal cells induced by cell-damaging agents possibly causes a failure of the defensive function of lipids in the mucous.

The pretreatment with GGA protected the TCA/HCl-induced change in the labeling of PC and enhanced the incorporation of fatty acids and glycerol into PC as compared with the saline control within 15 min after the instillation of TCA/HCl. In addition, the incorporation of palmitic acid and of oleic acid into the *sn*-1 and *sn*-2 positions of PC was increased. These results indicate that GGA stimulates the synthesis of PC immediately after the instillation of TCA/HCl. However, the enhanced incorporation of glycerol into PC exerted by GGA was less than those of fatty acids; therefore, the deacylation-reacylation pathway may be activated by the pretreatment with GGA in addition to the stimulation of PC syntheses. Morphological studies indicated that acidified TCA instilled into the stomach rapidly induces injury to the gastric surface cells, and these changes are correlated with the change in potential difference [27, 28]. Experiments to examine the degradation of mucosal lipids indicated that mucosal lipids labeled with fatty acids prior to the TCA/HCl instillation were removed into the gastric lumen by TCA/HCl, suggesting that the surface cells of the stomach were injured by TCA/HCl and passed into the gastric lumen. GGA had no effect on the degradation of mucosal lipids induced by TCA/HCl, while it enhanced the incorporation of fatty acids into PC in the gastric mucosa. Since the gastric mucosal damage induced by TCA/HCl was inhibited almost completely by GGA, when examined 15 min after the treatment with TCA/HCl, the enhancement of cellular synthesis of PC elicited by GGA may be involved in the prevention of gastric damage.

The incorporation of fatty acids into the PI + PS fraction was affected slightly by the instillation of TCA/HCl, suggesting that the metabolic turnover of the PI + PS fraction is regulated in a different way from that of other phospholipids in the gastric mucosa. On the other hand, GGA significantly inhibited the uptake of fatty acids into the PI + PS fraction in the normal rats. It has been recognized that the turnover of PI is involved in cellular responses to stimulation by hormones and transmitters [29–31]. Considering that HCl secretion is inhibited by GGA in non-stimulated rats, and the effect is ascribed at least in part to the stimulation of the secretion of secretin [32, 33], further study seems necessary on the relationship between the inhibition by GGA of phospholipid (especially PI) turnover and the functions of gastric mucosal cells.

The instillation of TCA/HCl increased the radioactivity present in the FFA fraction about 7-fold over the saline control. The labeling in DG also increased rapidly after the TCA/HCl instillation. These changes seem due to the leakage of fatty acids from blood vessels to the gastric lumen and the penetration of extracellular fatty acids into mucosal cells. In addition, the inhibition of phospholipid synthesis by TCA/HCl may also cause the increase in intracellular fatty acids and the increase in the labeling in DG. The pretreatment with GGA reduced the TCA/HCl-increased labeling in the FFA fraction to the level of the saline control and significantly inhibited the increase in labeling in DG, indicating that GGA completely protected the gastric blood vessels against damage by TCA/HCl. This view is supported by the observation that the pretreatment with GGA almost completely inhibited the gastric bleeding after the instillation of TCA/HCl.

GGA enhanced the uptake of fatty acids into mucosal TG in the normal rats and TCA/HCl-injured rats as compared with the saline control. Similar results were obtained in our experiments in which rats were pretreated with 20% ethanol (a mild irritant) and then injured by the instillation of absolute ethanol into the stomach [10]. Although the mechanism of enhanced uptake of fatty acids into TG induced by GGA remains to be resolved, it may be correlated with the stimulation of prostaglandin generation by GGA. The mild irritant stimulates the generation of endogenous prostaglandins, and thereby exerts a protective effect on the gastric mucosa [34]. GGA was also demonstrated to enhance prostaglandin generation in rats [35]. Several investigators have observed in other tissues, such as kidney, endothelial cells, and endometrium cells, that the stimulation of prostaglandin synthesis by hormones and other agents enhances the incorporation of fatty acids into TG [36–39].

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