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# EFFECTS OF 15(*S*)-15-METHYL PROSTAGLANDIN $E_2$ METHYL ESTER ON PHOSPHOLIPID METABOLISM IN RAT GASTRIC MUCOSA

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**Abstract**—The effects of 15(*S*)-15-methyl prostaglandin $E_2$ ($PGE_2$) methyl ester on gastric mucosal metabolism of phospholipids in intact rats and rats injured by intragastric instillation of acidified taurocholic acid were examined by using radioisotope-labeled precursors. The incorporation of palmitic, oleic and arachidonic acids into phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was reduced by treatment with 15(*S*)-15-methyl $PGE_2$ methyl ester in the intact rats, but the incorporation of glycerol was unaffected or affected only slightly. Instillation of acidified taurocholic acid resulted in decreased incorporation of palmitic acid and glycerol into PC and PE, whereas pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester caused the incorporations of these precursors to be maintained after acidified taurocholic acid treatment. These results suggest that 15(*S*)-15-methyl $PGE_2$ methyl ester may reduce the incorporation of fatty acids into PC and PE by inhibition of the deacylation–reacylation cycle either directly or indirectly, whereas acidified taurocholic acid decreases *de novo* synthesis of PC and PE, and probably also the reacylation of fatty acid into phospholipids. Pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester protected the PC- and PE-synthesizing activity against the injury induced by acidified taurocholic acid, and this effect may be involved in the prevention of mucosal damage.

There are several lines of evidence that some prostaglandins not only inhibit the gastric secretion of HCl [1–3] but also protect the gastric mucosa at lower dose levels than the antisecretory doses against damage induced by cell-damaging agents such as HCl, bile acids, ethanol and other necrotizing agents [4–6]. The protection of the mucosal cells by prostaglandins has been ascribed to prevention of gastric mucosal barrier disruption, stimulation of mucus secretion, enhancement of mucosal blood flow, and stimulation of alkaline secretion as reviewed by Miller and Jacobson [7] and Miller [8]. Lipids present in the mucosal surface of the stomach have been reported to act as a defensive factor protecting the mucosal cells against injury [9–13]. In addition, Lichtenberger *et al.* [14] demonstrated that a prostaglandin $E_2$ derivative increases the phospholipid content in the stomach surface. These studies were focused on the lipids present in the surface layer of the stomach and on the role of lipids as a protective barrier. We have found recently that absolute ethanol instilled into the stomach cavity reduces the incorporations of labeled fatty acids and glycerol into phospholipids in the mucosal cells, and that pretreatment with 20% ethanol prevents the decrease in the incorporations of the precursors into phospholipids on subsequent treatment with absolute ethanol [15]. Robert *et al.* found that the exposure of the gastric mucosa to a mildly damaging concentration of an agent (such as 20% ethanol) increases mucosal resistance to subsequent exposure to more damaging concentrations of the same agent (such as absolute ethanol) [16], and they proposed that these protective effects are mediated by stimulation of the release of prostaglandins by the stomach [17]. These observations, therefore, raise the possibility that prostaglandins may affect the intracellular metabolism of phospholipids in the gastric mucosa. In the present study we evaluated the effects of a prostaglandin $E_2$ derivative, 15(*S*)-15-methyl prostaglandin $E_2$ methyl ester [15(*S*)-15-methyl $PGE_2$ methylester‡], on the mucosal metabolism of phospholipids in intact rats, and also examined the effects of pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester on the changes in phospholipid metabolism induced by intragastric instillation of a mucosa-damaging agent, acidified taurocholic acid.

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‡ Abbreviations: 15(*S*)-15-methyl $PGE_2$ methyl ester, 15(*S*)-15-methyl prostaglandin $E_2$ methyl ester; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; and PS, phosphatidylserine.

## MATERIALS AND METHODS

*Chemicals.* [1-$^{14}$C]Palmitic acid (58 mCi/mmol), [9,10-$^3$H]oleic acid (4.3 Ci/mmol), and [5,6,8,9,11,12,14,15-$^3$H]arachidonic acid (163 Ci/mmol) were purchased from Amersham Inc., U.K., and [2-$^3$H]glycerol (2 Ci/mmol) from ICN Radiochemicals (California, USA). Bovine serum albumin and pancreatic phospholipase $A_2$ were obtained from the Sigma Chemical Co., and taurocholic acid sodium salt was from the Calbiochem-Behring Co.; other chemicals used were of reagent grade. 15(*S*)-15-Methyl $PGE_2$ methyl ester was prepared by the method of Yankee *et al.* [18].

*Administration of labeled precursors*. Labeled precursor was dissolved in 5% (w/v) bovine serum albumin solution, and 0.5 ml of the solution (40 $\mu$Ci for fatty acids, 100 $\mu$Ci for glycerol) was injected into the femoral vein of rats. Male Sprague–Dawley rats aged 7 or 8 weeks were fasted for 24 hr before use. 15(*S*)-15-Methyl $PGE_2$ methyl ester (4 or 40 $\mu$g/kg, dissolved in 1.2 ml of saline) or saline (1.2 ml) was administered orally, and 15 min later 80 mM taurocholic acid in 0.1 M HCl or saline (1.2 ml) was administered intragastrically. The labeled precursors were injected 20 sec before or 15 min after the instillation of acidified taurocholic acid, and the rats were killed 15 min or 45 min after the treatment respectively. The rats treated with 15(*S*)-15-methyl $PGE_2$ methyl ester alone, without the instillation of acidified taurocholic acid, were injected with labeled precursors 15 min after oral administration of 15(*S*)-15-methyl $PGE_2$ methyl ester, and killed 30 min after the injection.

*Extraction of mucosal lipids and determination of incorporated radioactivity*. The stomach was excised, opened with scissors along the greater curvature, and washed in ice-cold saline. The gastric mucosa was then scraped with a metal spatula on an ice-cold glass plate. Mucosal cells were homogenized in 0.5 mM EDTA and total lipids were extracted by the method of Bligh and Dyer [19]. Determination of radioactivity incorporated into lipid fractions was carried out by a method reported previously [15, 20].

*Analysis of the positional distribution of incorporated fatty acids*. The positional distribution of radioactivity incorporated into phospholipids was analyzed by TLC after digestion with phospholipase $A_2$ as described previously [15, 20].

*Histological examination*. 15(*S*)-15-Methyl $PGE_2$ methyl ester (40 $\mu$g/kg) or saline was administered orally, and 15 min later 80 mM taurocholic acid in 0.1 M HCl or saline was administered intragastrically. The rats were killed 30 min after the treatment with acidified taurocholic acid, and the gastric wall was cut from the forestomach to pylorus. The glandular mucosa was fixed in formalin and stained with hematoxylin (H) and eosin (E). Mucosal specimens were evaluated under light microscopy.

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

## RESULTS

*Effects of 15(S)-15-methyl $PGE_2$ methyl ester on the incorporation of labeled precursors into phospholipids in intact rats*. 15(*S*)-15-Methyl $PGE_2$ methyl ester was administered orally to rats at a dose of 4 or 40 $\mu$g/kg and the incorporation of labeled precursors into mucosal phospholipids was measured to evaluate the effects of the prostaglandin $E_2$ derivative on phospholipid metabolism in intact rats (Fig. 1). The incorporations of [$^{14}$C]palmitic, [$^3$H]oleic, and [$^3$H]arachidonic acids into phosphatidylcholine (PC) were reduced significantly dose-dependently by the treatment with 15(*S*)-15-methyl $PGE_2$ methyl ester, whereas the incorporation of [$^3$H]glycerol into PC was affected only slightly. 15(*S*)-15-Methyl $PGE_2$ methyl ester also decreased the incorporation of labeled fatty acids into phosphatidylethanolamine (PE), but it did not produce any significant effect on the incorporation of glycerol into PE. On the other hand, 15(*S*)-15-methyl $PGE_2$ methyl ester did not affect the incorporation of fatty acids into the phosphatidylinositol plus phosphatidylserine (PI + PS) fraction, and the incorporation of glycerol into the PI + PS fraction was increased by treatment with 15(*S*)-15-methyl $PGE_2$ methyl ester. The incorporation of fatty acids into triacylglycerol was also decreased significantly ($P < 0.01$) by treatment with 15(*S*)-15-methyl $PGE_2$ methyl ester at a dose of 40 $\mu$g/kg to 44.1 and 45.7% of the saline control (mean value of four rats) for palmitic acid and oleic acid, respectively, whereas the incorporation of arachidonic acid into triacylglycerol was not affected (104.1%). Palmitic acid and oleic acid were incorporated into the *sn*-1 and *sn*-2 positions of PC and the incorporation into both positions was decreased by treatment with 15(*S*)-15-methyl $PGE_2$ methyl ester. The incorporation of oleic acid into the *sn*-1 and *sn*-2 positions of PE was also reduced by this treatment. Arachidonic acid was incorporated only into the *sn*-2 position of PC and PE in all rats, and its incorporation was decreased by the treatment with 15(*S*)-15-methyl $PGE_2$ methyl ester.

*Incorporation of labeled precursors into phospholipids after intragastric instillation of acidified taurocholic acid*. The damage to the gastric mucosa induced by acidified taurocholic acid instilled into the gastric cavity and the effects of pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester on the changes by acidified taurocholic acid were examined (Fig. 2). Acidified taurocholic acid induced the desquamation of the surface epithelium (indicated by arrow), necrotic lesions deep in the mucosa, and the formation of edema in the submucosa. Pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester appeared to protect the mucosa against the damage induced by acidified taurocholic acid, and it was particularly noticeable that the deep necrotic lesions were reduced significantly by this pretreatment.

The incorporation of [$^{14}$C]palmitic acid into mucosal phospholipids was determined to elucidate the effects of acidified taurocholic acid on the metabolism of phospholipids (Fig. 3). The effects of pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester (4 and 40 $\mu$g/kg) on the changes induced by acidified taurocholic acid were also evaluated. The instillation of acidified taurocholic acid reduced significantly the incorporation of palmitic acid into PC, PE and PI + PS within 15–45 min as compared with the saline control; the decrease in the incorporation into PI + PS was less than in the case of PC and PE. Although the incorporation of labeled palmitic acid into PC and PE was decreased by the treatment with 15(*S*)-15-methyl $PGE_2$ methyl ester alone, as mentioned previously, the incorporations into PC and PE increased immediately after the instillation of acidified taurocholic acid in the rats pretreated with 15(*S*)-15-methyl $PGE_2$ methyl ester at both doses, and the incorporations in these rats were significantly higher at 15–45 min than those in the rats pretreated with saline. The

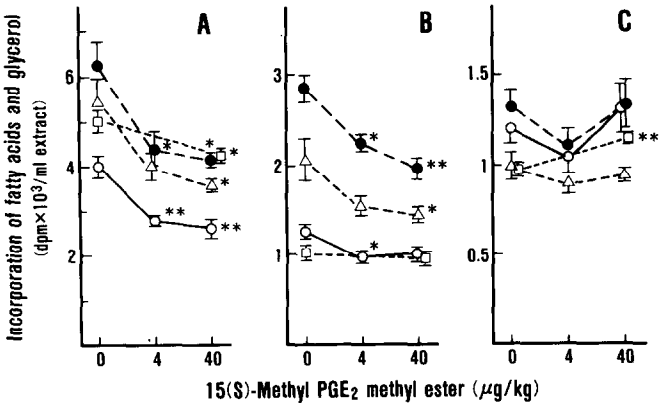


Fig. 1. Effects of 15(*S*)-15-methyl $PGE_2$ methyl ester on the incorporation of labeled fatty acids and glycerol into mucosal phospholipids in intact rats. 15(*S*)-15-Methyl $PGE_2$ methyl ester was administered orally to rats at a dose of 4 or 40 μg/kg. The rats were injected with [$^{14}$C]palmitic acid, [$^3$H]oleic acid, [$^3$H]arachidonic acid or [$^3$H]glycerol 15 min later, and killed 30 min after the injection of labeled precursors. Radioactivity incorporated into PC (A), PE (B) and PI + PS (C) was measured. Key: palmitic acid (○); oleic acid (●); arachidonic acid (△); and glycerol (□). Each value is the mean ± SE of four or five rats. Significant differences: * $P < 0.05$, and ** $P < 0.01$ vs saline control.

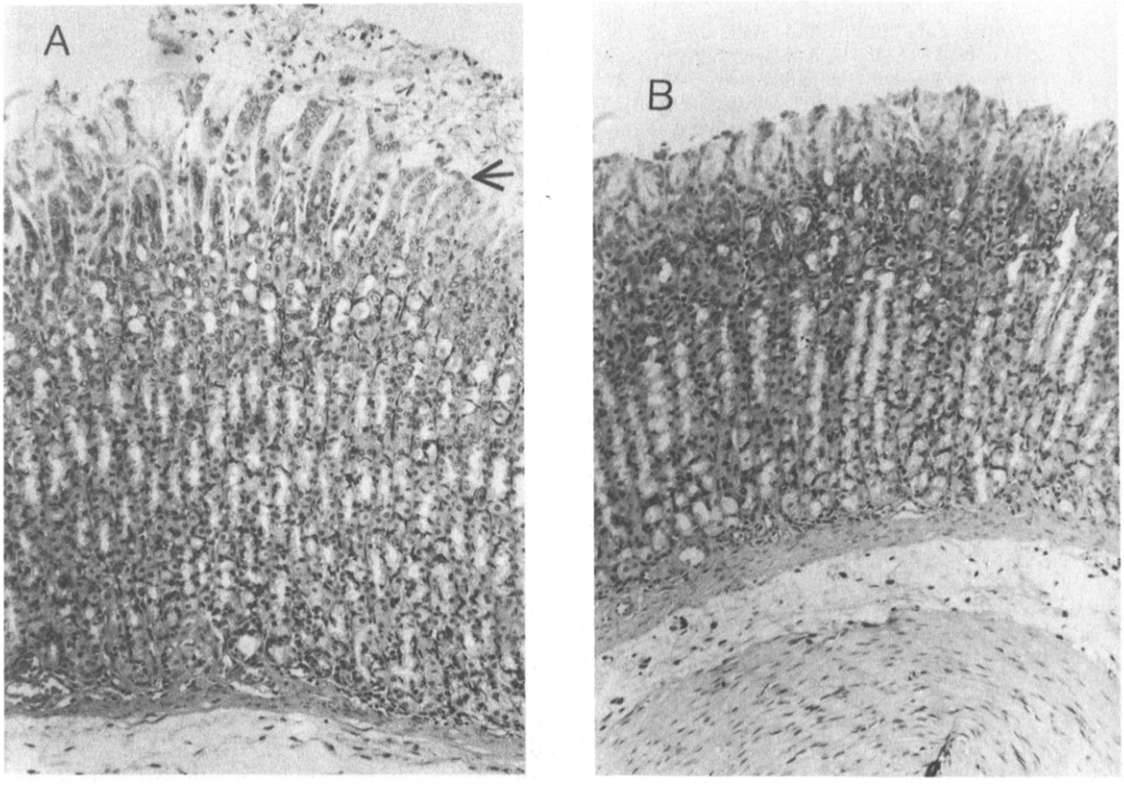


Fig. 2. Light micrographs of the gastric fundic mucosa after instillation of acidified taurocholic acid (H and E, × 100). (A) 30 min after acidified taurocholic acid in saline-pretreated rat; (B) 30 min after acidified taurocholic acid in the rat pretreated with 15(*S*)-15-methyl $PGE_2$ methyl ester (40 μg/kg).

incorporation of palmitic acid into PI + PS was maintained by the pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester at a dose of 40 μg/kg.

The incorporation of [$^3$H]glycerol into mucosal phospholipids was further measured to examine whether the intragastric instillation of acidified taurocholic acid might affect the *de novo* synthesis of phospholipids (Table 1). The treatment with acidified taurocholic acid produced a significant decrease in the incorporation of glycerol into PC as compared

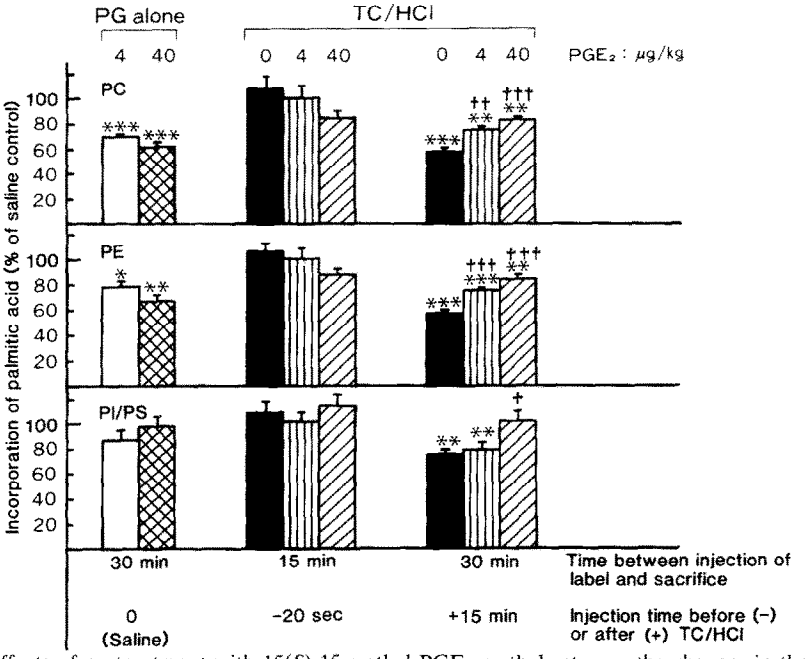


Fig. 3. Effects of pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester on the changes in the incorporation of labeled palmitic acid into mucosal phospholipids induced by instillation of acidified taurocholic acid (TC/HCl). Rats were injected with [$^{14}$C]palmitic acid intravenously and killed at the time points depicted in the figure. 15(*S*)-15-Methyl $PGE_2$ methyl ester was administered orally 15 min before the instillation of acidified taurocholic acid. Amounts of radioactivity incorporated into PC, PE, and PI + PS were measured. Each value is the mean ± SE from five to nine rats. Significant differences: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs saline control; † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ vs saline pretreatment. The variations of incorporated radioactivity among the saline control groups were 3458–5067, 1140–1206, and 1061–1310 dpm/ml extract (mean value of five rats) for PC, PE, and PI + PS respectively.

with the saline control, whereas incorporation into PE was not affected significantly. On the other hand, the incorporation of glycerol into PI + PS was increased by the instillation of acidified taurocholic acid. The pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester enhanced the incorporation of labeled glycerol into PC, PE and PI + PS as compared with the rats pretreated with saline.

Table 1. Effects of pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester on the changes in the incorporation of [$^3$H]glycerol into mucosal phospholipids induced by intragastric instillation of acidified taurocholic acid

| | Incorporation of [$^3$H]glycerol (% of saline control*) | |
|---|---|---|
| | Pretreatment with saline | Pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester |
| PC | 78.5 ± 6.1† | 106.5 ± 7.5‖ |
| PE | 89.0 ± 4.8 | 114.1 ± 7.9‖ |
| PI + PS | 123.7 ± 3.4‡ | 142.9 ± 6.8§‖ |

Rats were pretreated with 15(*S*)-15-methyl $PGE_2$ methyl ester (40 μg/kg) or saline, instilled with acidified taurocholic acid 15 min later, injected with [$^3$H]glycerol, and killed 15 min and 45 min after the instillation respectively. The radioactivity incorporated into phospholipids was measured. Each value is the mean ± SE of four rats.

* The incorporation of [$^3$H]glycerol into the lipid fractions in the saline control rats was 5046, 1015 and 980 dpm/ml extract (mean value of four rats) for PC, PE, and PI + PS respectively.

†–‖ Significant differences: † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$ vs saline control; and ‖ $P < 0.05$ vs saline pretreatment.

## DISCUSSION

The present studies demonstrated that 15(*S*)-15-methyl $PGE_2$ methyl ester reduced the incorporation of labeled fatty acids into PC and PE in intact, uninjured rats, but had no effect or affected only slightly the incorporations of labeled glycerol into these lipids, indicating that the *de novo* synthesis of PC and PE is little affected by treatment of rats with 15(*S*)-15-methyl $PGE_2$ methyl ester. It is well established that, in the liver and other tissues, arachidonic acid is specifically incorporated into the *sn*-2 position of phospholipids by acyltransferase [21], and our results on the incorporation of arachidonic acid into gastric mucosal PC and PE confirmed this [15]. Therefore, the reduced incorporation of arachidonic acid induced by 15(*S*)-15-methyl $PGE_2$ methyl ester may be due to an inhibitory effect on the deacylation–reacylation cycle either directly, or indirectly through a response mediated by a prostaglandin $E_2$ receptor, which is present in the plasma membrane of the gastric mucosa [22, 23]. Alternatively, since 16,16-

dimethyl $PGE_2$ was found to reduce the gastric mucosal blood flow by 15% in intact, anesthetized rats [24], the reduced incorporation may be produced by reduced transport of exogenously supplied arachidonic acid from the blood into the mucosal cells. Considering that the incorporation of arachidonic acid into the PI + PS fraction and traicylglycerol was not affected by treatment with 15(*S*)-15-methyl $PGE_2$ methyl ester, and that the incorporation of glycerol into PC and PE was little affected by this treament, the former mechanism seems more likely. Although the contribution of the deacylation–reacylation cycle to the uptake of palmitic acid and oleic acid into the *sn*-1 and *sn*-2 positions of PC and PE in the gastric mucosa is not clear at present, the reduced incorporation of these fatty acids into PC and PE induced by 15(*S*)-15-methyl $PGE_2$ methyl ester can probably be ascribed to the same mechanism as in the case of arachidonic acid.

Another finding is that acidified taurocholic acid reduced the incorporation of palmitic acid into phospholipids, especially PC and PE. The treatment with acidified taurocholic acid also decreased the incorporation of glycerol into PC, although the decrease was less than those with fatty acid. These results indicate that acidified taurocholic acid instilled into the stomach cavity inhibited the *de novo* synthesis of PC in the mucosal cells, and probably the reacylation of fatty acids into phospholipids. Bile acids have been shown to break the mucosal barrier of the stomach, resulting in an increase in back-diffusion of $H^+$ and net efflux of $Na^+$ into the gastric lumen [25–27]. Taurocholic acid as a protonated form can be absorbed by gastric mucosa [28], and may disrupt the intracellular integrity of phospholipid turnover in the mucosal cells.

Pretreatment with 15(*S*)-15-methyl$PGE_2$ methyl-ester increased the incorporation of glycerol into PC and PE, as compared with that in rats pretreated with saline, after the treatment with acidified taurocholic acid, indicating that the pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester protected the *de novo* synthesis of PC and PE. In addition, the incorporation of palmitic acid into PC and PE was increased in the rats pretreated with 15(*S*)-15-methyl $PGE_2$ methyl ester after the instillation of acidified taurocholic acid, as compared with that in the intact rats given 15(*S*)-15-methyl $PGE_2$ methyl ester alone. It is therefore likely that the suppressed deacylation–reacylation cycle was restored in these rats after the injury by acidified taurocholic acid. The doses of 15(*S*)-15-methyl $PGE_2$ methyl ester used in the present studies (4 and 40 $\mu$g/kg) are not antisecretory doses [29], but were sufficient to protect against damage by acidified taurocholic acid [30, 31]. Indeed, our histological examination confirmed that pretreatment with 15(*S*)-15-methyl $PGE_2$ methyl ester reduced the damage to the gastric mucosa induced by acidified taurocholic acid. Since the integrity of gastric mucosal phospholipid synthesis can be maintained in rats pretreated with 15(*S*)-15-methyl $PGE_2$ methyl ester, this effect of the prostaglandin $E_2$ derivative may be involved in the prevention of mucosal damage induced by acidified taurocholic acid. These results support the hypothesis that phospholipids may play an important role in the gastric cytoprotection elicited by prostaglandins [14].

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