Molecular Heterogeneity of Platelet-Activating Factor (PAF) in Rat Glandular Stomach Determined by Gas Chromatography/Mass Spectrometry. PAF Molecular Species Changes upon Water-Immersion Stress[1]

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The molecular heterogeneity of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (alkylacetyl-GPC) and 1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine (acylacetyl-GPC) in normal rat glandular stomach was studied by gas chromatography/mass spectrometry (GC/MS) and tandem mass spectrometry. The percentage compositions of the molecular species of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC in the antrum were, respectively, 1-alkyl [16:0 (34%) and 18:0 (66%)]-2-acetyl-GPC and 1-acyl [16:0 (60%), 18:0 (14%) and 18:1 (26%)]-2-acetyl-GPC. The alkyl chain composition of 1-alkyl-2-acetyl-GPC was quite different from that of 1-alkyl-2-acyl-GPC in both the antrum and corpus, demonstrating a high degree of selectivity of alkyl chain utilization in PAF biosynthesis. The amount of 1-acyl-2-acetyl-GPC was much greater than that of 1-alkyl-2-acetyl-GPC. The molecular heterogeneity of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC in the corpus was similar to that in the antrum. Water-immersion stress affected not only the amount of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC, but also their molecular heterogeneity in the antrum and corpus. Whereas the amounts of 1-hexadecyl-2-acetyl-GPC and 1-acyl [16:0, 18:0 and 18:1]-2-acetyl-GPC decreased markedly (to less than one-fifth) in the antrum after such stress for 1 hr, the amount of 1-octadecyl-2-acetyl-GPC increased markedly (up to 4-fold) in the corpus and severe lesions were observed after stress for 7 hr. The changes may be associated with the pathogenicity of gastric ulcers.
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Platelet-activating factor [PAF, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (1)] is a chemical mediator with a wide spectrum of biological activities, *e.g.*, stimulation of platelets and neutrophils, and enhancement of vascular permeability and smooth muscle contraction (2). PAF is not only generated in a variety of cells upon chemical or immune stimulation associated with inflammation and anaphylaxis (2), but is also found in normal animal tissues [bovine brain (3), rat uterus (4) and fetal rabbit lung (5)]. In a previous study, we found a significant amount of PAF in normal stomach (especially in the antral mucosa), and observed an alteration in the level of PAF upon water-immersion stress, as determined by a bioassay based on the release of [^{3}H]serotonin from washed rabbit platelets (6). Over the last several years, data have been accumulating that suggest a molecular heterogeneity of PAF in cells (7–9) and tissues (3,4) which is dependent on the pathophysiological state. Evidence exists that the different molecular species of PAF homologs and analogs may exert different biological activities (1,10). In the present study, we determined the molecular species of rat gastric PAF homologs [1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (alkylacetyl-GPC)] and analogs [1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine (acylacetyl-GPC)], and compared the compositions of their alkyl and acyl chains with those of alkyl and acyl chains at the 1-position of 1-akyl-2-acyl-GPC and 1,2-diacyl-GPC in order to investigate the selectivity of alkyl and acyl chain utilization in PAF biosynthesis. The influence of water-immersion stress on the molecular heterogeneity of PAF was also elucidated by gas chromatography/mass spectrometry (GC/MS).

MATERIALS AND METHODS

Materials. Male Wistar rats were deprived of food, but not water, for 24 hr before the experiments. Animals were placed in a stress cage and immersed in water (23°C ± 1°C) to the level of xiphoid process, in accordance with the method of Takagi and Okabe (11). Control rats were placed in a cage under no restraint at 25°C ± 1°C. The rats were then sacrificed by decapitation under light diethyl ether anesthesia unless otherwise stated.

Chemicals. 1-Hexadecyl-GPC, 1-hexadecyl- and 1-octadecyl-2-acetyl-GPC and 1-hexadecanoyl-GPC were purchased from Bachem Feinchemikalien AG, Bubendorf, Switzerland. 1-Octadecenyl-2-acetyl-GPC was prepared from human polymorphonuclear leukocytes (12). 1-Octadec-*cis*-9′-enoyl- and 1-octadecanoyl-GPC were obtained from Serdary Research Laboratories Inc., Ontario, Canada. Perdeuterated acetic anhydride(d_6) was a product of E. Merck, Darmstadt, Germany. 1-Hexadecyl-2-perdeuteroacetyl-GPC (internal standard for 1-akyl-2-acetyl-GPC) and 1-hexadecanoyl-, 1-octadec-*cis*-9′-enoyl- and 1-octadecanoyl-2-acetyl-GPC were synthesized by acetylating 1-hexadecyl-GPC and 1-hexadecanoyl-, 1-octadec-*cis*-9′-enoyl- and 1-octadecanoyl-GPC with perdeuterated acetic anhy-

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Abbreviations: GC/MS, gas chromatography/mass spectrometry; GPC, *sn*-glycero-3-phosphocholine; PAF, platelet-activating factor; SIM, selected ion monitoring; *t*-BDMS, *tert*-butyldimethylsilyl; TLC, thin-layer chromotography; TNS, 6-*p*-toluidine-2-naphthalenesulfonic acid.

dride and acetic anhydride, respectively, in the presence of perchloric acid as a catalyst (13). 1-Heptadecanoyl-2-perdeuteroacetyl-GPC (internal standard for 1-acyl-2-acetyl-GPC) was synthesized as described previously (9). The *tert*-butyldimethylchlorosilane/imidazole reagent and all fatty acid methyl ester standards were obtained from Applied Science (State College, PA). Phospholipase C (EC 3.1.4.3) was partially purified from *Bacillus cereus* (14). Phospholipase A_2 (EC 3.1.1.4) from *Agkistrodon halys blomhoffi* was purified to homogeneity as reported previously (15).

Isolation of PAF, phosphatidylcholine and phosphatidylethanolamine from rat glandular stomach. The total lipids of antrum and corpus, respectively, were extracted by the method of Bligh and Dyer (16) at 4°C in the presence of 50 mM glycine-HCl buffer (pH 2.8) in a single-phase system to inactivate PAF acetylhydrolase, as described previously (6). The biological activity before and after the weak-acid treatment of the PAF extracted without acid treatment, as determined by rabbit platelet activation (17), was not altered, showing that only traces of the 1-alk-1′-enyl-type PAF, if any, were present. Lipid phosphorus was determined by the method of Bartlett (18). The lipids (*ca.* 2.5 mg phosphorus) were applied to a silic AR CC-7 column (8 × 90 mm). The neutral lipids were separated by elution with 40mL of chloroform. Phospholipids (*ca.* 500 μg phosphorus), eluted with 80 mL of methanol, were separated by thin-layer chromatography (TLC) on silica gel 60H plates (20 × 20 cm, 0.6 mm thick) using the solvent system chloroform/methanol/water (65:35:6, v/v/v). After spraying the plate with 1 mM 6-*p*-toluidine-2-naphthalenesulfonic acid (TNS) (19), the bands between lysophosphatidylcholine and sphingomyelin, and those of phosphatidylcholine and phosphatidylethanolamine, were localized under ultraviolet light, scraped off, and extracted. In order to separate acidic phospholipids, the phosphatidylcholine fraction (*ca.* 1 mg phosphorus) dissolved in chloroform was applied to a column of activated alumina 200 (8 × 16 mm) and eluted with 60 mL of chloroform/methanol (1:1, v/v).

Derivatization of PAF for mass spectrometry (MS) analysis. The isolated PAF fractions were digested with phospholipase C, and the resulting diradylglycerols were converted to *tert*-butyldimethylsilyl (*t*-BDMS) derivatives essentially as described previously (20). The *t*-BDMS derivatives were purified further by TLC (10 × 20 cm, 0.3 mm thick) using the solvent system hexane/diethyl ether (9:1, v/v).

Determination of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC by GC/MS coupled with selected ion monitoring (SIM). For measurement of 1-akyl-2-acetyl-GPC, the total lipids were mixed with 1-hexadecyl-2-perdeuteroacetyl-GPC ($4.2–4.8 \times 10^{-7}$ mol/mol total antrum phospholipds and 1.2×10^{-7} mol/mol total corpus phospholipids) as internal standard. For measurement of 1-acyl-2-acetyl-GPC, 1-heptadecanoyl-2-perdeuteroacetyl-GPC ($3.8–4.4 \times 10^{-6}$ mol/mol total antrum phospholipids and 2.4×10^{-7} mol/mol total corpus phospholipids) was used as internal standard.

A JEOL (Tokyo, Japan) JMS-DX300 instrument (ionization energy, 70 eV) with a JMA 3100 computer was coupled to a gas chromatograph equipped with a 1 m × 2 mm glass spiral packed with 1% OV-1 on Chromosorb W (80–100 mesh). The temperatures of the column, injection port, separator, inlet and ionization chamber were kept at 240, 290, 290, 290, and 270°C, respectively. The flow rate of helium was 55 mL/min. Ions $[M-57]^+$, produced by cleavage of the *tert*-butyl radical from the parent ion, and $[CH_3CO + 74]^+$ and $[RCO + 74]^+$, formed by rearrangements of the acetyl or acyl residue and dimethyl silanol, were selected for SIM. The abundance of each molecular species was calculated from the ratios of their peak areas to that of the internal standard.

Tandem mass spectrometry (MS/MS). MS/MS was performed on a JEOL HX110-HX110 tandem mass spectrometer equipped with a JMA-DA5000 computer. The instrument was used in the electron impact mode, and the sample was directly inserted into the ion source. The ionization energy, ionization current, ionization chamber temperature and accelerating voltage were 70 eV, 300 μA, 200°C and 10.0 kV, respectively. The selected $[M-57]^+$ ion was collided with helium.

Determination of the composition of ether-linked choline and ethanolamine glycerophospholipids. Choline and ethanolamine glycerophospholipids (358–587 μg phosphorus) were hydrolyzed with 2 mL of 0.1 N KOH in 90% methanol at room temperature for 60 min. The ether-linked type was recovered in the chloroform–soluble phase (32.2 and 28.7 μg phosphorus from choline glycerophospholipids of the antrum and corpus, and 154.7 and 113.6 μg phosphorus from ethanolamine glycerophospholipids of the antrum and corpus, respectively), and that derived from the diacyl type was recovered in the water-soluble phase (372.8 and 557.8 μg phosphorus from choline glycerophospholipids of the antrum and corpus, and 203.2 and 329.6 μg phosphorus from ethanolamine glycerophospholipids of the antrum and corpus, respectively) by the extraction method of Bligh and Dyer (16). Then the chloroform-soluble fraction was incubated in a mixture of 1 mL of chloroform, 2 mL of methanol and 0.8 mL of 0.5 N HCl at 37°C for 15 min. The products of alk-1′-enyl-GPC and alk-1′-enyl-*sn*-glycero-3-phosphoethanolamine (GPE) were recovered in the water-soluble phase (9.7 and 13.2 μg phosphorus from ether-linked choline lysoglycerophospholipids of the antrum and corpus, and 112.4 and 89.4 μg phosphorus from ether-linked ethanolamine lysoglycerophospholipids of the antrum and corpus, respectively), and alkyl-GPC and alkyl-GPE were recovered in the chloroform-soluble phase (20.9 and 13.8 μg phosphorus from ether-linked choline lysoglycerophospholipids of the antrum and corpus, and 31.2 and 20.7 μg phosphorus from ether-linked ethanolamine lysoglycerophospholipids of the antrum and corpus, respectively) in a two-phase system (16).

Analysis of the composition of acyl and alkyl chains at the 1-position of choline and ethanolamine glycerophospholipids. The composition of acyl and alkyl chains at the 1-position was determined by analysis of lysoglycerophospholipids obtained by phospholipase A_2 hydrolysis and of ether-linked lysoglycerophospholipids obtained by treatment with weak alkali and acid as described above, respectively. Choline and ethanolamine glycerophospholipids (6–7 μg phosphorus) were completely hydrolyzed with 50 μg purified phospholipase

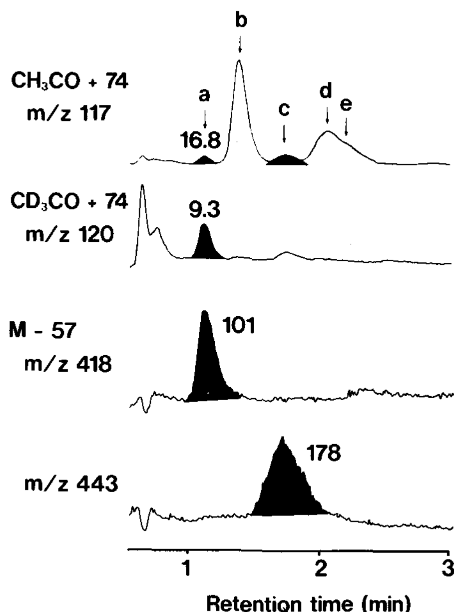

FIG. 1. Qualitative and quantitative analysis of 1-alkyl-2-acetyl-GPC in rat antrum by SIM analysis. Rats weighing 251 ± 31 g (157 animals), age 9–10 wk, were used (Experiment 1). The total antrum lipids were mixed with 1-hexadecyl-2-perdeuteroacetyl-GPC as internal standard. Peak areas are shown to the right of each of the peaks. Peaks of a, b, c, d and e correspond to those of *t*-BDMS derivatives of authentic 1-hexadecyl-2-acetyl-GPC and 1-hexadecanoyl-2-acetyl-GPC, 1-octadecyl-2-acetyl-GPC, 1-octadec-*cis*-9′-enoyl-2-acetyl-GPC and 1-octadecanoyl-2-acetyl-GPC, respectively. The result from one of 3 separate measurements is shown in the figure.

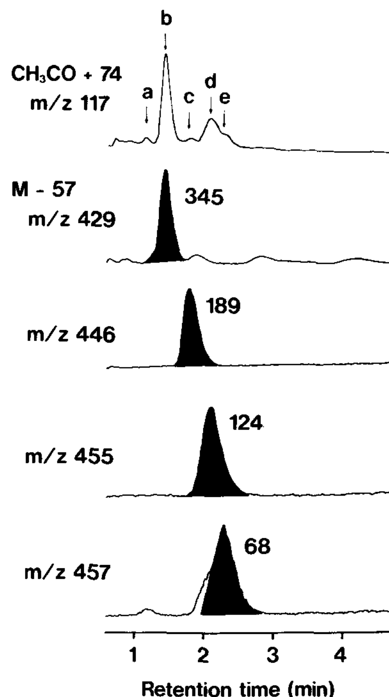

FIG. 2. Qualitative and quantitative analysis of 1-acyl-2-acetyl-GPC in rat antrum by SIM analysis. Rats weighing 223 ± 12 g (81 animals), age 9 wk, were used (Experiment 2). The total antrum lipids were mixed with both 1-hexadecyl-2-perdeuteroacetyl-GPC and 1-heptadecanoyl-2-perdeuteroacetyl-GPC as internal standards. Peak areas are shown to the right of each of the peaks. Peaks of a, b, c, d and e are as in Figure 1. The result from one of 3 separate measurements is shown in the figure.

A_2 (*Agkistrodon halys blomhoffi*) in a mixture of 3 mL of diethyl ether, 0.4 mL of 0.125 M Tris-HCl buffer (pH 8.2), 0.05 mL of 1.0 M NaCl and 10 mM $CaCl_2$ with vigorous shaking at room temperature for 16 hr. The lipid products were extracted and separated on a silica gel G plate in the solvent system chloroform/methanol/water (65:35:6, v/v/v). For analysis of the fatty acid composition of the purified lysoglycerophospholipids, the methyl esters derived by acid methanolysis were prepared (21). In order to determine the alkyl chain composition, the purified ether-linked lysoglycerophospholipids were mixed with 0.3 mL of acetic anhydride and 0.2 mL of glacial acetic acid at 150°C for 5 hr for conversion to 1-alkyl-2,3-diacetyl-glycerol (22). The fatty acid methyl esters and glyceryl ether diacetates were analyzed in a Shimadzu GC-7AG gas chromatograph (Kyoto, Japan) equipped with glass columns, packed 10% Silar 10C on Gas-Chrom Q (100-200 mesh) and 1% OV-1 on Chromosorb W (80–100 mesh) (both 2 m × 3 mm), respectively. The columns were operated at 185–215°C for analysis of fatty acid methyl esters and at 240°C for analysis of glyceryl ether diacetates. The peaks of each chromatogram were assigned on the basis of their retention times relative to those of standards.

RESULTS

Identification of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC in rat antrum. For GC/MS analysis, the *t*-BDMS derivative of the 'diglyceride' prepared enzymatically was used. The presence of both 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPG in rat antrum was demonstrated by SIM analysis (Figs. 1 and 2). Upon monitoring the ion at *m/z* 117, $[CH_3CO + 74]^+$, five peaks were detected with retention times of 1′07″, 1′24″, 1′44″, 2′04″ and 2′12″, which corresponded respectively to those of authentic 1-hexadecyl-2-acetyl-GPC, 1-hexadecanoyl-2-acetyl-GPC, 1-octadecyl-2-acetyl-GPC, 1-octadec-*cis*-9′-enoyl-2-acetyl-GPC and 1-octadecanoyl-2-acetyl-GPC. For determining the molecular species of 1-alkyl-2-acetyl-GPC, MS/MS analysis was performed. The $[M-57]^+$ ion was selected and collided with helium because this ion became more prominent than the $[M]^{+\cdot}$ ion in the electron ionization mass spectrum of the *t*-BDMS derivatives of the diglycerides (23). The collision-induced fragmentation of ions at *m/z* 415 and 443, due to $[M-57]^+$, yielded daughter ions at *m/z* 355 and 383, due to the characteristic $[R + 130]^+$ ion for the alkyl residue, and an ion at *m/z* 117, due to $[CH_3CO + 74]^+$ for the acetyl residue, for 1-hexadecyl-2-acetyl-3-*t*-BDMS-glycerol and 1-octadecyl-2-acetyl-3-*t*-BDMS-glycerol, respectively (Fig. 3). The collision-induced fragmentation of an ion at *m/z* 441 did not yield the

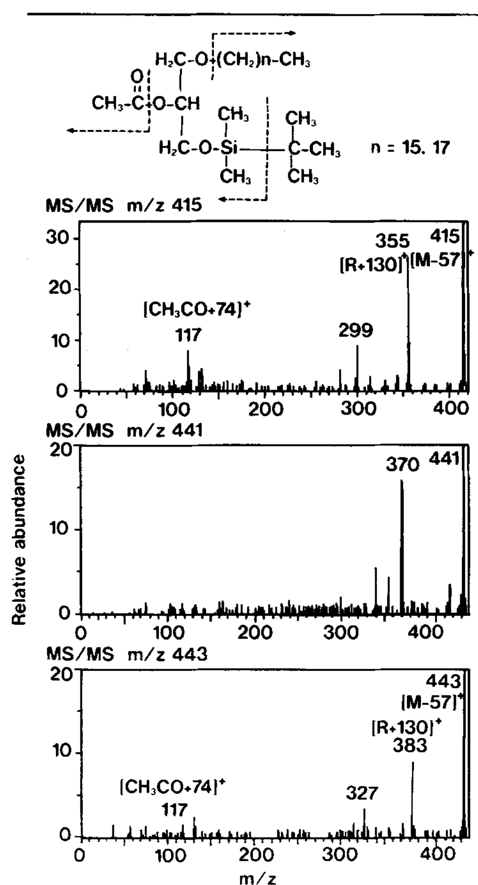

FIG. 3. MS/MS spectra of *t*-BDMS derivatives of antral 1-alkyl-2-acetyl-GPC in Experiment 2.

characteristic daughter ions for 1-octadecenyl-2-acetyl-3-*t*-BDMS-glycerol (Fig. 3). The observation indicates that 1-alkyl-2-acetyl-GPC was composed of 1-hexadecyl-2-acetyl-GPC and 1-octadecyl-2-acetyl-GPC. For the characterization of 1-acyl-2-acetyl-GPC, the ions at *m/z* 429, 455 and 457, due to $[M-57]^+$, and the ions at *m/z* 313, 339 and 341, due to $[RCO + 74]^+$, were selected. As shown in Figure 2, the peaks obtained by monitoring the ions at *m/z* 429, 455 and 457 were located at the same positions as those of authentic 1-hexadecanoyl-2-acetyl-GPC, 1-octadec-*cis*-9′-enoyl-2-acetyl-GPC, and 1-octadecanoyl-2-acetyl-GPC, respectively. Also, for the trace ions at *m/z* 313, 339 and 341, peaks were obtained at the same positions as those of 1-hexadecanoyl-2-acetyl-GPC, 1-octadec-*cis*-9′-enoyl-2-acetyl-GPC and 1-octadecanoyl-2-acetyl-GPC (data not shown).

Quantitation of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC in rat antrum. The amount of each molecular species was calculated from the ratios of their peak areas at $[M-57]^+$ relative to those of deuterated internal standards (1-hexadecyl-2-perdeuteroacetyl-GPC for measurement of 1-alkyl-2-acetyl-GPC and 1-heptadecanoyl-2-perdeuteroacetyl-GPC for measurement of 1-acyl-2-acetyl-GPC) using calibration curves (Figs. 1 and 2). In order to determine the amount of 1-hexadecyl-2-acetyl-GPC, the peak area at *m/z* $[CH_3CO + 74]^+$ relative to the internal standard was measured. The regression equations were $Y = 1.088X + 0.145$ and $Y = 0.583X + 0.121$ with correlation coefficients of $r = 1.000$ and 0.993 for 1-hexadecyl-2-acetyl-GPC and 1-octadecyl-2-acetyl-GPC, respectively, where X is the ratio by weight and Y is the ratio of peak areas. The regression equation for 1-hexadecanoyl-2-acetyl-GPC was $Y = 1.040X - 0.038$ with $r = 1.000$, which was coincident with the regression equation reported previously (14). Hence, for the calibration of 1-octadec-*cis*-9′-enoyl-2-acetyl-GPC and 1-octadecanoyl-2-acetyl-GPC, $Y = 0.871X - 0.044$ and $Y = 0.812X + 0.003$ were used as the regression equations, respectively, as reported previously (14). The results, presented as mol per mol total antrum phospholipids, are shown in Table 1. Thus, 1.9 ng of 1-alkyl [16:0 and 18:0]-2-acetyl-GPC and 15.6 ng of 1-acyl [16:0, 18:0 and 18:1]-2-acetyl-GPC were detected per rat antrum. The main species of 1-alkyl-2-acetyl-GPC was 1-octadecyl-2-acetyl-GPC, followed by 1-hexadecyl-2-acetyl-GPC. In contrast, the main species of 1-acyl-2-acetyl-GPC was 1-hexadecanoyl-2-acetyl-GPC, followed by 1-octadecenoyl-2-acetyl-GPC and 1-octadecanoyl-2-acetyl-GPC.

The composition of alkyl and acyl chains at the 1-position of choline and ethanolamine glycerophospholipids in perfused rat antrum and corpus. On analysis of choline and ethanolamine glycerophospholipids, rats were perfused with saline since it was necessary to remove phospholipids which might derive from blood cells and lipoproteins. As shown in Table 2, the composition of the diacyl, alkyl and alk-1′-enyl types of choline glycerophospholipids was quite different from that of ethanolamine glycerophospholipids in both the antrum and corpus. The amount of the alkylacyl type of both choline and ethanolamine glycerophospholipids was low (less than 5% of total phospholipids). The amount of the alkylacyl type in the antrum was about 2-fold greater than that in the corpus, which might be related to the localization of 1-alkyl-2-acetyl-GPC since the amount of PAF in the antrum was about 4-fold greater than that in the corpus (6). The alkyl chain distribution of choline glycerophospholipids in the antrum and corpus was 16:0, 18:0 and 18:1 with other components in less than trace amounts (Table 3). On the other hand, there was a significant difference in the fatty acid distribution between choline and ethanolamine glycerophospholipids. Whereas the predominant fatty acid in ethanolamine glycerophospholipids was 20:4 (28.9% in the antrum and 27.3% in the corpus), the predominant fatty acid in choline glycerophospholipids was 16:0 (25.8% in the antrum and 27.7% in the corpus) (data not shown). The acyl chain at the 1-position of diacyl type choline glycerophospholipids was composed mainly of 16:0 (36.3% in the antrum and 37.1% in the corpus) (Table 3), in contrast to the predominance of 18:0 (35.0% in the antrum and 28.8% in the corpus) in those of ethanolamine glycerophospholipids (data not shown).

Alteration in molecular species of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC associated with rat gastric ulcer induced by water-immersion stress. In a previous paper, we have reported that water-immersion stress for 1 hr and 3 hr, before the development of mucosal damage, produces a significant decrease in the gastric PAF level (39% of untreated controls), as determined by bioassay (6). The present study was done to confirm this observation physicochemically by GC/MS analysis. As shown

TABLE 1

Contents of Antral 1-Alkyl-2-acetyl-GPC and 1-Acyl-2-acetyl-GPC in Normal and Water-Immersion-Stressed Rats[a]

	Control		Water-immersion stress
	Experiment 1 9–10 wk old	Experiment 2 9 wk old	Experiment 3 9 wk old
	mol/mol total phospholipids		
1-Alkyl-2-acetyl-GPC			
1-Hexadecyl-2-acetyl-GPC	7.22×10^{-7} [b]	5.90×10^{-7} [b]	1.16×10^{-7} [b]
1-Octadecyl-2-acetyl-GPC	13.6×10^{-7}	11.3×10^{-7} [b]	7.00×10^{-7}
1-Octadecenyl-2-acetyl-GPC	not detected	not detected	not detected
1-Acyl-2-acetyl-GPC			
1-Hexadecanoyl-2-acetyl-GPC	not determined	8.20×10^{-6}	1.64×10^{-6}
1-Octadecanoyl-2-acetyl-GPC	not determined	1.94×10^{-6}	0.24×10^{-6}
1-Octadecenoyl-2-acetyl-GPC	not determined	3.60×10^{-6}	0.51×10^{-6}

[a]Rats used in experiments 1 and 2 are shown in Figures 1 and 2. In Experiment 3, rats weighing 233 + 17 g (189 animals), age 9 wk, were restrained in water maintained at 23°C ± 1°C for 1 hr. The antral lipid-extracts from 157, 81 and 189 animals were combined and analyzed in Experiments 1, 2 and 3, repsectively. The lipid-phosphorus contents were 484.4, 467.5 and 458.9 μg phosphorus/g antrum tissue in Experiments 1, 2 and 3, respectively.

[b]$[CH_3CO + 74]^+$ was selected for the quantitation.

TABLE 2

Composition of Choline and Ethanolamine Glycerophospholipids in Perfused Rat Antrum and Corpus[a]

Phospholipids	Antrum	Corpus
	mol%	
Ethanolamine glycerophospholipids[b]	26.8, 34.0	28.8, 31.8
Diacyl type[c]	56.8	74.4
Alkylacyl type[c]	9.4	4.8
Alk-1′-enylacyl type[c]	33.8	20.8
Choline glycerophospholipids[b]	44.9, 39.6	42.5, 40.5
Diacyl type[c]	92.1	95.1
Alkylacyl type[c]	5.4	2.5
Alk-1′-enylacyl type[c]	2.5	2.4

[a]Rats weighing 242 ± 17 g (means ± SD) (39 animals), age 9 wk, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and perfused with saline warmed at 37°C *via* the ventral aorta at a rate of 42.5 mL per min for 90 sec. The total lipids were extracted in a single phase of chloroform/methanol/water (1:2:0.8, v/v/v) in order to avoid cleavage of the alk-1′-enyl linkage. The lipid-phosphorus contents were 586.1 ± 13.5 μg phosphorus/g corpus tissue and 455.7 ± 27.0 μg phosphorus/g antrum tissue.

[b]The phospholipid fraction (60 μg phosphorus) after silic AR CC-7 column chromatography, as described in Material and Methods, was separated into phospholipid subclasses on a silica gel G plate (3 × 20 cm, 0.25 mm thick) (Analtech, Newark, NJ) in the solvent system chloroform/methanol/acetic acid/water (50:25:8:4, by vol). After spraying the plate with TNS reagent, PAF, phosphatidylcholine and phosphatidylethanolamine were scraped-off and extracted. Data are from combined samples from 19 and 20 rats.

[c]Diacyl, alkyl, and alk-1′-enyl types were determined as alkali-labile, alkali-stable and acid-stable, and alkali-stable and acid-labile phospholipids, respectively. Data are from combined samples from 39 rats.

in Figures 4-1 and 4-2, an alteration in the ratio of each molecular species of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC was observed 1 hr after commencement of stress. The levels of 1-hexadecyl-2-acetyl-GPC and 1-acyl [16:0, 18:0 and 18:1]-2-acetyl-GPC in the antrum (Experiment 3) decreased to 12–20% of those in untreated controls 1 hr after stress began (Experiment 2) (Table 1). Interestingly, the degree of decrease in the level of 1-octadecyl-2-acetyl-GPC was insignificant (62% of untreated control), in contrast to a remarkable decrease in the level of 1-hexadecyl-2-acetyl-GPC. Stress for 7 hr resulted in severe mucosal damage mainly in the corpus. The previous study, based on bioassay, demonstrated that the PAF content 7 hr after the stress was still decreased in the antrum (49% of the level in untreated controls), but was restored to normal in the corpus (6). The present SIM analysis revealed a remarkable alteration in the composition and amount of molecular species of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC during the 7-hr period of stress, as shown in Figures 4-3 and 4-4. In the corpus after 7 hr of stress, the amounts of 1-hexadecyl-2-acetyl-GPC and 1-hexadecanoyl-2-acetyl-GPC decreased. Surprisingly, the amount of 1-octadecyl-2-acetyl-GPC increased 4-fold (1.01×10^{-6} mol/mol total phospholipids). The EC_{50} values of 1-hexadecyl-2-acetyl-GPC and 1-octadecyl-2-acetyl-GPC for rabbit platelet activation ([^{3}H] serotonin release) were 1.0×10^{-10} M and 2.7×10^{-10} M, respectively. Therefore, the discrepancy between the results obtained by the bioassay and those by GC/MS analysis can be considered to result from the different biological activities of each of the molecular species of PAF.

DISCUSSION

In the present study we elucidated the molecular heterogeneity of PAF in the rat glandular stomach by GC/MS analysis. It was observed that odd-numbered 1-alkyl-2-acetyl-GPC eluted at the same position as even-numbered 1-acyl-2acetyl-GPC upon simultaneous

TABLE 3

Composition of Acyl and Alkyl Chains at the 1-Position of Choline Glycerophospholipids in Perfused Rat Antrum and Corpus After Digestion with Phospholipase A_2 and Treatment with Weak Alkali and Acid[a]

	Antrum		Corpus	
Chain length and number of double bonds	Acyl[b]	Alkyl[c]	Acyl[b]	Alkyl[c]
	weight%			
14:0	0.8, 2.1		0.3, 1.5	
14:1	2.8, 4.8		1.8, 1.5	
16:0	37.6, 35.0	42.0	34.1, 40.0	44.3
16:1	2.0, 1.3		0.5, 1.1	
18:0	21.8, 22.9	18.2	17.6, 15.6	16.7
18:1	15.0, 14.8	33.0	15.1, 14.1	32.4
18:2	7.6, 4.4		11.3, 9.5	

[a]For details, see legend to Table 2 and Materials and Methods.

[b]Data are from combined samples from 19 and 20 rats.

[c]Data are from combined samples from 39 rats.

analysis of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl GPC. In the SIM analysis of fractions corresponding to 1-alkyl-2-acetyl-3-*t*-BDMS-glycerol purified by TLC using hexane/diethyl ether (9:1, v/v), the peaks at 1′24″, 2′04″ and 2′12″ almost disappeared whereas the peaks at 1′07″ and 1′44″ were mostly retained. The observation indicates that there are only trace components, if any, of odd-numbered alkyl and acyl chains. As a consequence of this finding, it was possible to determine 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC simultaneously by SIM. The amount of 1-acyl [16:0, 18:0 and 18:1]-2-acetyl-GPC was about 8-fold greater than that of 1-alkyl [16:0 and 18:0]-2-acetyl-GPC (Table 1). A preponderance of 1-acyl-2-acetyl-GPC over 1-alkyl-2-acetyl-GPC is a common feature of tissues such as bovine brain (3) and rat uterus (4). However, this is not true in stimulated cells such as polymorphonuclear leukocytes, in which the amount of 1-alkyl-2-actyl-GPC is five times higher than that of 1-acyl-2-acetyl-GPC (24). There was a great degree of similarity in the alkyl chain distributions of 1-alkyl-2-acyl-GPC between the antrum and corpus (Table 3). Furthermore, the molecular heterogeneity of 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC in the corpus was quite similar to that in the antrum (Fig. 4). These observations suggest that the cells producing PAF in the corpus might be the same as those in the antrum. The acyl chain distribution at the 1-position of 1-acyl-2-acetyl-GPC remained more or less the same as that at the 1-position of 1,2-diacyl-GPC (Tables 1 and 3). However, the alkyl chain composition of 1-alkyl-2-acetyl-GPC did not seem to be dictated by that of 1-alkyl-2-acyl-GPC (Tables 1 and 3). Whereas 1-hexadecyl-2-acyl-GPC was the major molecular species of 1-alkyl-2-acyl-GPC (42% in the antrum and 44% in the corpus), 1-octadecyl-2-acetyl-GPC (66% in the antrum and 59% in the corpus) was the major molecular species of 1-alkyl-2-acetyl-GPC. This suggests that enzymes in PAF biosynthesis may have an extraordinary selectivity of alkyl chain utilization or that substrates located in an extraordinary position of stomach tissues (cells) may be utilized for PAF biosynthesis.

In a previous study (6), we found that water-immersion stress affected the gastric PAF content based on bioassay. The present GC/MS analysis revealed that such stress was associated with both qualitative and quantitative alterations in the molecular species of

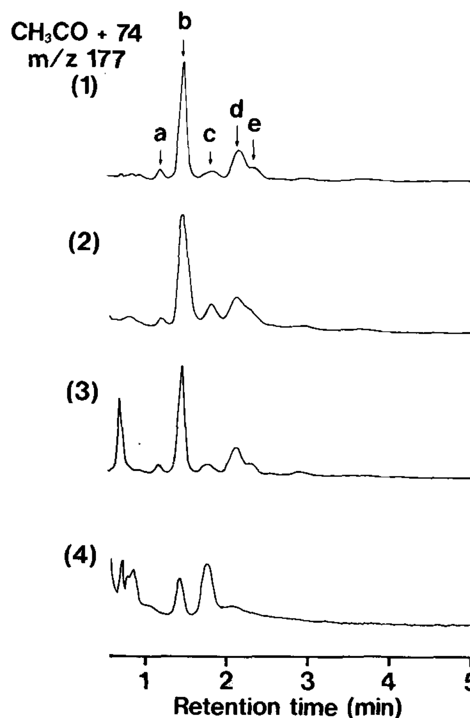

FIG. 4. SIM chromatograms of *t*-BDMS derivatives of gastric 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC in control and water-immersion-stressed rats. 1. Control rat antrum (Experiment 1); 2. Antrum from 1-hr water-immersion-stressed rat (Experiment 3); 3. Control rat corpus (Experiment 4); 4. Corpus from 7-hr water-immersion-stressed rat (Experiment 5). In Experiments 4 and 5, rats weighing 225 ± 31 g (26 animals) and rats weighing 228 ± 14 g (22 animals) (age 9 wk) were used, respectively. The lipid-phosphorus contents were 702.5 and 722.8 μg phosphorus/g corpus tissue in Experiments 4 and 5, respectively. Peaks of a, b, c, d and e are as in Figure 1.

1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC in both the antrum and corpus. 1-Hexadecyl-2-acetyl-GPC and 1-acyl [16:0, 18:0 and 18:1]-2-acetyl-GPC showed a significant decrease before the development of mucosal damage in the glandular stomach upon application of water-immersion stress for 1 hr (Table 1 and Fig. 4). By contrast, 1-octadecyl-2-acetyl-GPC increased markedly in the corpus during a 7-hr period of stress, which resulted in severe mucosal damage to the corpus, characterized by hemorrhage and inflammatory infiltrates. The increased amount of 1-octadecyl-2-acetyl-GPC in the corpus may have been produced by stimulated resident cells or circulating blood cells. However, rat polymorphonuclear leukocytes and rat basophilic leukemia cells, either unstimulated or stimulated, have been reported to produce 1-hexadecyl-2-acetyl-GPC as the predominant molecular species of PAF (25). The cells producing endogenous 1-alkyl-2-acetyl-GPC and 1-acyl-2-acetyl-GPC in the rat glandular stomach and their biological significance still need to be clarified. However, our present observations may help in elucidating the biological function of gastric PAF. 1-Hexadecyl-2-acetyl-GPC and 1-octadecyl-2-acetyl-GPC may possibly play different roles in the stomach. The qualitative and quantitative alteration of specific molecular species of PAF might be associated with the pathogenesis of gastric ulceration. In order to clarify these aspects, it will be necessary to understand the intrinsic biological function of each molecular species of PAF homologs and analogs.

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