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MODULATION BY DRUGS ACTING ON THE AUTONOMIC NERVOUS SYSTEM OF PLATELET-ACTIVATING FACTOR IN THE STOMACH OF RATS

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Abstract—Platelet-activating factor (PAF), an ether linked choline glycerophospholipid, is a potent initiator of diverse physiological and pathological processes. We have reported that gastric endogenous PAF levels were reduced and the contents of each of its molecular species changed during water-immersion stress in rats (Sugatani J *et al.*, *FASEB J* **3**: 65–70, 1989 and Sugatani J *et al.*, *Lipids* **26**: 1347–1353, 1991). In this study, we determined the effects of autonomic drugs on the level of gastric PAF, its molecular heterogeneity and formation of gastric erosions in unstressed rats and those subjected to water-immersion stress. Atropine, an anticholinergic drug, suppressed both the stress-induced changes and development of gastric lesions. 6-Hydroxydopamine-induced sympathectomy induced a small decrease in the gastric PAF levels and the addition of stress further decreased the PAF levels and development of gastric lesions. Carbamylcholine induced a transient decrease in the gastric PAF level of normal rats, which was not associated with gastric erosion formation. In contrast, the endogenous gastroprotective factor dopamine evoked transient dose- and time-dependent increases in the gastric PAF levels. These observations indicate that cholinergic muscarinic-receptor activation in rats led to decreases in gastric PAF levels and a prolonged and marked decrease in its level was associated with the development of gastric lesions, and that dopamine increases gastric PAF levels. Gastric endogenous PAF levels are closely associated with the autonomic nervous system and should be considered further in investigations of gastric function.

Platelet-activating factor (PAF§, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) [1, 2] has a wide spectrum of biological activities. It activates a variety of cells, induces systemic hypotension, increases vascular permeability and smooth muscle contraction and functions as a potent chemical mediator in pathophysiological processes [3]. Although exogenous PAF delivered to the stomach via the bloodstream is a potent ulcerogen [4], which causes extensive gastric mucosal damage and hemorrhage associated with septic shock [5], normal rat stomach contains a considerable amount of endogenous PAF [6, 7] and possesses characteristic enzymes for its metabolism [6, 8]. Endogenous PAF is also found in tissues, such as guinea-pig parotid gland and pancreas, chick retina, rat brain, fetal rabbit lung, human thymus, rat uterus and rabbit spermatozoa [9, 10]. The results of other studies suggest that PAF generated in polymorphonuclear leukocytes, eosinophils, monocytes/macrophages and endothelial cells is an important mediator of inflammation and allergic diseases [3, 11]. However, the role of endogenous PAF in normal tissues has yet to be elucidated fully.

In an earlier study, we found that the gastric endogenous PAF level and contents of various molecular species of PAF were changed under the condition of slight gastric mucosal damage caused by water-immersion stress for 1 hr [6, 7]. The effects of changes in the PAF levels on normal gastric mucosal function are, however, unknown. Disturbance of the gastric mucosal autonomic nervous system appears to be involved in gastric erosion induced by water-immersion stress [12], although the neuromodulator dopamine and dopamine-receptor agonists have been shown to protect the gastric mucosa against stress-induced ulceration [13]. Therefore, in this study, we investigated whether autonomic drugs affect the gastric PAF levels. We also determined the effect of dopamine on the gastric PAF level in normal and stressed rats.

MATERIALS AND METHODS

Experimental animals. Male Wistar rats (9 weeks old, weighing 220–260 g) were deprived of food, but not water, for 24 hr before carrying out the experiments. The water-immersion stress test was carried out by placing the animals in wire-mesh restraint cages and immersing them in water at

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§ Abbreviations: PAF, platelet-activating factor, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; acylPAF, 1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine; 16:0 acylPAF, 1-hexadecanoyl-2-acetyl-*sn*-glycero-3-phosphocholine; 18:0 acylPAF, 1-octadecanoyl-2-acetyl-*sn*-glycero-3-phosphocholine; 18:1 acylPAF, 1-octadecenoyl-2-acetyl-*sn*-glycero-3-phosphocholine; lysoPAF, 1-alkyl-*sn*-glycero-3-phosphocholine; 16:0 PAF, 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine; 18:0 PAF, 1-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine; 18:1 PAF, 1-octadecenyl-2-acetyl-*sn*-glycero-3-phosphocholine.

23 ± 1° up to the sternal midpoint for 1, 3 and 7 hr, as described by Takagi and Okabe [12]. The control rats were kept in cages at room temperature (about 23°) for 1, 3 and 7 hr. Atropine sulfate (Merck, Darmstadt, F.R.G.) dissolved in saline (0.3, 1, 2 and 4 mg/kg/2 mL), or saline (2 mL/kg), was injected subcutaneously (s.c.) 0.5 hr before experiments. To induce chemical sympathectomy, 6-hydroxydopamine hydrobromide [the Sigma Chemical Co., St Louis, MO, U.S.A.; 100 mg/kg/mL, injected intraperitoneally (i.p.)] dissolved in saline containing 5 mg/mL ascorbic acid to prevent autoxidation was administered for 3 consecutive days and the animals used for experiments on the fourth day [14]. On completion of each experiment the rats were killed by decapitation under diethylether anesthesia, the stomachs removed, opened along the greater curvature, rinsed thoroughly with cold isotonic saline solution and treated as described below.

Determination of stress-induced lesions. On completion of the stress test, the rats were killed and their stomachs removed, as described above, and then treated with formalin solution to fix the inner and outer gastric wall layers. The gastric mucosa was examined for lesions under a dissecting microscope (×10) and the length and width of each lesion were measured by two skilled observers, who were unaware of the experimental regimen. The total lesion area was calculated as the sum of the individual lesion areas. For histological evaluation, gastric mucosal tissues were fixed in buffered formalin, processed routinely and embedded in paraffin. Sections (3 μm thick) were cut and stained with hematoxylin and eosin for light microscopic examination. All assessments of gastric damage were performed without knowledge of the treatment regimen to avoid observer bias.

Extraction and purification of PAF from rat corpus and antrum. On completion of the relevant experiment, each rat was killed, the stomach removed promptly, as described above, and the corpus and antrum were separated and frozen rapidly by immersion in liquid nitrogen. The frozen tissues were weighed and homogenized in 10 vol. of $CHCl_3$:CH_3OH:glycine/HCl buffer (50 mM, pH 2.8) to inactivate PAF acetylhydrolase in an UltraTurrax homogenizer (IKA-WERK, Staifen, F.R.G.) for 1 min at 4°. The total lipids were obtained from the samples by extracting three times using a modified procedure of that described by Bligh and Dyer [15]. The supernatants were pooled and $CHCl_3$ and glycine/NaOH buffer (50 mM, pH 9.0) were added to produce a $CHCl_3$:CH_3OH:H_2O ratio of 1:1:0.9. The $CHCl_3$ phase was removed and an equal volume of $CHCl_3$ was added to the aqueous phase. After vigorously mixing, the $CHCl_3$ phase was separated and combined with the first $CHCl_3$ fraction and the lipid–phosphorus content was determined using Bartlett's method [16].

The lipids were applied to a Silic AR CC-7 column; the neutral lipids were removed with 30 mL of $CHCl_3$ and the phospholipids were eluted with 60 mL of CH_3OH followed by separation using TLC on silica gel 60 H with $CHCl_3$:CH_3OH:H_2O (65:35:6, v/v/v) as solvent. The PAF, which was located on the thin-layer plate between the areas corresponding to sphingomyelin and lysophosphatidylcholine, was detected by UV fluorescence after spraying the plate with 1 mM 6-*p*-toluidine-2-naphthalenesulfonic acid. The PAF fraction was scraped off and extracted, as described by Bligh and Dyer [15]. The recovery of PAF using these purification procedures was 79.0 ± 0.6% (mean ± SE of 125 different experiments), which was determined by radiotracer studies using 1-[^{3}H]hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine ([^{3}H]16:0 PAF, Du Pont-New England Nuclear, Tokyo, Japan).

Quantitation of PAF. PAF was measured by radioimmunoassay (RIA) using a scintillation proximity assay kit (Amersham International plc, U.K.) with PAF antisera of previously established specificity [17]. A problem with determining PAF in tissues is that substances contaminating the preparation, such as sphingomyelin and lysophosphatidylcholine, result in overestimation of the PAF content. Therefore, to correct for contaminants in our PAF preparation, we deacetylated samples with phospholipase A_2 from *Agkistrodon halys blomhoffii* (purified essentially as described by Kawauchi *et al.* [18] to homogeneity, assessed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis) in the presence of 1 mM $CaCl_2$. The reaction was stopped by adding ethylenediaminetetraacetic acid (13 mM, final concentration). Phospholipase A_2-treated or untreated samples (100 μL) or a known amount (0.03–2 pmol) of 16:0 PAF (Bachem AG, Bubendorf, Switzerland) were added, followed by 100 μL of 1-[^{3}H]octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine ([^{3}H]18:0 PAF, about 14,000 cpm; Amersham International plc), 100 μL of PAF antiserum in Tris–HCl buffer (50 mM, pH 7.4) containing 0.9% NaCl, 0.5% lactose, 0.1% gelatin and 100 μL of fluomicrosphere suspension and incubated in polyethylene vials on an orbital shaker at room temperature (about 23°) for 20 hr. Phospholipase A_2-treated and untreated and the 16:0 PAF samples containing [^{3}H]18:0 PAF and fluomicrospheres with protein A were dispersed in Tris–HCl buffer (50 mM, pH 7.4) containing 0.9% NaCl, 0.01% Triton X-100, 0.1% gelatin and 0.0057% thimerosal. The amount of radiolabeled PAF bound to the fluomicrospheres was determined by direct counting in a Packard Tri-Carb 460 liquid scintillation counter, keeping the window open from 0 to 999 for 4 min. The total binding (Bo) and non-specific binding (NSB) were 3300–3500 and 60–70 cpm, respectively. The actual PAF content was calculated by subtracting the value obtained after deacetylation of the sample from the apparent value of the untreated sample containing PAF plus contaminants. The amounts of corporal PAF in unstressed rats and those subjected to stress for 7 hr obtained using this RIA were 270 and 669 nmol/mol phosphorus of total lipids, respectively and those in unstressed and stressed (for 7 hr) rats, measured in nmol/mol phosphorus of total lipids by gas chromatography/mass spectrometry (GC/MS) analysis, were 101 and 61 for 16:0 PAF, 284 and 499 for 18:0 PAF and a trace and 285 for 1-octadecenyl-2-acetyl-*sn*-glycero-3-phosphocholine (18:1 PAF), respectively. As the sensitivity of the PAF antibody to 18:0 PAF is about half that to 16:0 PAF [17], these

data indicate that determination of PAF using this RIA was reliable with a degree of accuracy comparable to that obtained with GC/MS analysis.

Determination of PAF and 1-acyl-2-acetyl-sn-glycero-3-phosphocholine (acylPAF) by GC/MS coupled with selected ion monitoring. 1-Hexadecyl-2-perdeuteroacetyl-*sn*-glycero-3-phosphocholine and 1-heptadecanoyl-2-perdeuteroacetyl-*sn*-glycero-3-phosphocholine were synthesized by acetylating 1-hexadecyl-*sn*-glycero-3-phosphocholine (Bachem AG) and 1-heptadecanoyl-*sn*-glycero-3-phosphocholine (Serdary, Ontario, Canada) with perdeuterated acetic anhydride (d_6, Merck) in the presence of perchloric acid as a catalyst [19]. They were then added to the total lipids as internal standards (500 nmol perdeuterated compounds/mol phosphorus of total lipids) for measurement of PAF and acylPAF, respectively. The PAF was purified essentially as described by Oda *et al.* [20] by activated alumina (200 mesh) column chromatography followed by silica gel H TLC. The isolated PAF fractions were digested with *Bacillus cereus* phospholipase C and the resulting diglycerides were converted to *tert*-butyldimethylsilyl (*t*-BDMS) derivatives by incubation with *tert*-butyldimethylchlorosilane/imidazole reagent (Applied Science, State College, PA) at 110° for 10 min. The *t*-BDMS derivatives were purified further by silica gel H TLC (0.3 mm thick) using hexane/diethylether (9:1, v/v) as solvent. A JEOL JMS-DX300 instrument supplying 70 eV ionization energy 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 maintained at 240, 290, 290, 290 and 270° respectively and the helium gas flow rate was 55 mL/min. The amount of each molecular species was calculated from the ratio of its peak area to that of the deuterated internal standard (determined by monitoring the ions of $[CH_3CO + 74]^+$ formed by rearrangement of the acetyl residue and dimethyl silanol and $[M-57]^+$ produced by cleavage of the *tert*-butyl radical from the parent ion) using calibration curves, as described by Oda *et al.* [20].

Extraction and measurement of catecholamines. On completion of the relevant experiment, each rat was killed, the stomach removed promptly, as described above, and the corpus and antrum were separated and frozen rapidly by immersion in liquid nitrogen. The frozen tissues were weighed, homogenized in 5 vol. of 0.4 M perchloric acid in an Ultra-Turrax for 1 min at 4° and the catecholamines obtained by two extraction procedures, as follows. The homogenate was centrifuged (10,000 *g*, 30 min, 4°) and the supernatant stored at −70° until analysed. The catecholamines in the supernatant were isolated by alumina absorption and elution with 0.5 M acetic acid and then fractionated by HPLC (Nucleosil 5 C18 column, 150 × 4 mm to separate norepinephrine and epinephrine and 150 × 6 mm to separate dopamine) on a Hitachi 638-30 liquid chromatograph. The purified norepinephrine and epinephrine were measured fluorophotometrically using the trihydroxyindole method [21] and dopamine using the ethylenediamine method [22] with a Hitachi 650-10LC fluorescence spectrophotometer. The recoveries of known amounts of norepinephrine, epinephrine and dopamine with these assay systems were 91.9 ± 1.6%, 98.9 ± 0.2% and 97.2 ± 2.6%, respectively (means ± half-ranges of duplicate samples).

Statistical analysis. The data are expressed as means ± SE. Statistical significance was evaluated using unpaired Wilcoxon rank sum test to compare the scores of lesions and unpaired Student's *t*-test for other data. Differences at a probability value of less than 0.05 were considered to be significant.

RESULTS

Effect of atropine on changes in gastric levels of PAF and its molecular species and gastric erosion formation in water-immersion-stressed rats

In the corpus, stress for periods of 1 and 3 hr induced a decrease in the PAF levels to 30.5–34.6% of those in the control rats, but the levels returned to normal after 7 hr of stress (Fig. 1A). In the antrum, however, the decreased PAF level persisted during a 7-hr period of stress (13.6–18.9% of the control rat levels) (Fig. 1C). The lipid–phosphorus level did not change significantly in stressed rats compared with the controls, although stress for 1 and 3 hr caused slight stomach damage and for 7 hr resulted in severe mucosal damage characterized by hemorrhage and inflammatory cells infiltration, mainly in the corpus (Figs 1E and 2).

To elucidate the correlation between the reduction of the PAF level and development of gastric lesions, we examined the effect of an antiulcer drug atropine (0.3, 1, 2 and 4 mg/kg, s.c.). At a dose of 4 mg/kg, it suppressed the PAF level decreases markedly; the corporal and antral PAF levels in atropine-pretreated rats stressed for 1 hr were 70.4 and 61.3% of those in unstressed atropine-pretreated rats, respectively (Fig. 1B and D) and suppressed the formation of gastric erosions during a 7-hr period of stress completely (Fig. 2A and B).

We also investigated the effect of atropine on changes in the gastric contents of various molecular species of PAF and acylPAF in water-immersion-stressed rats. In control rats, the major molecular species of PAF in both the corpus and antrum was 1-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (18:0 PAF), followed by 16:0 PAF, and the major acylPAF species was 1-hexadecanoyl-2-acetyl-*sn*-glycero-3-phosphocholine (16:0 acylPAF), followed by 1-octadecanoyl-2-acetyl-*sn*-glycero-3-phosphocholine (18:1 acylPAF) and 1-octadecanoyl-2-acetyl-*sn*-glycero-3-phosphocholine (18:0 acylPAF), as reported previously [7]. After stress for 7 hr, the amount of corporal PAF, particularly 18:1 PAF, increased and that of acylPAF decreased to 64% of the control rat levels, whereas the amounts of antral 16:0 PAF and 16:0 acylPAF both decreased markedly (to less than 17% of those in the control rat levels, Table 1). The amounts of corporal PAF and acylPAF in rats pretreated with atropine (4 mg/kg) and then subjected to water-immersion stress for 7 hr were similar to those in unstressed atropine-pretreated rats. Atropine (4 mg/kg) had a similar

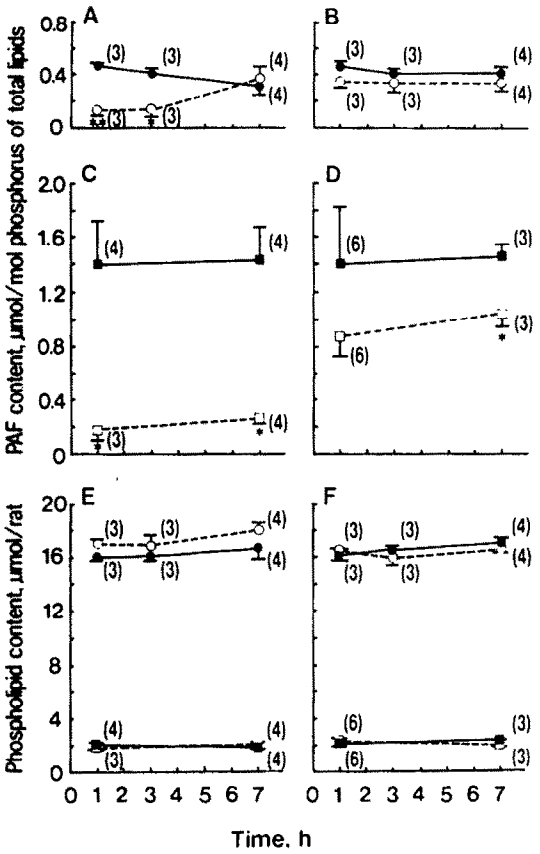

Fig. 1. Effects of water-immersion stress and pretreatment with atropine on the PAF and phospholipid contents of rat corpus and antrum. Atropine sulfate dissolved in saline (4 mg/kg/2 mL) or saline (2 mL/kg) was injected s.c. 0.5 hr before the onset of water-immersion stress. The amounts of PAF in pooled samples from at least two rats were determined by RIA, as described in Materials and Methods, and the amounts of phospholipids are expressed as the phosphorus content of the total lipids. Results are means ± SE of the numbers of experiments shown in parentheses. *P < 0.05, **P < 0.01, vs the corresponding control group (Student's *t*-test for unpaired data). (A), (C) and (E), vehicle-pretreated rats; (B), (D) and (F), atropine-pretreated rats; (—●—), control rat corpus; (--○--), water-immersion-stressed rat corpus; (—■—), control rat antrum; (--□--), water immersion-stressed rat antrum.

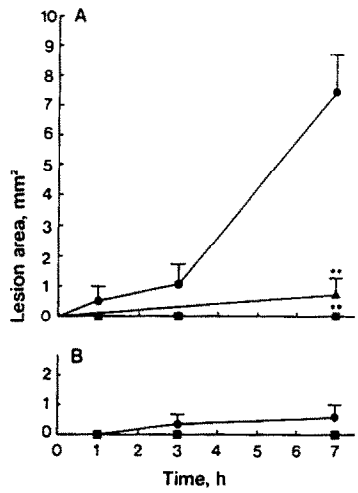

Fig. 2. Effect of atropine pretreatment on gastric mucosal lesions induced by water-immersion stress in rats. Atropine sulfate dissolved in saline (2 and 4 mg/kg/2 mL) or saline (2 mL/kg) was injected s.c. 0.5 hr before the onset of water-immersion stress. The erosion indices in the corpus (A) and antrum (B) are shown as total lesion areas (mm^2). Points and bars are means ± SE of four experiments. Treatment: (●), vehicle; (▲), atropine (2 mg/kg); (■), atropine (4 mg/kg). **P < 0.01, vs the corresponding vehicle-pretreated group (Wilcoxon rank sum test for unpaired data).

effect on changes in the antral contents of 16:0 PAF and 16:0 acylPAF.

Effect of 6-hydroxydopamine on gastric PAF levels and gastric erosion formation in water-immersion-stressed rats

Subjecting rats to water-immersion stress was associated with increases in the levels of norepinephrine, epinephrine and dopamine in stomach tissue (Table 2). In order to elucidate the relationship between increases in the catecholamine and dopamine levels and changes in the PAF levels (i.e. to determine the influence of the sympathetic nervous system on gastric PAF levels), we studied the effect of 6-hydroxydopamine, which induces chemical sympathectomy by destroying sympathetic nerve endings [23]. After administration of 100 mg/kg/day of 6-hydroxydopamine (i.p.) to rats for 3 days, the respective corporal norepinephrine and epinephrine contents were reduced to 13.2 and 20.0% and the antral contents to 7.6 and 30.4% of those in the control rats (Table 2). In contrast, the corporal PAF level was reduced to 53.0% and the antral to 56.3% of those in the control rats. Exposure of the 6-hydroxydopamine-pretreated rats to water-immersion stress for 1 and 3 hr resulted in a further decrease in the corporal and antral PAF levels (17.8–55.7% and 7.8–38.1% of those in unstressed 6-hydroxydopamine-pretreated rats, respectively, Fig. 3), whereas their norepinephrine and epinephrine contents remained low during stress in 6-hydroxydopamine-pretreated rats (4.9–14.9% of those in stressed control rats, Table 2). However, the gastric lesions were not aggravated in 6-hydroxydopamine-pretreated rats stressed for 1, 3 and 7 hr; the lesion areas after stress for 7-hr in rats pretreated with 6-hydroxydopamine or vehicle were 3.82 ± 0.95 mm^2 and 5.60 ± 1.26 mm^2 in the corpus and 0.60 ± 0.35 mm^2 and 0.63 ± 0.46 mm^2 in the antrum, respectively (N = 5).

Effect of carbamylcholine on gastric PAF levels and gastric erosion formation in normal rats

Next, we determined the effect of parasympathomimetic stimulation by carbamylcholine (0.8, 8 and 80 μg/kg, s.c.) on the gastric PAF levels. The greatest effect was observed with the 8 μg/kg

Table 1. Effect of atropine on corporal and antral contents of various molecular species of PAF and acylPAF in water-immersion-stressed rats

	Corpus*				Antrum†			
	Vehicle		Atropine		Vehicle		Atropine	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
	(nmol/mol phosphorus of total lipids)							
PAF								
16:0	101	61	235	269	883	Trace	476	293
18:0	284	499	336	336	ND	ND	ND	ND
18:1	Trace	336	Trace	Trace	ND	ND	ND	ND
AcylPAF								
16:0	2167	1110	1919	2074	13538	2278	6742	4665
18:0	37	42	92	97	ND	ND	ND	ND
18:1	230	404	467	218	ND	ND	ND	ND

The rats were kept in cold water or in the cage at room temperature for 7 hr. Atropine sulfate dissolved in saline (4 mg/kg/2 mL) or saline (2 mL/kg) was injected s.c. 0.5 hr before the onset of water-immersion stress.

* Results are for combined samples from 27–30 rats. The initial lipid–phosphorus contents were 430, 522, 466 and 465 μmol in vehicle-pretreated control rats (N = 30), vehicle-pretreated rats after 7 hr of stress (N = 30), atropine-pretreated control rats (N = 28) and atropine-pretreated rats after 7 hr of stress (N = 27), respectively.

† Results are for combined samples from 31–51 rats. The initial lipid–phosphorus contents were 63.9, 87.8, 62.2 and 102.2 μmol in vehicle-pretreated control rats (N = 36), vehicle-pretreated rats after 7 hr of stress (N = 47), atropine-pretreated control rats (N = 31) and atropine-pretreated rats after 7 hr of stress (N = 51), respectively.

ND, not done.

Table 2. Effect of water-immersion stress on gastric catecholamine and dopamine contents in control and chemically sympathectomized rats

		Corpus*		Antrum†	
	Stress (hr)	Control	Sympathectomy	Control	Sympathectomy
		(ng/g tissue)			
Norepinephrine	0	199.7 ± 39.5	26.3 ± 10.0‖	239.2	18.2
	1	226.9 ± 58.3	19.6 ± 4.6‖	288.2	14.1
	3	228.0 ± 48.5	18.9 ± 7.8‖	216.1	14.5
Epinephrine	0	2.5 ± 0.4	0.5 ± 0.0‖	2.3	0.7
	1	15.4 ± 1.4§	2.3 ± 0.6‖	18.7	2.2
	3	36.2 ± 5.1‡	4.0 ± 0.7‡‖	34.1	3.9
Dopamine	0	8.7 ± 0.7	8.0 ± 0.6	8.0	7.0
	1	21.4 ± 3.1‡	7.0 ± 1.0‖	17.0	5.0
	3	24.0 ± 1.5§	7.3 ± 0.9‖	10.0	5.0

* Results are means ± SE of three different experiments.

† Results are for pooled samples from five to six rats.

‡ $P < 0.05$ vs time 0 hr within group (Student's *t*-test for unpaired data).

§ $P < 0.001$ vs time 0 hr within group (Student's *t*-test for unpaired data).

‖ $P < 0.05$ vs the corresponding control group (Student's *t*-test for unpaired data).

dose, 30 min after administration of which the corporal and antral PAF levels (166 ± 22 and 817 ± 87 nmol/mol phosphorus of total lipids) decreased transiently to 44.0% and 51.8% of those in vehicle-treated rats (377 ± 65 and 1577 ± 254 nmol/mol phosphorus of total lipids) ($P < 0.05$, Fig. 4), respectively. However, 3 hr after carbamylcholine administration, the PAF levels were increased and after 7 hr they had returned to normal (Fig. 4). No gastric lesions developed in these rats during the 7-hr period after carbamylcholine administration. These results suggest that the PAF level reduction was not due to gastric lesion formation, but was associated with vagal fiber stimulation.

Effect of dopamine on gastric PAF levels and gastric erosion formation in normal and water-immersion-stressed rats

We also investigated the effects of dopamine on gastric PAF levels. The amount of gastric PAF in rats given dopamine (s.c.) was determined after removal of the intravascular blood components by

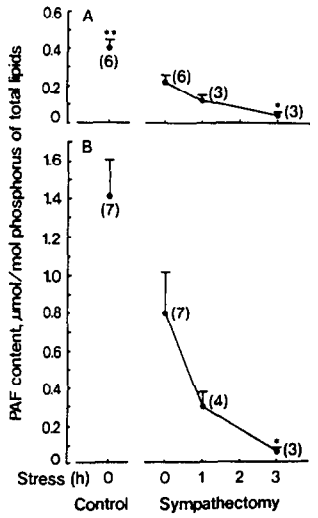

Fig. 3. Effect of 6-hydroxydopamine-induced sympathectomy on gastric PAF contents in water-immersion-stressed rats. The amounts of PAF were determined as μmol/mol phosphorus of total lipids by RIA, as described in Materials and Methods. Results are means ± SE of the numbers of experiments shown in parentheses. The respective gastric lipid-phosphorus contents in control rats and sympathectomized rats after 0, 1 and 3 hr of stress were respectively 16.00 ± 0.36, 14.68 ± 0.45, 15.17 ± 0.70 and 13.77 ± 0.58 μmol phosphorus/rat in the corpus (A) and 1.97 ± 0.09, 1.77 ± 0.06, 1.60 ± 0.03 and 1.88 ± 0.05 μmol phosphorus/rat in the antrum (B). *P < 0.05, **P < 0.01, vs unstressed sympathectomized group (Student's *t*-test for unpaired data).

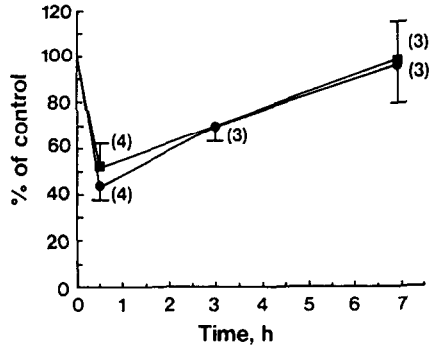

Fig. 4. Effect of carbamylcholine treatment on gastric PAF contents in normal rats. Carbamylcholine chloride (Sigma) dissolved in saline (8 μg/kg/2 mL) or saline (2 mL/kg) was injected s.c. and the stomach was removed at the indicated times. The PAF contents were determined by RIA, as described in Materials and Methods. Results are expressed as percentage change between vehicle- and carbamylcholine-treated rats at the indicated times. Numbers of experiments are shown in parentheses. The gastric lipid-phosphorus contents of vehicle- and carbamylcholine-treated rats did not differ significantly. (●), Corpus; (■), antrum.

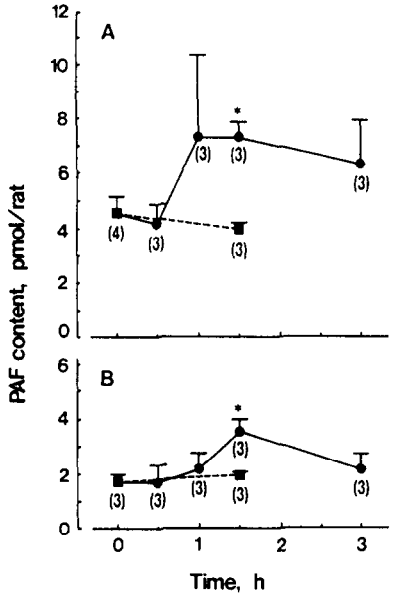

Fig. 5. Effects of dopamine treatment on gastric PAF contents. Dopamine hydrochloride (Sigma) dissolved in saline (3 mg/kg/2 mL, —●—) or saline (2 mL/kg, --■--) was injected s.c. At the indicated times, the rats were anesthetized with Nembutal (50 mg/kg, i.p.), laparotomized and perfused with saline at 37° via the descending aorta at a flow rate of 24 mL/min for 90 sec. The gastric lipid-phosphorus contents in control rats (0 hr) were 19.13 ± 0.63 μmol phosphorus/rat in the corpus (A) and 2.03 ± 0.22 μmol phosphorus/rat in the antrum (B). The weights and lipid-phosphorus contents of the corpus and antrum in vehicle- and dopamine-treated rats did not differ significantly. The PAF in pooled samples from two rats was determined by RIA, as described in Materials and Methods. Results are means ± SE of the numbers of experiments shown in parentheses. *P < 0.05 vs time 0 hr (Student's *t*-test for unpaired data).

perfusion with saline at 37°, in order to avoid any influence of PAF in blood. The PAF levels 1.5 hr after the administration of dopamine increased in a dose-related manner; they were 203 ± 12, 205 ± 13, 531 ± 154 and 730 ± 325 nmol/mol phosphorus of total lipids in the corpus and 1047 ± 229, 1086 ± 69, 1917 ± 27 and 1184 ± 83 nmol/mol phosphorus of total lipids in the antrum at doses of 0 (control vehicle), 1, 3 and 10 mg/kg, respectively (N = 2). Furthermore, the gastric PAF contents changed in a time-dependent manner after dopamine administration (Fig. 5). The corporal and antral PAF contents increased to 7.28 ± 0.62 pmol/rat (1.9-fold that of the vehicle-treated rats) and 3.56 ± 0.45 pmol/rat (1.8-fold that of the vehicle-treated rats), respectively 1.5 hr after administration of 3 mg/kg of dopamine.

The effects of dopamine on changes in the gastric PAF levels and gastric erosion formation were also studied in water-immersion-stressed rats. Subcutaneous administration of dopamine 30 min before the onset of stress suppressed the decreases in corporal and antral PAF levels in a dose-dependent

manner; in rats pretreated with 100 mg/kg of dopamine subjected to stress for 1 and 7 hr, the corporal PAF levels were 56 and 85% and the antral PAF levels were 76 and 41% of those in unstressed dopamine-pretreated rats, respectively. Furthermore, dopamine prevented the development of gastric lesions in a dose-dependent manner; the lesion areas after stress for 7 hr in rats pretreated with vehicle, or 30 and 100 mg/kg of dopamine were $7.54 \pm 1.25\ mm^2$, $3.35 \pm 1.16\ mm^2$ and $0.03 \pm 0.01\ mm^2$ ($P < 0.05$) in the corpus and $1.20 \pm 1.04\ mm^2$, $0\ mm^2$ and $0\ mm^2$ in the antrum, respectively ($N = 4$). Administration of 3 and 10 mg/kg of dopamine induced the transient increases in the gastric PAF levels as described above, but was not sufficient to prevent the decreases in the gastric PAF levels and gastric erosion formation during a 7-hr period of stress.

DISCUSSION

In a previous study, we found that the stomach of normal rats contained a considerable amount of endogenous PAF, the level of which decreased during water-immersion stress prior to the development of mucosal damage [6, 7]. There is evidence in rats that endogenous PAF in the central nervous system acts as a neurotransmitter/neuromodulator, stimulating the secretion of hypothalamic-pituitary-adrenal axis products [24, 25] and inducing neuronal differentiation [26]. PAF in the lobules of guinea pig exocrine secretory glands, such as the pancreas and parotid gland, has been reported to stimulate exocytosis [27]. However, little is known about the function of endogenous gastric PAF. Therefore, information on whether reduction of endogenous PAF is associated with the pathogenesis of gastric lesions and what factor(s) may be involved in changes in the levels of PAF and each of its molecular species could provide clues to the physiological role(s) of endogenous PAF in the stomach.

The present study revealed that application of 7 hr of water-immersion stress, at a stage of severe gastric erosion formation, affected not only the amounts of PAF and acylPAF but also their molecular heterogeneity in the stomach (Table 1). In rat stomach, the major molecular species of PAF and acylPAF are 18:0 PAF and 16:0 acylPAF, and acylPAF is predominant over PAF, which is likely to be a common feature in normal tissue PAF [28]. In contrast, in polymorphonuclear leukocytes, the major molecular species of PAF synthesized is 16:0 PAF followed by 18:1 PAF and 18:0 PAF, and PAF is predominant over acylPAF [29]. Although it is possible that PAF produced by inflammatory cells in pathological lesions partly affected these alterations, they might be due mainly to disturbance of PAF metabolism in the stomach itself. Endogenous PAF in normal tissues, such as chick retina [30], rat renal medulla [31], rat brain [32] and rat stomach [8], is synthesized by a *de novo* pathway in which 1-alkyl-2-acetyl-*sn*-glycerol is converted to PAF by a reaction catalysed by 1-alkyl-2-acetyl-*sn*-glycerol: CDP-choline cholinephosphotransferase. Furthermore gastric mucosa [8] has been reported to lack the acetyltransferase activity required for the remodeling pathway, in which 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine is hydrolysed initially by phospholipase A_2 to the intermediate 1-alkyl-*sn*-glycero-3-phosphocholine (lysoPAF) and free fatty acids (predominantly arachidonic acid) and then lysoPAF is acetylated at the 2-position by acetyltransferase to form PAF. The PAF acetylhydrolase activity in rat corporal and antral homogenates was not affected by water-immersion stress for 1, 3 and 7 hr [6]. These results suggest that the level of endogenous PAF may remain low and suppression of PAF biosynthesis may occur during prolonged periods of hypothermic stress, which are associated with sympathomimetic, parasympathomimetic and/or hypothalamic-pituitary-adrenal axis stimulation.

The pathogenesis of stress-induced gastric mucosal lesions has been suggested to be associated with the autonomic, in particular the parasympathetic, nervous system [33]. In fact, atropine, a muscarinic receptor antagonist, has been shown to suppress water-immersion stress-induced lesions [12]. In this study, we found that atropine prevented the stress-induced reduction in gastric PAF levels and changes in the contents of each molecular species of PAF (Fig. 1 and Table 1). The dose (4 mg/kg) of atropine that provided protection against the gastric lesions was similar to that which inhibited the reduction in the endogenous gastric PAF levels (Figs 1 and 2). When the contribution of the peripheral sympathetic nervous system was investigated, treatment with 6-hydroxydopamine induced significant reductions in the gastric norepinephrine, epinephrine and dopamine contents (Table 2) and slight reductions in that of endogenous gastric PAF (Fig 3). In addition, treatment of spontaneously hypertensive rats with 6-hydroxydopamine has been reported to result in the enhancement of gastric choline acetyltransferase activity [34]. The exposure of the sympathectomized rats to stress elicited a further decrease in the gastric PAF levels (Fig. 3), whereas the muscarinic agonist carbamylcholine (8 μg/kg), which did not induce gastric lesions, resulted in a transient decrease in the gastric PAF levels of normal rats (Fig. 4). These findings indicate that the decreases in the endogenous PAF levels resulted from enhancement of the gastric cholinergic, muscarinic-receptor mediated mechanism(s), which may be associated with physiological functions such as gastric HCO_3^- secretion [35], glycoprotein secretion [36] and stomach tissue contraction [37].

The total amount of PAF in the corpus (6.42 ± 1.98 pmol/rat, $N = 10$) was about 2.3-fold that in the antrum (2.84 ± 1.28 pmol/rat, $N = 8$) and stress-induced reductions in the corporal PAF content (3.98–5.23 pmol/rat) were greater than those in the antrum (2.08–2.57 pmol/rat) (Fig. 1). After prolonged reduction of the gastric PAF levels, mucosal damage occurred mainly in the corpus. Pretreatment with atropine and dopamine suppressed the reduction in the gastric PAF levels and the concomitant lesion development induced by stress, whereas the transient decrease in the gastric PAF levels induced by carbamylcholine (8 μg/kg) was not associated with gastric lesion development. These observations suggest that a prolonged decrease in the gastric PAF levels (to 19–29% of those in the

control rats) may be one of the factors associated with gastric lesion development. In contrast, intravenous infusion of PAF at a dose of 4.8 pmol/min for 10 min to rats has been reported to induce dysfunction of the gastric microcirculation [4], characterized by ischemia with congestion [38], leading to lesion formation. PAF antagonists CV3988, BN52021 and Ro193704 inhibited the gastrointestinal damage induced by intravenous infusion of PAF [39]. Macroscopically visible gastric damage induced by water-immersion stress was significantly reduced by the PAF structural analog CV3988 (40 mg/kg, i.v., about 85% inhibition) [40, 6], whereas PAF analogs structurally dissimilar to PAF, WEB2086 (30 mg/kg, i.v.) and BN52021 (50 mg/kg, p.o.), exhibited no protective effect [41, 42]. In order to interpret the discrepancy between the effects of PAF antagonists, we hypothesize that endogenous PAF is essential to the regulation of gastric function and CV3988 may function partly as a PAF agonist in water-immersion-stressed rats. On the other hand, WEB2086 administered at a dose of 5×10^{-5} M in the drinking water for 16 days (Sugatani *et al.*, unpublished result) or BN52021 administered orally at a dose of 50 mg/kg [42] did not aggravate the stress-induced lesions and did not cause gastric injury. These structurally dissimilar PAF antagonists can act on target cells of exogenous PAF, but they may be unable to prevent the function of endogenous PAF and/or intracellular PAF in the stomach. In other words, the mode of action of gastric endogenous PAF may be different from that of exogenous PAF [43].

In this study, we found that dopamine (3–10 mg/kg) induced increases in the gastric PAF levels. It has been suggested that dopamine protects the rat gastric mucosa against stress- or ethanol-induced gastric lesions via inhibition of gastric motor activity mediated by α_2-adrenoceptor stimulation [44, 45]. The dose (3–10 mg/kg) of dopamine that provided protection against gastric lesions induced by ethanol and inhibiting gastric motor activity in rats was similar to that which enhanced the endogenous gastric PAF levels. It has yet to be determined whether endogenous PAF is involved in the gastric cytoprotective action of dopamine. However, our results indicate that the level of endogenous gastric PAF is profoundly affected by the autonomic nervous system and endogenous PAF may have a physiological role in the stomach. We are now studying the possible functions and mechanism(s) of action of endogenous PAF in the stomach.

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