Synthesis of 1-O-Alkyl-2-acetylglycero-3-phosphocholine (Platelet-activating Factor) in Exocrine Glands and Its Control by Secretagogues*

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1-O-Alkyl-2-acetylglycero-3-phosphocholines (platelet-activating factor (PAF)) stimulate exocytosis in isolated lobules from guinea pig parotid glands or pancreas by an acetylcholine-like mechanism (Soling, H. D., Eibl, H. J., and Fest, W. (1984) Eur. J. Biochem. 144, 65–72). We show here that both tissues are able to synthetize PAF themselves. Isolated guinea pig parotid gland acini incorporate labeled acetate into the 2-position of PAF. Stimulation with A23187 or carbamoylcholine lead to a significant stimulation of this process. The newly synthetized PAF is partially released into the medium. Addition of lyso-PAF to the incubation medium does not significantly affect the rate of incorporation of labeled acetate into PAF in the absence or presence of carbamoylcholine. Isolated pancreatic lobules are also able to incorporate labeled acetate into PAF, and cholecystokinin and caerulein lead to a strong stimulation of this process. Incorporation of radioactive lyso-PAF into PAF, but not into 1-O-alkyl-2-long chain acyl-sn-glycero-3-phosphocholine was also significantly stimulated by carbamoylcholine in isolated parotid acini. Under these conditions, the time-dependent stimulation of amylase release paralleled that of lyso-PAF incorporation into PAF. The same holds for the concentration dependency of the carbachol effect on these two parameters. In isolated pancreatic lobules, caerulein also stimulated the incorporation of lyso-PAF into PAF. Pulse-chase experiments with radioactive lyso-PAF indicate that stimulation of incorporation of radioactively labeled PAF into PAF represents increased net synthesis of PAF rather than increased PAF-turnover. Using the platelet aggregation test, substantial amounts (0.79 nmol/g) of PAF could be determined in isolated acini from guinea pig parotid glands.

As these compounds stimulate secretion and aggregation of rabbit platelets at concentrations of 10⁻¹¹ M and lower, they were given the name “platelet-activating factor” (PAF) (1, 6).

However, more recently, it has been shown that PAF also has effects on smooth muscle (7) and on liver metabolism (8). In our experiments, we could demonstrate that PAF can stimulate exocytosis in exocrine glands like the parotid gland and the exocrine pancreas (9). We could also show that PAF had more or less exactly the same effects as carbamoylcholine, an acetylcholine analogue, in that it increased 40Ca⁺ influx, the turnover of polyphosphoinositides, and the formation of diacylglycerol and inositol 1,4,5-trisphosphate (9). In view of the increasing number of extra platelet effects of PAF, we have questioned the justification of the name platelet-activating factor as it seems more likely that PAF represents, in addition to the biologically active products of arachidonate metabolism, another group of lipid metabolites with wide spread biological activity. Nevertheless, the question remained whether PAF might be synthetized by the above-mentioned blood cells followed by transport via the bloodstream to the target organs or whether the organs themselves might be able to synthetize PAF for their own requirements. In the latter case, how might PAF synthesis in the organ be regulated? We have therefore determined the amount of PAF and studied the synthesis of PAF in intact guinea pig parotid gland acini. Acini were chosen instead of lobules in order to avoid as much as possible contamination of the preparation with PAF-synthetizing blood cells.

The results show that guinea pig parotid gland acinar cells do not only contain substantial amounts of biologically active PAF-like material, but are also able to synthetize PAF. The synthesis is stimulated strongly by a calcium ionophore and by the acetylcholine analogue carbamoylcholine. Stimulation of PAF synthesis occurs also in the exocrine pancreas following stimulation by cholecystokinin or caerulein.

EXPERIMENTAL PROCEDURES

Methods—Male guinea pigs (250–320 g body weight) were obtained from Winkelmann, Dormbach, FRG. They were fed ad libitum a standard laboratory diet (Altromin R) supplemented with ascorbic acid. Parotid gland lobules were prepared according to Leslie et al. (10). The lobules were used for the preparation of acini according to Schmidt et al. (11). Microscopically, the preparation contained almost exclusively parotid acinar cells. When a sedimented preparation of

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1The abbreviations used are: PAF, platelet-activating factor; 1-O-alkyl-2-acetylglycero-3-phosphocholines; lyso-PAF, 1-O-alkylglycero-3-phosphocholines; 1-0-alkyl-PC, 1-0-alkyl-2-long chain acyl-sn-glycero-3-phosphocholines; Hepes, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.
Incorporation of Radioactive Acetate into PAF—Parotid acini were incubated under 100% O₂ in minimal essential Eagle's medium for 60 min, pancreatic lobules in Krebs-Ringer-Tris buffer containing 5 mM glucose and 200 µCi of carrier-free [³H]acetate. Agonists were added 2 min later. After 20 min of incubation in the presence of 100 µg of PAF, the acini were homogenized in glass/glass Potter homogenizers. The chloroform acid-treated samples were centrifuged, and the pellets were resuspended in 3 ml of chloroform/methanol (1:2, v/v). After vigorous vortexing, 1 ml of chloroform and 1 ml of H₂O were added to all samples to achieve phase separation. The chloroform phase was removed and evaporated under N₂. This treatment reduced contamination with remaining [³H]acetate to less than 0.5% of the radioactivity in total lipids. The dried material was dissolved in 50 µl of chloroform, and an aliquot was removed for the determination of [³H]radioactivity (= radioactivity in "total lipids"). To the remaining fraction, 20 µg of 1-O-hexadecyl-2-acyl-sn-glycero-3-phosphocholine was added, and the sample was applied to silica gel thin layer plates. The plates were developed in a mixture of chloroform/methanol/acet acid (10:60:50 v/v/v). The lipid fractions were stained by exposure to iodine vapor. The spots corresponding to PAF were scraped off, re-extracted with chloroform/methanol/H₂O (1:2:0.8; v/v/v), and dissolved in 0.5 ml of ethyl ether/ethanol (98:2; v/v), followed by the addition of 0.2 ml of 25 mM Tris-Cl, 30 mM CaCl₂, and 1000 units of phospholipase C. The mixture was incubated for 3 h at 37 °C. Ether was removed by evaporation, and 1 ml of chloroform was added. After vortexing and centrifugation, the lower phase was removed. This was repeated twice. The combined chloroform phases were dried under N₂, dissolved in 20 µl of CHCl₃/CH₃OH (1:2, v/v), and applied again to a silica thin layer plate which was developed in heptane/diisopropyl ether/acetic acid (60:40:4, v/v). Staining with 2,7-dichlorofluorescein revealed that, in this system, long chain diacylglycerols were completely separated from 1-O-alkyl-2-acyl-glycerol. (The Rf values were as follows: 1,3-diacylglycerol 0.25 ± 0.01, 1,2-diacylglycerol 0.23 ± 0.01, 1-O-alkyl-2-acylglycerol, 0.17 ± 0.005; monoacylglycerols, 0.04 ± 0.008.) The spots corresponding to 1-O-alkyl-2-acylglycerol were removed and extracted as described above. After transfer to the upper part (flask) of a Bartley microdistillation vessel (13), the material was dried under N₂ and redissolved in 0.2 ml of ethanol. 2 M KOH (0.5 ml) was added, and the mixture was kept at room temperature for 1 h. After this incubation period, 0.5 mM sodium citrate, pH 3.0 (0.5 ml), and 2 M HCl (0.49 ml) were added next, and the mixture was immediately frozen in dry ice/ether. 1 M acetic acid (10 µl) was added as carrier material. To the lower part of the distillation apparatus, 0.2 ml of 2 M KOH was added and similarly frozen. The two parts of the microdistillation apparatus were connected, and the whole device was kept for 90 min at −80 °C in a freezer. Microdistillation of acetic acid was then performed as described by Bartley (13). Recovery of acetate during the microdistillation procedure was determined with [³H]acetate and found to be between 97 and 99%. It should be noted that PAF synthesis refers always to the radioactive PAF found in the mixture of cells or lobules plus incubation medium, except in the experiments given in Fig. 1. Lactate dehydrogenase activity was determined at 334 nm and 30 °C under the following conditions (final concentrations): triethanolamine chloride, 0.2 M, pH 7.6, NADH, 0.36 mM, pyruvate, 10 mM.

Incorporation of Radioactive Lyso-PAF into PAF and 1-O-Alkly-2-acyl-sn-glycero-3-phosphocholine (1-O-AKP)—The preparation and incubation of isolated guinea pig parotid acini or pancreatic lobules were performed as given above. The experimental details are presented in Figs. 2 and 3. Incorporation of lyso-PAF into PAF was measured by isolation of the 1-O-[³H]octadecyl-1-acylglycerol following phospholipase C treatment.

Extraction of PAF from Acini from Guinea Pig Parotid Glands and Determination of Biological PAF Activity in a Platelet Aggregation System—Acini were homogenized in 0.1 M NaCl, 0.01 M sodium cacodylate, pH 7.4, and centrifuged for 10 min at 13,600 g, and used for the preparation of phospholipase C. Phospholipase C from Bacillus cereus (800 units/mg) and carboxyzylocine were purchased from Sigma. A23187 from Calbiochem. Caerinlein was from Serva, Heidelberg, FRG, cholecystokinin from Kabi, Stockholm, Sweden. Thin layer plates (Silica 60) and all chemicals not mentioned were obtained from Merck.

Materials—1-O-Hexadecyl-2-acyl-sn-glycero-3-phosphocholine and 1-O-hexadecyl-2-acyl-sn-glycerophosphorylcholine were synthesized as reported previously (9) and kindly donated by Dr. H. Eibl, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, FRG. 1-O-Hexadecyl-2-acyl-sn-glycero-3-phosphocholine was obtained from 1-O-hexadecyl-2-acyl-sn-glycerophosphorylcholine by treatment with phospholipase C. Phospholipase C from Bacillus cereus (800 units/mg) and carboxylzylocine were purchased from Sigma. A23187 from Calbiochem. Caerinlein was from Serva, Heidelberg, FRG, cholecystokinin from Kabi, Stockholm, Sweden. Thin layer plates (Silica 60) and all chemicals not mentioned were obtained from Merck.

PAF Synthesis in Exocrine Glands

Fig. 1. Distribution of newly formed radioactive PAF between cells and extracellular space in experiments with isolated guinea pig parotid gland acini. The acini were prepared and incubated in the presence of [³H]acetate as given under "Methods." They were stimulated with 10⁻⁶ M carbamoylcholine. Twenty min later, the acinar cells were sedimented by centrifugation for 5 min at 200 × g. PAF was separately extracted from the sedimented cells and the supernatants and analyzed for incorporation of [³H]acetate into the 2-position of PAF. The columns give the radioactivity incorporated within 20 min per 50 mg of wet tissue and represent mean values from three experiments. The asterisks mark the results from single experiments. A, supernatant from unstimulated cells; B, supernatant from stimulated cells; C, unstimulated sedimented cells; D, stimulated sedimented cells.

The biological sample to 270 µl of platelet-rich plasma. Materials—1-O-Hexadecyl-2-acyl-sn-glycero-3-phosphocholine and 1-O-hexadecyl-2-acyl-sn-glycerophosphorylcholine were synthesized as reported previously (9) and kindly donated by Dr. H. Eibl, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, FRG. 1-O-Hexadecyl-2-acyl-sn-glycero-3-phosphocholine was obtained from 1-O-hexadecyl-2-acyl-sn-glycerophosphorylcholine by treatment with phospholipase C. Phospholipase C from Bacillus cereus (800 units/mg) and carbamoylcholine were purchased from Sigma. A23187 from Calbiochem. Caerinlein was from Serva, Heidelberg, FRG, cholecystokinin from Kabi, Stockholm, Sweden. Thin layer plates (Silica 60) and all chemicals not mentioned were obtained from Merck. [³H]Acetate (4 Ci/mmole) and 1-O-[³H]octadecyl-lyso-PAF (80–120 Ci/mmole) came from Amersham. The PAF antagonist SR16-072 was a kind gift of Dr. D. A. Handley, Sandoz Research Institute, Sandoz Inc., East Hanover, NJ.
RESULTS

Experiments with Guinea Pig Parotid Gland Acini—We have examined the effects of the divalent cation ionophore A23187 and the acetylcholine analogue carbamoylcholine on the incorporation of \(^{3}H\)acetate into the 2-position of PAF and compared this with the incorporation obtained in the absence of these compounds.

The results are summarized in Table I. Acetate was incorporated into PAF already under control conditions indicating a permanent turnover of this compound. The incorporation was between 0.9 and 3% of the incorporation into total lipids. As we do not know the specific radioactivity of the intracellular acetyl-CoA pool over the incubation time, we cannot quantify the absolute rate of PAF synthesis under our conditions. The incorporation of acetate into total lipids was not significantly affected by the agonists tested, indicating that the changes in PAF synthesis observed had not resulted from shifts in the specific radioactivity of the acetyl-CoA precursor pool but represented true changes of PAF synthesis or turnover.

The ionophore A23187 at a concentration of \(2 \times 10^{-6} \text{ M}\) more than tripled the incorporation of acetate into PAF. Carbamoylcholine at a concentration of \(5 \times 10^{-5} \text{ M}\) was almost as effective as the ionophore.

Theoretically, stimulation of PAF synthesis can be enhanced not only by increasing the incorporation of acetate into lyso-PAF, but also by increasing the supply of lyso-PAF. Were formation of lyso-PAF the rate-limiting step, addition of external lyso-PAF by itself should stimulate incorporation of acetate into PAF and diminish the degree of stimulation by carbamoylcholine. We have, therefore, conducted experiments in which we incubated parotid acini first in the presence of 50 \(\mu\text{M}\) 1-O-hexadecyl-sn-glycero-3-phosphocholine (lyso-PAF) before adding radioactive acetate plus or minus \(10^{-5} \text{ M}\) carbamoylcholine. As summarized in Table II, the addition of lyso-PAF did not significantly affect the incorporation of \(^{3}H\)acetate into PAF, and the absence or presence of carbamoylcholine, although exogenous lyso-PAF is immediately entering cellular metabolism (see below). The results indicate that the guinea pig parotid acinar cell is not only responsive to PAF with respect to a variety of parameters including exocytosis (9), but also is able to synthesize this biologically active compound itself.

We have also studied whether PAF labeled in the 2-position with \(^{3}H\)acetate during stimulation with carbamoylcholine remains inside the cells or whether at least some of the synthesized PAF leaves the cell. For this purpose, the acini were incubated as described under "Methods." \(^{3}H\)Acetate was added 2 min before adding the stimuli. The incubations were stopped 20 min after the beginning of the stimulation. Results from three separate experiments are shown. The numbers in parentheses give the relative radioactivity incorporated (percent of control).

\[\text{PAF Synthesis in Exocrine Glands}\]

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<table>
<thead>
<tr>
<th>Experiment</th>
<th>Radioactivity in 1-O-alkyl-2-acetyl-PC (PAF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>dpm/200 mg wet wt</td>
</tr>
<tr>
<td>Cholecystokinin</td>
<td></td>
</tr>
<tr>
<td>Caerulein</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1739 (189)</td>
</tr>
<tr>
<td>2</td>
<td>3467 (321)</td>
</tr>
<tr>
<td>3</td>
<td>3298 (388)</td>
</tr>
</tbody>
</table>

\(\ast\) The double values in the experiments with caerulein represent separate preparations of pancreatic lobules.

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TABLE II

<table>
<thead>
<tr>
<th>Effect of exogenous lyso-PAF on the incorporation of radioactive acetate into the 2-position of PAF in isolated guinea pig parotid gland acini</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-0-Cie-Lyso-PAF (final concentration, 50 (\mu\text{M})) was added 5 min before, (^{3}H)acetate 2 min before stimulation with 10^{-5} \text{ M} carbamoylcholine. The incubations were stopped 20 min after the addition of carbamoylcholine. Mean values ± S.D. from five separate experiments. The numbers in parentheses give the relative incorporation of radioactivity as percent of control which was set as 100%.</td>
</tr>
<tr>
<td>Radioactivity in 1-O-alkyl-2-acetyl-PC (PAF)</td>
</tr>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>dpm/50 mg wet tissue</td>
</tr>
<tr>
<td>3,200 ± 480</td>
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TABLE III

<p>| Effect of cholecystokinin (0.08 (\mu\text{M})) and caerulein (0.08 (\mu\text{M})) on the incorporation of radioactive acetate into the 2-position of PAF in experiments with isolated guinea pig pancreatic lobules |
|---|---|---|
| Pancreatic lobules were prepared and incubated as given under &quot;Methods.&quot; (^{3}H)Acetate was added 2 min before adding the stimuli. The incubations were stopped 20 min after the beginning of the stimulation. Results from three separate experiments are shown. The numbers in parentheses give the relative radioactivity incorporated (percent of control). |</p>
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Radioactivity in 1-O-alkyl-2-acetyl-PC (PAF)</th>
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<tbody>
<tr>
<td>Control</td>
<td>dpm/200 mg wet wt</td>
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<tr>
<td>Cholecystokinin</td>
<td></td>
</tr>
<tr>
<td>Caerulein</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1711 (186) (192) (193)</td>
</tr>
<tr>
<td>2</td>
<td>2408 (223) (269) (262)</td>
</tr>
<tr>
<td>3</td>
<td>2831 (353) (403) (357)</td>
</tr>
</tbody>
</table>

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Radioactivity recovered in the total lipid fraction amounted to 240,000 dpm/50 mg of wet tissue and was not affected by the different agonists used. Accordingly, incorporation of \(^{3}H\)acetate into the 2-position of PAF amounted to about 2.3% of the incorporation into total lipids. The values given in the table represent mean values ± S.D. The numbers in parentheses give the relative radioactivity (percent of control).

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Exocrine Glands

To examine further our working hypothesis that receptor-mediated regulation of PAF synthesis is a quite general phenomenon, we have studied the effects of cholecystokinin and caerulein on the incorporation of \(^{3}H\)acetate into PAF in isolated guinea pig pancreatic lobules. In this case, we have renounced the use of acini as the existence of receptors for cholecystokinin or caerulein on any type of blood cells seemed highly unlikely. As indicated in Table III, both agonists en-
enhanced significantly the incorporation of $[^3]$Hacetate into the 2-position of PAF.

We have also measured the incorporation of radioactive lyso-PAF into PAF and into 1-O-alkyl-PC. The results obtained with isolated parotid gland acini are depicted in Fig. 2. Carbamoylcholine (10^{-5} M) enhanced in all four experiments the incorporation of lyso-PAF into PAF. During 15 min following carbamoylcholine, the incorporation of radioactive lyso-PAF increased by 303 ± 21%, whereas in the absence of the agonist the incorporation increased only by 42 ± 13% (mean values ± S.D.). This is equivalent to a more than 7-fold increase. As demonstrated also in Fig. 2, carbamoylcholine did not affect the incorporation of labeled lyso-PAF into 1-O-alkyl-PC.

Assuming that the stimulation of PAF synthesis by carbamoylcholine proceeds via occupation of the same receptors as during stimulation of amylase release, one would expect a similar time and dose relationship for amylase secretion and for PAF synthesis. As indicated in Fig. 3, a and b, this is indeed the case.

The possible contribution of an increased PAF turnover to the increased incorporation of radioactive lyso-PAF into PAF in the presence of carbamoylcholine was studied in the following way. Isolated parotid acini were incubated with $[^14]$Clyso-PAF and stimulated with 10^{-5} M carbamoylcholine for the indicated times. For details of incubation and analysis of $[^3]$HPAF formation, see “Experimental Procedures.” Open circles, PAF synthesis; closed circles, amylase secretion.

**Fig. 2.** Incorporation of radioactive 1-O-octadecyl-lyso-PAF into PAF and 1-O-alkyl-2-sn-long chain acyl-glycero-3-phosphocholine (1-O-alkyl-PC) by isolated guinea pig parotid gland lobules. After a preincubation of 30 min, acini (equivalent to about 50 mg wet weight/ml) were transferred to incubation vessels containing 10 μCi of 1-O-[3H]octadecyl-lyso-PAF. The incubation medium contained 0.1% (w/v) bovine serum albumin. After 2 min in the presence of radioactive lyso-PAF, carbamoylcholine (10^{-5} M) was added to one sample, a second sample served as control. Aliquots were removed immediately before and 5 and 15 min after addition of carbamoylcholine and analyzed for incorporation of radioactive lyso-PAF into PAF and into 1-O-alkyl-PC. The mean counts in PAF and in 1-O-alkyl-PC at zero time were 8,666 and 36,667 dpm/50 mg wet weight, respectively. The results of four independent experiments are shown.

**Fig. 3.** a, time course of release of amylase and incorporation of radioactive lyso-PAF into PAF following stimulation of isolated guinea pig parotid gland acini with carbamoylcholine. The points represent mean ± S.E. from three experiments. Acini equivalent to about 35 mg wet weight were incubated in the presence of 5 μCi of carrier-free 1-O-[3H]octadecyl-lyso-PAF and stimulated with 10^{-5} M carbamoylcholine for the indicated times. For details of incubation and analysis of $[^3]$HPAF formation, see “Experimental Procedures.” b, dependency of amylase release and stimulation of incorporation of radioactive lyso-PAF into PAF on the concentration of carbamoylcholine in experiments with isolated guinea pig parotid gland acini. Each point represents the mean ± S.E. from three experiments. Acini equivalent to about 50 mg wet weight were incubated, stimulated with the indicated concentrations of carbamoylcholine, and analyzed for amylase release and $[^3]$HPAF formation as given under “Experimental Procedures.” Open circles, PAF synthesis; closed circles, amylase secretion.
that for the duration of the experiments the rate of formation of radioactive PAF could be substantially affected by changes in PAF breakdown.

In three experiments, we have examined the effect of caerulein (10^{-8} M) on the incorporation of [3H]-lyso-PAF into PAF in isolated pancreatic lobules. As indicated in Table IV, caerulein did significantly enhance the incorporation of the labeled lyso-PAF into PAF. In contrast to the experiments with parotid gland acini, caerulein led also to a slight stimulation of incorporation of lyso-PAF into 1-O-alkyl-PC.

Biologically Active PAF-like Material in Isolated Guinea Pig Parotid Gland Acini—Under our conditions, PAF could be quantified in the human platelet aggregation assay in the range from 10^{-9} to 5 \times 10^{-7} M. Human platelets corresponded in the range from 10^{-7} to 10^{-9} M in a dose-dependent way. The extracts from parotid gland acini were diluted such that they fitted within this range. An example showing calibration measurements with different concentrations of synthetic PAF and measurements with material from parotid acini is shown in Fig. 5. Extracts from TLC plates developed without parotid gland material served as controls. They had no measurable effects on platelet aggregation. The PAF antagonist SRI 63-072 inhibited the effects of PAF as well as of the material from parotid acini (Fig. 6), but did not affect thrombin-induced aggregation (results not shown here). Incubation of the PAF-like material with phospholipase C destroyed completely its capacity to induce platelet aggregation (results not shown here). The concentration of PAF-like material in the biological probes was estimated from a comparison with concentrations of PAF which exerted a comparable effect on platelet aggregation. On this basis, we found PAF-like material equivalent to 0.79 \pm 0.33 nmol/g of acini (mean value \pm S.D.; range from 0.40-1.32 nmol/g; n = 5). In three experiments, stimulation with 10^{-5} M carbamoylcholine for 20 min did not lead to a change in the total amount of PAF-like material (cells + surrounding medium) (results not shown here).

**DISCUSSION**

The results show that exocrine glands cannot only respond to PAF with an increased exocytosis, but also are able to synthesize and to release PAF. Therefore, it seems possible that PAF receptors on the plasma membrane of the secretory cells react mainly or to a large extent with PAF synthetized in the gland.

<table>
<thead>
<tr>
<th>TABLE IV</th>
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<tr>
<td><strong>Stimulation of incorporation of radioactive 1-O-octadecyl-lyso-PAF into PAF and 1-O-alkyl-2-sn-long chain acylglycero-3-phosphocholine (1-O-alkyl-PC) in isolated guinea pig parotid acini</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incorporation of radioactivity into</th>
<th>PAF</th>
<th>1-O-Alkyl-PC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td>19,500 \pm 3,750</td>
<td>50,115 \pm 12,980</td>
</tr>
<tr>
<td><strong>Caerulein</strong></td>
<td>92,000 \pm 12,391</td>
<td>145,360 \pm 35,880</td>
</tr>
</tbody>
</table>

The means in parentheses give the relative radioactivity as percent of the respective controls.
increasing concentrations of added to possibility that acetylcholine, by increasing the formation of 0.25% "Methods." The reaction was started by the addition of like material extracted from isolated guinea pig parotid gland acini and purified by TLC as given under "Methods." (I) and cases, PAF-like material extracted and purified from was diluted 10-fold with protein and added to give the final concentrations indicated in the figure.

FIG. 5. A, aggregation of human platelets in the presence of increasing concentrations of 1-O-octadecyl-2-sn-acetylglycero-3-phosphocholine (PAF). Platelet-rich plasma was prepared as given under "Methods." The reaction was started by the addition of 30 μl of the PAF solution to 270 μl of platelet-rich plasma, which had been thermostimulated to 37°C. The final concentrations of PAF are indicated. For the matter of clarity, a scale is only given for the experiment with the highest PAF concentration. B, addition of PAF-like material extracted from isolated guinea pig parotid gland acini and purified by PLC as given under "Methods." (I) and (II) represent PAF-like material from two different preparations of acini. In both cases, PAF-like material extracted and purified from 160 mg wet weight was dissolved in 100 μl of ethanol. An aliquot of this fraction was diluted 10-fold with 0.9% NaCl, 25 mM Hepes/KOH, pH 7.4, 0.25% bovine serum albumin. Thirty μl of this diluted material was added to 270 μl of platelet-rich plasma to initiate the aggregation reaction. Extracts from thin layer plates which had been developed under identical conditions had no aggregating activity.

We do not know yet whether and how the relatively large amount of PAF detected in parotid acinar cells is compartmentalized. It is, however, noteworthy in this context that Cox et al. (19) have reported the existence of PAF in saliva. This would point to the possibility that at least some PAF might be sequestered in the secretory granules.

It is not clear yet which step is regulated under conditions where PAF synthesis is stimulated in exocrine cells (calcium ionophore secretagogues). Synthesis of PAF could be limited as well by the provision of lyso-PAF for the acetyltransferase reaction as by the activity of the acetyltransferase itself. Although our experiments with radioactive lyso-PAF show that exogenous lyso-PAF can be rapidly utilized by parotid and pancreatic acinar cells, the addition of exogenous lyso-PAF to isolated parotid gland acini did neither enhance basal nor carbamoylcholine-stimulated synthesis of PAF (Table II). Our experiments with radioactive lyso-PAF demonstrate that exogenous lyso-PAF has access to that compartment where receptor-stimulated PAF synthesis takes place. Therefore, the inefficiency of exogenous lyso-PAF to stimulate the incorporation of acetate into PAF supports the idea that the acetyltansferase itself is regulated. This would also be in line with data reported by Lenihan and Lee (20). These authors concluded from their experiments with isolated spleen microsomes that lyso-PAF acetyltransferase might be regulated by reversible phosphorylation.

We have taken the fact that acetate incorporation into total lipids was not significantly affected by the secretagogues as an indication that the increased incorporation of acetate into PAF was not simply the result of an increase in the specific radioactivity of the intracellular acetyl-CoA pool. The incorporation of acetate into PAF increased 3- to 4-fold in the presence of the secretagogues. If this had resulted only from a change of the specific radioactivity of cellular acetyl-CoA pool, one would be forced to conclude that the secretagogues had depressed fatty acid synthesis by 66-75% as [3H]acetate incorporation into total lipids stayed the same. There is no report in the literature that secretagogues inhibit fatty acid synthesis. In addition, the experiments with labeled lyso-PAF also show clearly that secretagogues do enhance synthesis of PAF. The chase experiments shown in Fig. 4 make it also unlikely that the increased incorporation of precursors into PAF reflected only increased turnover without increased net synthesis. During the 10 min before addition of the chase with cold lyso-PAF, the stimulated glands had formed more labeled PAF than the unstimulated controls. Even if the turnover of PAF in unstimulated and stimulated glands were about the same, one would expect a somewhat larger loss of radioactivity from the stimulated glands following the chase as the PAF already synthesized contained more radioactivity. This may be the reason for the slightly higher loss of radioactivity

We measured values from 1.25-4.7 nmol/g wet weight (H. D. Soling and W. Fest, unpublished observations.)
PAF Synthesis in Exocrine Glands

during the first 5 min following the chase in experiment III of Fig. 4. The loss of label from the radioactive pool was by no means such that it could explain the 5–7-fold increase in incorporation of lyso-PAF into PAF found in the experiments shown in Figs. 2 and 3 following stimulation by carbamoylcholine. Furthermore, as we have demonstrated by aid of the bioassay, the existence of a rather large pool of PAF already in unstimulated parotid acinar cells, it seems unlikely that changes of the turnover rate of this pool would greatly affect the rate of incorporation of radioactive lyso-PAF into PAF over the duration of the stimulation experiments.

Although cholecystokinin and caerulein act on pancreatic acinar cells by receptors which are different from those for acetylcholine, the postreceptor effects mediated by these receptors are the same: increased intracellular free calcium, increased turnover of polyphosphatidylinositides, and increased cellular cGMP (9 and (for a review), see Ref. 21). Therefore, it seems highly likely that cholecystokinin and caerulein in exocrine pancreas and carbamoylcholine in the parotid gland enhance PAF synthesis via the same mechanism.

The data reported in the literature together with those reported here make it highly likely that the 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholines represent, in addition to the biologically active metabolites of arachidonate metabolism, another wide spread group of “cell hormones” of lipid nature. Recently, Chilton et al. (22) have shown, in experiments with polymorphonuclear leucocytes, that in the unstimulated state most of the 1-O-alkyl-2-acyl-sn-glycero-3-phosphocholines contain arachidonate in the 2-position. Upon stimulation, arachidonate is preferentially removed from the 2-position by activation of a phospholipase A₂ giving rise to an increase in the concentration not only of the PAF precursor lyso-PAF, but also of arachidonate, the precursor for the cyclooxygenase and lipoxygenase pathways. Possibly, we are only at the beginning of our understanding of a new complicated regulatory network at the cellular level.

REFERENCES