JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1993, 44, 4, 365—381

J. JAWOREK, S. J. KONTUREK

EFFECTS OF PROSTAGLANDIN OF E, F AND I SERIES, LEUKOTRIENE C_4 AND PLATELET ACTIVATING FACTOR ON AMYLASE RELEASE FROM ISOLATED RAT PANCREATIC ACINI

Institute of Physiology, University School of Medicine, Cracow, Poland.

Exogenous eicosanoids were reported to affect pancreatic secretion, but it is unknown whether this effects is mediated by the changes in pancreatic circulation or by direct action on pancreatic secretory cells. In this study the effects of prostaglandins (PG), leukotriene C_4 (LTC_4) and platelet activating factor (PAF) and the blockers of their biosynthesis or receptor antagonists on amylase release from the isolated rat pancreatic acini were examined. The acini were incubated with pancreatic secretagogues, such as caerulein or urecholine in the presence or absence of various concentrations (10^{-9}-10^{-5} M) of PGE_2, Nocloprost (stable analog of PGE_2), PGI_2, $PGF_{1\alpha}$, LTC_4, PAF, indomethacin (inhibitor of endogenous PG formation) and A-63162 (blocker of endogenous LT biosynthesis). PGE_2, PGI_2 and $PGF_{1\alpha}$ inhibited secretagoguestimulated enzyme secretion from isolated pancreatic acini but their inhibitory potency was about 1000 times lower, on molar basis, than that of Nocloprost. PAF and LTC_4 produced concentration-dependent stimulation of amylase release from the unstimulated acini reaching about 50% of caerulein-induced maximal response and enhanced amylase release induced by caerulein or urecholine. The effects of PAF and LTC_4 were reversed by the addition of respective receptor antagonist for PAF (TCV-309) and for LTC_4 (FPL-55712). These results indicate that PG inhibit, while PAF and LTC_4 stimulate pancreatic enzyme secretion and thus could be implicated in the control of this secretion.

Key words: *pancreas, amylase, prostaglandins, caerulein, urecholine.*

INTRODUCTION

Prostaglandins (PG), leukotrienes (LT) and platelet activating factor (PAF) are generated in most gastrointestinal tissues as the result of phospholipase A_2-activation, and its effect on membrane-bound phospolipids (1). Pancreas is capable to release these autacoids spontaneously or during the stimulation by CCK (or caerulein) and secretin (2, 3, 4), but the role of pancreatic eicosanoids is still not established.

The reports concerning the effects of PG on exocrine pancreatic secretion are controversial. The earlier study from our laboratory demonstrated that PGE_2 may stimulate enzyme secretion in humans (6). Also Marshall and coworkers (7) reported that PG of D, E, F and I series increase amylase release from the whole mouse pancreas. On the other hand, PGI_2 was found in one study on conscious dogs to inhibit pancreatic protein secretion (8) and PGE_2 in the another study on perfused canine pancreas to inhibit this secretion (9). Blockade of cyclooxygenase activity by indomethacin was reported recently to abolish the stimulation of pancreatic bicarbonate secretion (10) suggesting that endogenous PG are essential for the hormonal stimulation of exocrine pancreas.

Results from the studies using pancreatic slices and isolated acinar cells suggested that PG are not involved in excitation-secretion coupling (2, 11). On the contrary, Mossner et al (12) demonstrated recently that PG are able to inhibit amylase release acting directly on pancreatic acinar cells. The reported differences in the action of PG on pancreatic secretion could be attributed to the species differences as well as to the techniques applied to examine the action of PG on the pancreas.

LT have been shown to decrease pancreatic exocrine secretion in conscious dogs, although these effects were not supported by the results of the *in vitro* studies (3).

The effect of PAF on the exocrine pancreas has been little studied and single report indicated that PAF may stimulate pancreatic enzyme secretion (13). Recently, Soling and Fest (5), have shown that exocrine pancreas can synthesize PAF-aceter and that this synthesis is stimulated by cholecystokinin or caerulein.

This study was undertaken to evaluate the direct effects of PG of E, F or I series, LTC_4, PAF and agents affecting their generation or receptors, on amylase release from isolated rat pancreatic acini.

MATERIAL AND METHODS

PGE_2 was the kind gift from the Upjohn Co. (Kalamazoo, Michigan, USA), Nocloprost-stable analog of PGE_2, was obtained from Asche AG. (Hamburg, Germany), PGI_2, $PGF_{1\alpha}$, LTC_4 were provided by the Hoechst Co. (Frankfurt, Germany), TCV-309, a specific PAF receptor antagonist was offered by Takeda (Japan), FPL-55712, a specific LTC_4 receptor antagonist was a gift obtained from Fisons Pharmaceutical Labs (England). A-63162 was obtained from Abbott Lab., (Chicago, I1). Following items were purchased from SIGMA Chemical Co. (St. Louis, MO); PAF, indomethacin, urecholine and trypsin inhibitor. Essential and nonessential amino acid mixtures were purchased from SERVA Feinbiochemica GMBH (Heidelberg, Germany), caerulein from Farmitalia (Milano, Italy) and purified collagenase CLSPA was purchased from Cooper Biomedical Co. (Freehold, N.J.).

Dispersed pancreatic acini were prepared from male Wistar rats (200–250 g), fed *at libitum* on standard chow diet before each experiment. Animals were killed, pancreas was removed and digestion was performed with collagenase according the method of Amsterdam et al. (14) as previously described. Briefly, the pancreas was injected with collagenase solution (100 U/ml), placed in Erlenmayer flask, saturated with oxygen and digested in the shaking bath (37°C) for 15 min, then washed with incubation buffer without collagenase, oxygenated and digested with fresh collagenase solution for the second period (20 min). After the digestion, pancreas was dissociated by sequential passage through three pipettes and passed by the filter. Acini were suspended in fresh incubation buffer (pH 7.4) containing; 24.5 mM Hepes, 98.0 mM NaCl, 4.0 mM KCl, 11.2 mM KH_2PO_4, 0.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.0 mM glucose, 1% w/v essential and nonessential amino acid mixture, 0.2% bovine serum albumin and 0.01% w/v trypsin inhibitor. The incubation medium was saturated with oxygen and maintained at 37°C. Acinar suspensions were incubated in shaking bath for 30 min in presence of various concentrations of PGE_2, PGI_2, PGF_{1a}, Nocloprost, Indomethacin, A-63162, LTC_4 and PAF (10^{-9}—$^{-5}$M) alone or in combination with constant dose of of caerulein (10^{-12}M), urecholine (10^{-5}M), FPL-55712 (10^{-5}M), and TCV-309 (10^{-5}M). In addition, a constant dose of Nocloprost (10^{-5}M), LTC_4 (10^{-6}M), or TCV-309 (10^{-5}M) was added to the incubation medium containing increasing concentrations of caerulein ($10^{-13}-10^{-9}$M), or urecholine ($10^{-7}-10^{-3}$M).

In the time-course experiments, constants concentrations of Nocloprost (10^{-7}M), LTC_4 (10^{-6}M), PAF (10^{-7}M) alone or in combination with submaximal contentrations of caerulein (10^{-12}M), TCV-309 (10^{-5}M), or FPL-55712 (10^{-5}M) were incubated in the medium for 5–45 min. In control tests secretagogues or antagonists were incubated without the addition of eicosanoids or PAF.

After incubation the acinar suspension was centrifuged at 1000 g for 5 min and the supernatant was separated from the pellet. The amylase contents in supernatant and in dissolved pellets were determined separately, using the method of Bernfeld (15). Amylase secretion was expressed as the percentage increment over basal value. Unstimulated amylase release during the entire experimental period was determined and presented as the control value.

After the incubation of acinar suspension without (control), or with investigated agens, the samples of incubation medium were taken for microscopic visualization. Hematoxilin-eosin preparates of acinar suspensions were examined under the light microscope.

Results are expressed as the means ± SEM. Statistical analysis of data was accomplished by Student's t test or more complex comparisons of the data. Analysis of variance (ANOVA) and Duncan's multiple range test were employed. Differences with $p < 0.05$ were considered significant.

RESULTS

The effects of PG, Nocloprost and indomethacin on amylase secretion

The effects of increasing concentrations of PGE_2, PGI_2 and $PGF_{1\alpha}$ ($10^{-7}-10^{-5}$M) on amylase release from isolated pancreatic acini under basal conditions and following stimulation by constant contentration of caerulein (10^{-12}M) or urecholine (10^{-5}M) are shown on *Table 1*.

Basal secretion of amylase was unaffected by any of PGs used at contentrations ranging from 10^{-5}M (*Table 1*).

Caerulein- or urecholine-stimulated amylase release was significantly inhibited by each prostaglandin tested (PGE_2, PGI_2 and $PGF_{1\alpha}$) when used at

10^{-6}M and 10^{-5}M contentrations. The strongest inhibition was observed at 10^{-5}M, and it averaged about 70% of the level obtained with secretagogue alone. These results indicated that the inhibitory potency of PGE_2, PGI_2 and $PGF_{1\alpha}$ was similar for each PG tested (*Table 1*).

Table 1. Effect of various concentrations of PGE_2, PGI_2 or $PGF_{1\alpha}$ on basal and caerulein- or urecholine-stimulated amylase release from the rat pancreatic acini (expressed as percent of total release per 30 min). Means ± SEM of 6 separate experiments on 6 preparations of acini. Asterisk indicates significant decrease ($p < 0.05$) below the value obtained with caerulein alone or urecholine alone.

	PGE_2 (M)				PGI_2 (M)			$PGF_{1\alpha}$ (M)		
	0	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}
Basal	3.8 ±0.8	2.9 ±0.5	3.9 ±0.3	4.1 ±0.5	3.7 ±0.7	3.9 ±0.6	4.1 ±0.8	4.6 ±1.3	4.3 ±0.8	4.8 ±1.4
Caerul. 10^{-12}M	14.4 ±1.0	12.4 ±1.1	10.9* ±0.4	10.3* ±0.9	13.9 ±1.2	11.9 ±0.5	11.0* ±1.0	13.8 ±1.4	12.3* ±0.3	10.7* ±0.7
Urechol. 10^{-5}M	13.7 ±0.5	12.5 ±1.0	11.5* ±0.6	10.3* ±1.1	13.5 ±1.0	11.6* ±1.0	10.8* ±0.6	11.2 ±1.3	10.5* ±0.8	9.7* ±1.0

Incubation of isolated rat pancreatic acini in the presence of a constant concentration of caerulein (10^{-12}M), or urecholine (10^{-5}M) resulted in a submaximal stimulation of amylase release, that reached respectively, about 14.8±1.7% and 13.5±1.4% of total release (*Fig. 1*). Addition of various concentrations of Nocloprost ($10^{-9}-10^{-5}$M) to the incubations solutions produced concentration-dependet inhibition of caerulein- or urecholine-stimulated enzyme secretion. The maximal inhibitory effect occurred at 10^{-6}M of Nocloprost, reaching about 55% of secretagogue-stimulated amylase release. Basal secretion of enzyme was unaffected by Nocloprost (*Fig. 1*).

In the time-course experiments, the dispersed acini were incubated in the presence of a submaximal concentration of caerulein (10^{-12}M) alone or in combination with a constant concentration of Nocloprost (10^{-7}M). Amylase release from the acini incubated for 45 min with caerulein combined with Nocloprost was significantly lower than that obtained with caerulein alone at all time periods tested (*Fig. 2*).

Amylase responses to increasing concentrations of caerulein or urecholine are shown on *Fig. 3*. The maximal secretory response was obtained with 10^{-11}M caerulein and 10^{-4}M urecholine. Addition of Nocloprost at 10^{-5}M to the incubation medium containing various concentrations of caerulein or urecholine resulted in a significant reduction of the entire response curves to caerulein or urecholine.

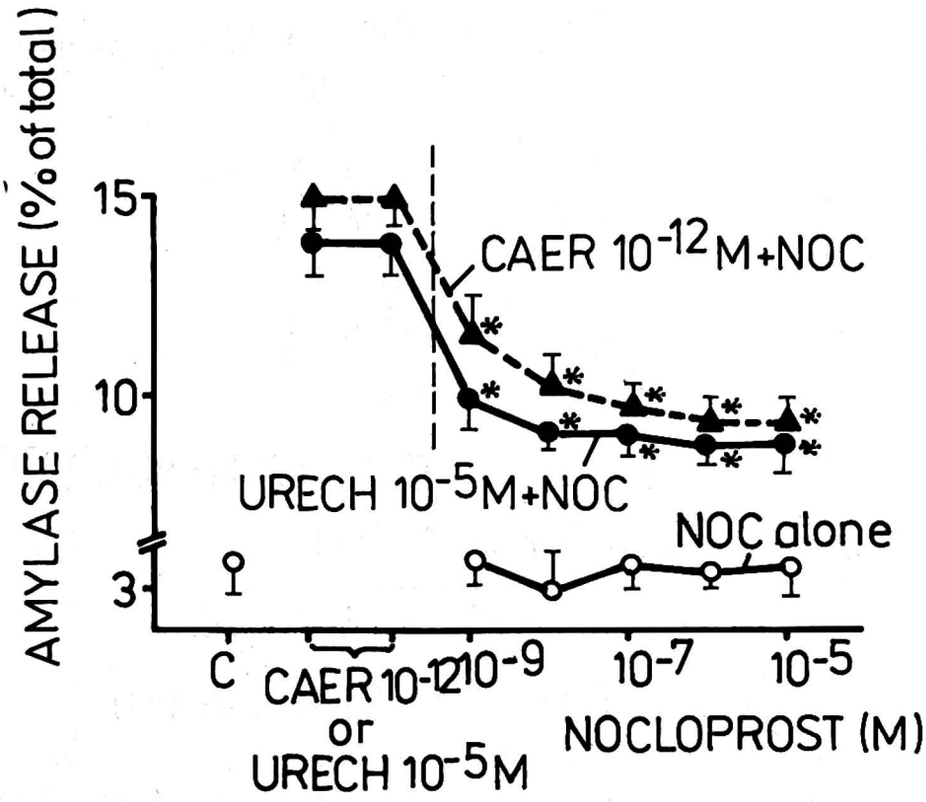

Fig. 1. Effect of various concentrations of Nocloprost on basal and secretagogue-induced amylase release from isolated rat pancreatic acini. Means ± SEM of 6 separate tests on 6 preparations of acini. Asterisk indicates significant inhibition ($p < 0.05$) below the value with secretagogue alone. C: control.

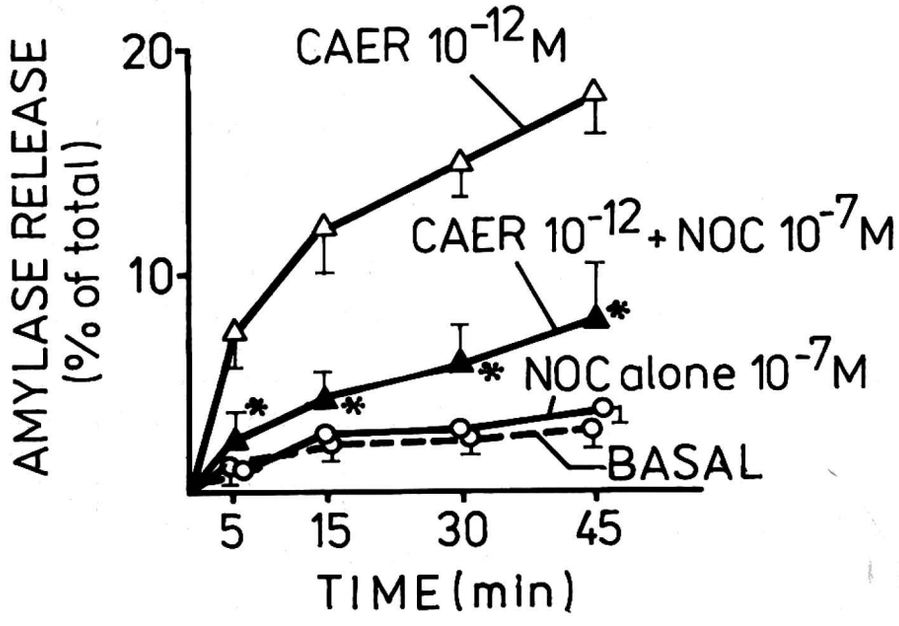

Fig. 2. The time course of amylase release from the rat pancreatic acini in test without (basal), and with addition of caerulein alone or combination of caerulein with Nocloprost. Means ± SEM of 6 separate experiments on 6 preparations of acini. Asterisk indicates significant ($p < 0.05$) decrease below the level obtained with caerulein alone.

Addition of indomethacin ($10^{-7} - 10^{-5}$M) to the incubation medium did not affect significantly basal or secretagogue-stimulated amylase release (*Table 2*).

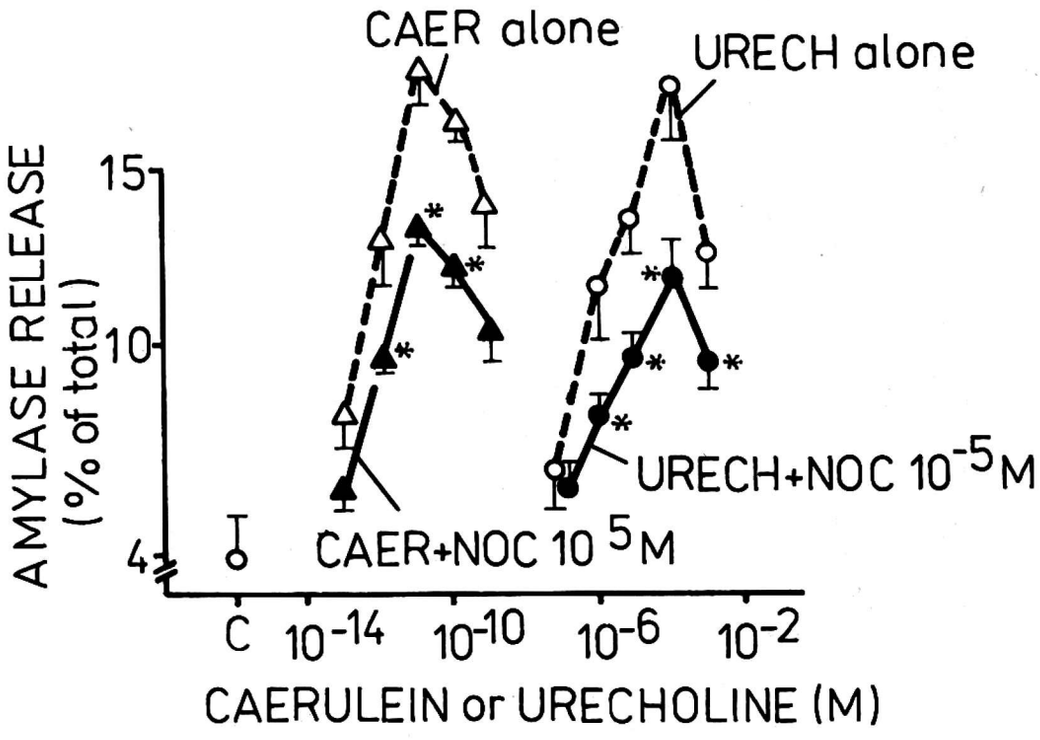

Fig. 3. Pancreatic amylase dose-response curves to graded concentrations of caerulein or urecholine alone or combined with a constant dose of Nocloprost. Means ± SEM of 6 separate tests on 6 preparations of acini. Asterisk indicates significant ($p < 0.05$) decrease below the value obtained with secretagogue alone.

Table 2. Effect of increasing concentrations of A-63162 or indomethacin on amylase release (expressed as percent of total per 30 min) from isolated rat pancreatic acini under basal conditions or after stimulation with constant concentration of caerulein or urecholine. Means ± SEM of 6 separate experiments on 6 preparations of acini.

	A-63162 (M)				Indomethacin (M)			FPL-55712 (M)		
	0	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}
Basal	4.5 ±0.7	5.5 ±1.5	5.1 ±1.6	5.4 ±0.2	3.9 ±0.3	4.0 ±0.2	4.7 ±0.3	4.8 ±0.5	3.8 ±0.6	4.8 ±1.2
Caerul. 10^{-12}M	13.5 ±2.6	11.1 ±2.6	12.8 ±0.8	13.4 ±2.1	14.5 ±1.0	14.9 ±0.5	15.6 ±1.5	15.4 ±0.7	13.5 ±0.4	15.5 ±0.8
Urechol. 10^{-5}M	13.0 ±1.0	14.4 ±0.7	12.6 ±1.6	12.7 ±0.6	14.3 ±0.7	15.4 ±1.5	15.6 ±1.6	14.0 ±0.7	12.9 ±1.1	15.4 ±2.3

The effects of LTC_4 and its antagonists, FPL-55712 and A-63162 on amylase release

Incubation of dispersed pancreatic acini in the presence of various concentrations of LTC_4 ($10^{-9} - 10^{-5}$M) resulted in a significant, and concentration-dependent increase of amylase secretion. The strongest stimulation of amylase release averaged about 8.8 ± 0.8% and was observed at 10^{-5}M of LTC_4, that was the highest concentration of LTC_4 examined in this study. The

addition of the LTC_4 receptor antagonist, FPL-55712, at the constant concentration 10^{-5}M completely abolished the stimulatory effect o LTC_4, whereas the addition of A-63162, an inhibitor of 5-lipoxygenase (10^{-4}M), failed to influence the amylase response to LTC_4 (*Fig. 4*). Neither A-63162 nor

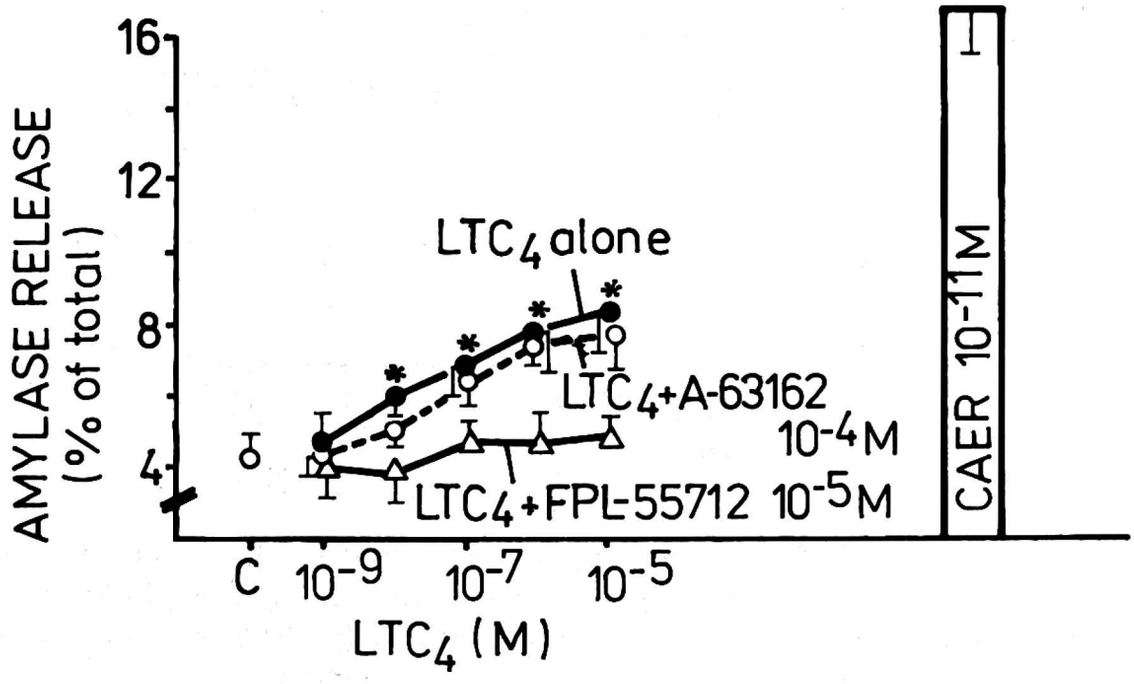

Fig. 4. Amylase release from dispersed rat pancreatic acini in response to various concentrations of LTC_4, and to combination of increasing concentrations of LTC_4 with a constant concentration of FPL-55712 and A-63162. Column on the right shows the amylase response to maximal caerulein stimulation. Means ± SEM of 6 separats experiments on 6 preparations of acini. Asterisk indicates significant ($p<0.05$) increase over the unstimulated control value. C: control.

FPL-55712, added in various concentrations ($10^{-7}-10^{-5}$) to the incubation medium of pancreatic acini in resting state or stimulated by caerulein (10^{-12}M) or urecholine (10^{-5}M), affected enzyme secretion (*Table 2*).

When the pancreatic acini were stimulated with a constant concentration of caerulein (10^{-12}M) or urecholine (10^{-5}M), the addition of LTC_4 (10^{-6} and 10^{-5}M) caused further significant increase in amylase release over that obtained with caerulein or urecholine alone (*Fig. 5*).

The time course of amylase secretion in response to a constant concentration of LTC_4, caerulein, combination of above or LTC plus FPL-55712 is presented on *Fig. 6*. LTC_4 given alone at 10^{-7}M produced significant stimulation in enzyme secretion over the basal value, to about 9.0 ± 1.0% of total release or about 52% of maximal caerulein-stimulated amylase release at 45 min of incubation. Combination of LTC_4 (10^{-7}M) and caerulein (10^{-12}M) produced the stimulation of amylase release similar to that obtained with caerulein alone. Addition of FPL-55712 (10^{-5}M) to the incubation medium containing LTC_4 resulted in the reduction in amylase secretion to the level not significantly different from that observed in unstimulated control (*Fig. 6*).

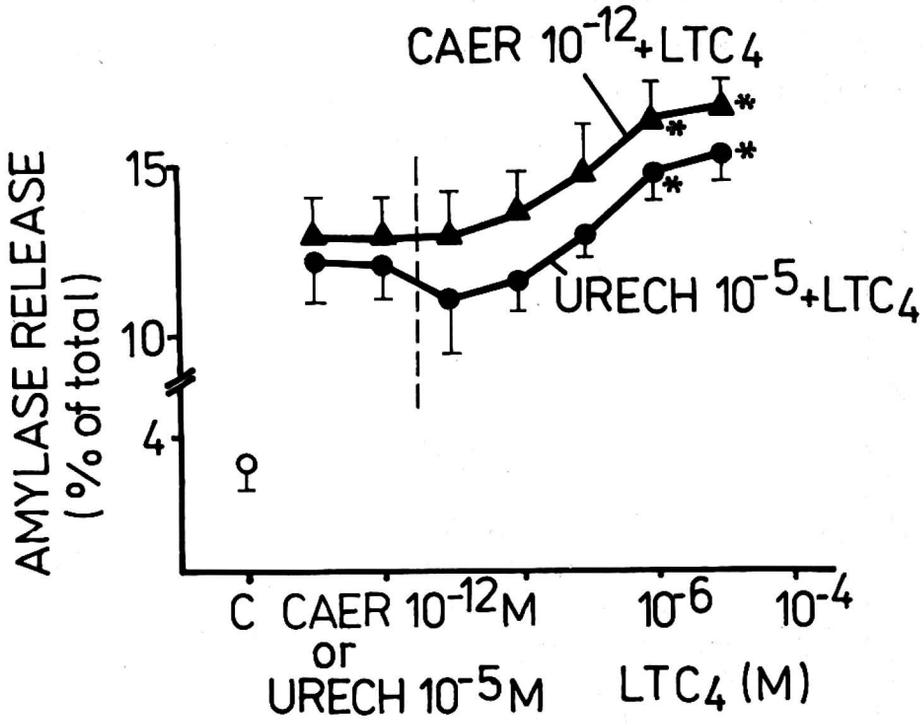

Fig. 5. Secretion of amylase from isolated pancreatic acini produced by various doses of LTC_4 combined with a constant concentrations of urecholine or caerulein. Means ± SEM of 5 separate tests on 5 preparations of acini. Asterisk indicates significant ($p < 0.05$) increase over the level obtained with secretagogue alone. C: control.

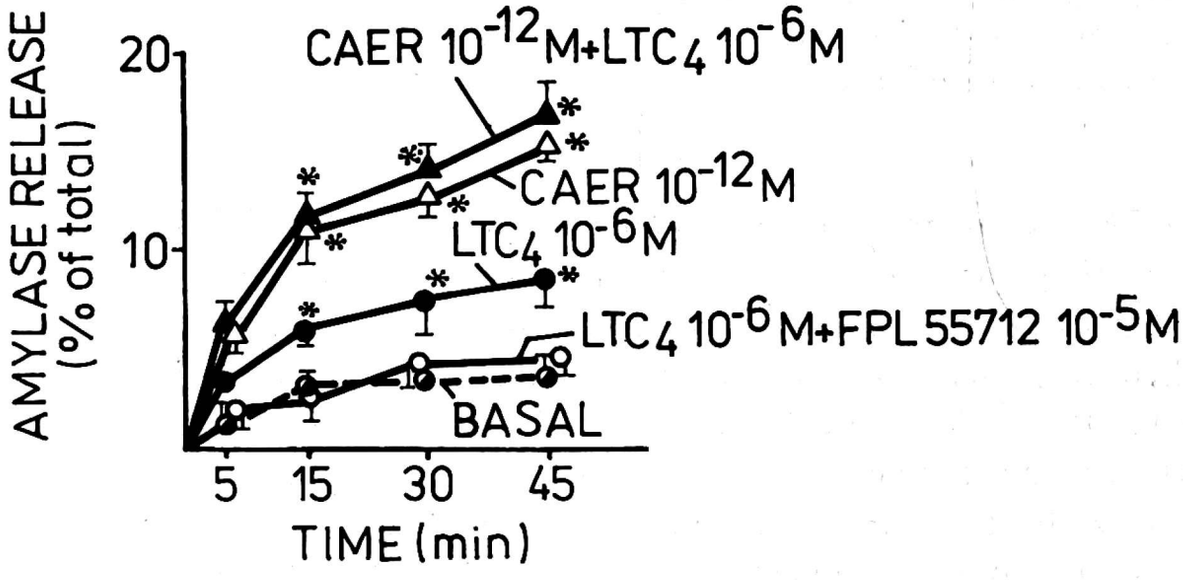

Fig. 6. Time course of amylase release from isolated pancreatic acini under basal conditions and following the stimulation with constant concentrations of LTC_4 alone, caerulein alone or with the combination of LTC_4 with caerulein or FPL-55712. Means ± SEM of 5 separate tests on 5 preparations of acini. Asterisk indicates significant stimulation over the basal value.

Constant concentration of LTC_4 (10^{-6}M) added to the acini stimulated with increasing concentrations of caerulein ($10^{-14} - 10^{-9}$M), or urecholine ($10^{-7} - 10^{-9}$M) caused further significant increase in enzyme secretion over the values obtained with secretagogue alone (*Fig. 7*).

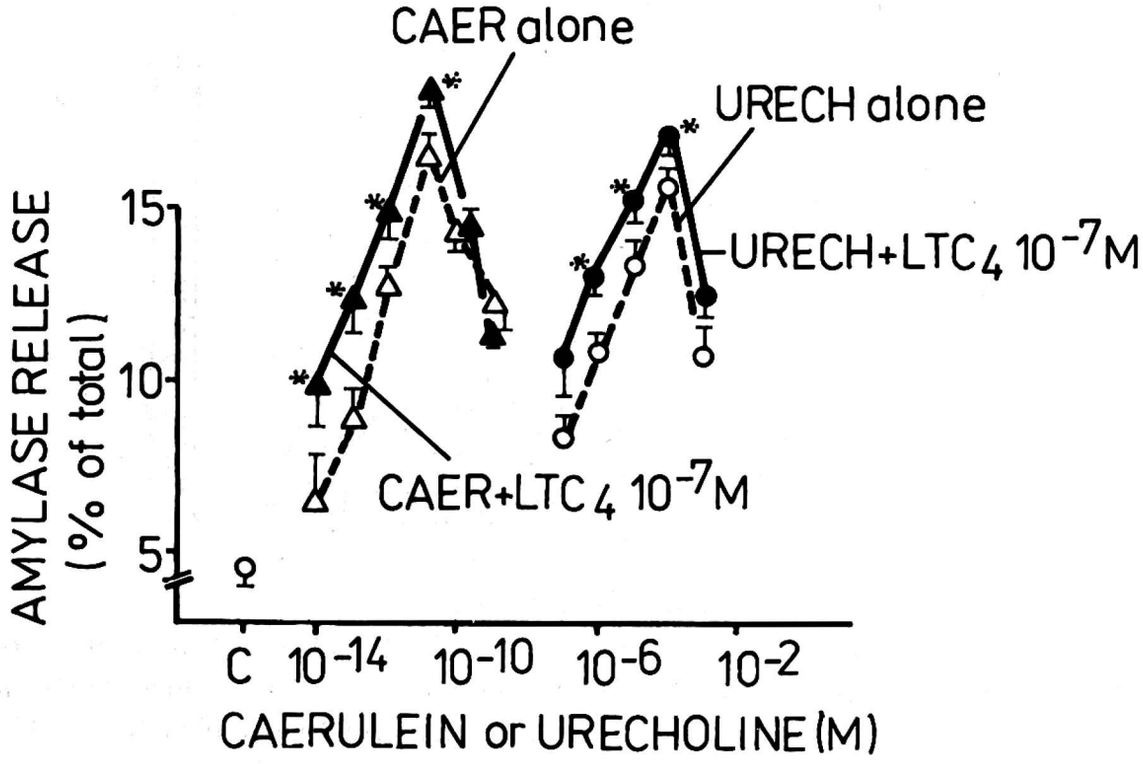

Fig. 7. Amylase release from isolated pancreatic acini in response to graded concentrations of caerulein or urecholine added to the incubation medium either alone or in combination with a constant concentration of LTC_4. Means ± SEM of 5 separate tests on 5 preparations of acini. Asterisk indicates significant ($p < 0.05$) increase over the values obtained with secretagogue alone. C: control.

The effects of PAF and its antagonist TCV-309 on amylase release.

When added to the incubation medium in gradually increasing concentrations, PAF ($10^{-10} - 10^{-5}$M) caused a concentration-dependent stimulation in basal amylase release (*Fig. 8*). The highest observed increase in enzyme release obtained with PAF (10^{-5}M) in these tests amounted to about 11.0 ± 1.0% of total. Such stimulation was completely reversed by the addition of PAF antagonist, TCV-309 at 10^{-5}M. Unstimulated amylase secretion was not altered by TCV-309 at any concentration ($10^{-10} - 10^{-5}$M) added to the incubation medium (*Fig. 8*). Increasing concentrations of PAF ($10^{-9} - 10^{-6}$M) added to the acini stimulated by caerulein (10^{-12}M), or urecholine (10^{-5}M) tended to raise enzyme release over the value obtained with secretagogues alone but this increase reached statistical significance only at the highest concentration (10^{-6}M) of PAF used in these experiments (*Fig. 9*).

The time course of amylase release from unstimulated dispersed acini and from the acini incubated in the presence of caerulein (10^{-12}M) alone, PAF (10^{-7}M) alone, or their combination is shown on *Fig. 10*. PAF produced significant stimulation in amylase secretion, that reached about 8.0 ± 0.5% of total, or about 47% of the caerulein-induced maximal release. Addition of

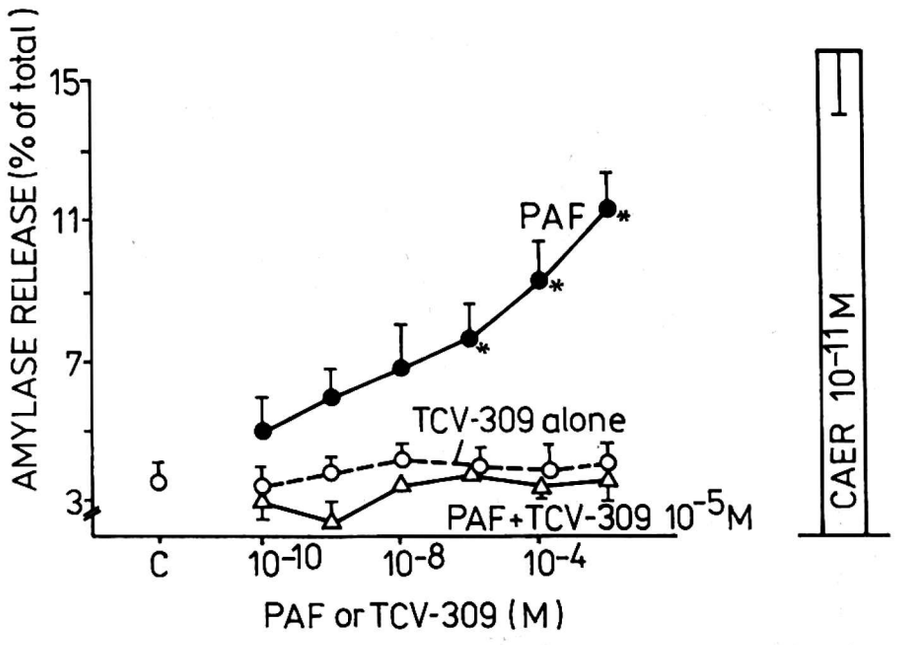

Fig. 8. Effect of increasing concentrations of PAF, or TCV-309 and combination of various doses of PAF with a constant concentration of TCV-309 on amylase release from isolated pancreatic acini. Column on the right shows maximal amylase response to caerulein from these acini. Means ± SEM of 6 separate tests on 6 preparations of acini. Asterisk indicates significant ($p < 0.05$) stimulation over the unstimulated control value. C: control.

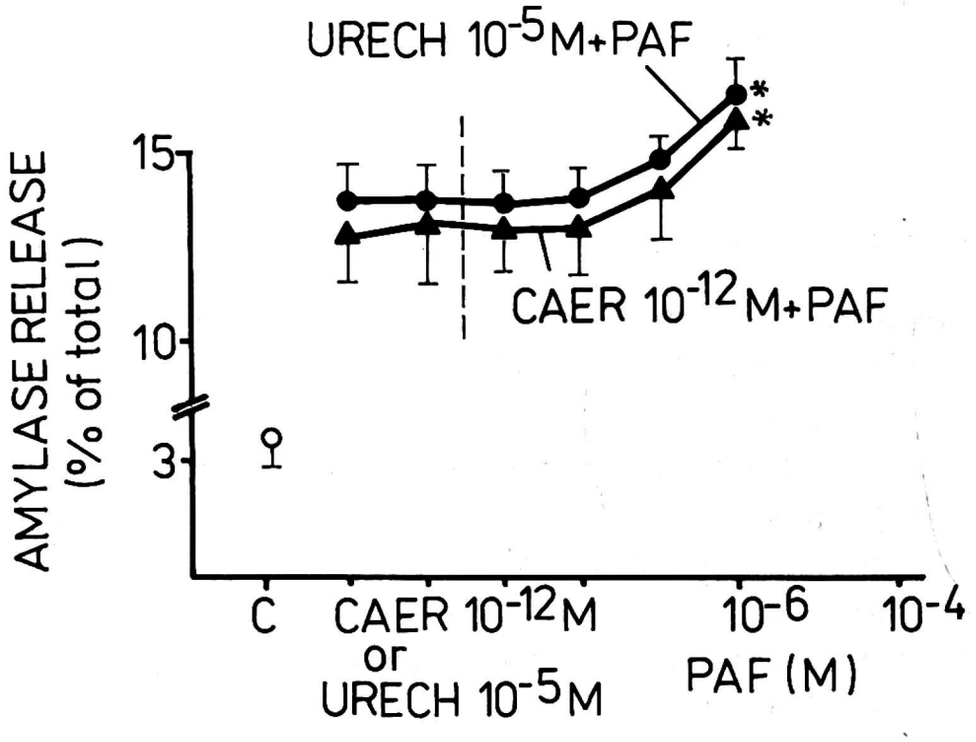

Fig. 9. Effect of PAF added in an increasing concentrations to the incubation medium of isolated pancreatic acini on cearulein- or urecholine-stimulated amylase release. Means ± SEM of 5 separate tests on 5 preparations of acini. Asterisk indicates significant ($p < 0.05$) increase over the value obtained with secretagogue alone. C: control.

PAF (10^{-7}M) to the acini stimulated by caerulein (10^{-12}M) failed to affect significantly amylase response to caerulein. Addition of TCV-309 (10^{-5}M) completely reversed the stimulatory effect of PAF on enzyme secretion, reducing the amylase release to the level not significantly different from the

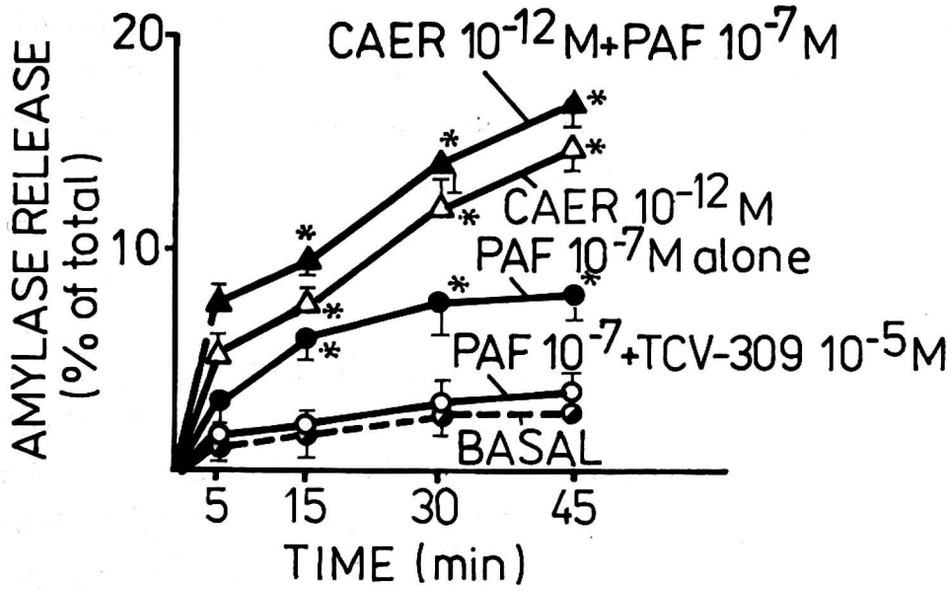

Fig. 10. The time course of amylase release from isolated acini under basal conditions and with addition of a constant concentrations of PAF or caerulein alone, or with combination of PAF + caerulein, or PAF + TCV-309. Means ± SEM of 6 separate tests on 6 preparations of acini. Asterisk indicates significant ($p < 0.05$) increase over the basal level.

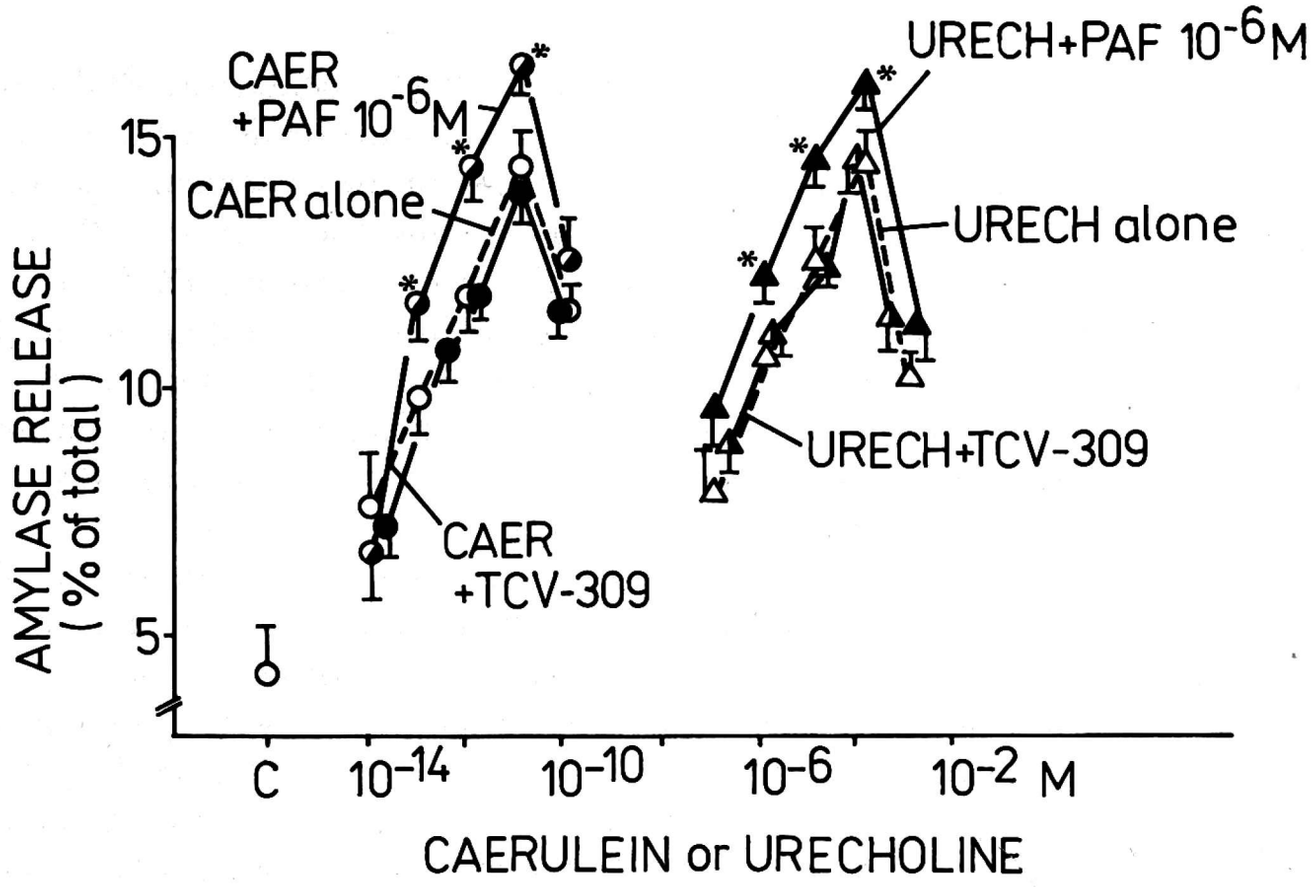

Fig. 11. Response curves of amylase release from isolated pancreatic acini to increasing concentration of caerulein and urecholine alone or combined with a constant concentration of PAF, or TCV-309. Means ± SEM of 5 separate tests on 5 preparations of acini. Asterisk indicates significant ($p < 0.05$) increase over the values obtained with secretagogues alone. C: control.

unstimulated basal control (*Fig. 10*). The addition of a constant concentration of PAF (10^{-6}M) to the acini stimulated by gradually increasing concentrations of caerulein ($10^{-14} - 10^{-9}$) or urecholine ($10^{-7} - 10^{-3}$M) produced the

responses that were significantly higher than those obtained with caerulein or urecholine alone (*Fig. 11*). TCV-309 (10^{-5}M) added to the acini stimulated by increasing concentrations of caerulein or urecholine, did not affect the response curves to these secretagogues (*Fig. 11*).

Morphological studies

Isolated pancreatic acini incubated for 30 min at 37°C, in presence of PGE_2, Nocloprost, LTC_4, PAF (10^{-5}M) alone, or in combination with urecholine (10^{-5}M) or caerulein (10^{-12}M) were not different from the controlled, untreated acini, when examined under the light microscope. Also TCV-309 (10^{-5}M) or FPL-55712 (10^{-5}M), added to the incubation medium did not affect the morphology of the pancreatic acini.

DISCUSSION

This study shows that PGE_2, its stable analog, Nocloprost, PGI_2 and $PGF_{1\alpha}$ inhibit amylase release from the isolated rat pancreatic acini, whereas LTC_4 and PAF stimulate this release in concentration-dependent manner.

The results reported in the literature, concerning the effects of PGs on pancreatic secretion are controversial. In the earlier reports PGE_2 or its analogs were shown to stimulate enzyme secretion in humans (6) and from the whole mouse pancreas (7) but it was further found that this stimulation result from the action on the pancreatic ducts and the facilitation of the enzyme transport out of the gland rather than from the direct stimulatory effect on pancreatic acinar cells (16).

Our present observation, that PG of E, F and I series produced the inhibition of the amylase release from the rat pancreatic acini, is in agreement with previous observations that PGI_2 (8) and PGE_2, or its stable analogs (17, 18) attenuated enzyme release when administered to the intact animals or humans. To the contrary, Homma and Malik (19) reported that PGE_2, PGI_2 and PGD_2 administered to anesthetized dogs had no effect on pancreatic exocrine secretion, but this may be due to the interference of anesthesia with the secretory activity of the pancreas in these animals. Also Chauvelot and coworkers (11) showed that in dispersed pancreatic acinar cells PGs did not affect the secretion of amylase both under basal conditions and following the stimulation by secretagogues such as carbachol or caerulein. However, in that study only natural PG were used and they are less potent and more rapidly degrading in the incubation medium than synthetic stable methylated analogs. Also the method used by Chauvelot and coworkers (11) to determine the

amylase release was different from that introduced by Bernfeld and used in our study (15).

It is well known, that different PG exhibit various, sometimes opposite, biological effects on the same organ (20). This is why, we decided to compare the action of various PG using the same model of isolated rat pancreatic acini. This allowed us to compare the secretory effects of investigated subsances directly on pancreatic acinar cells.

The results of present study confirmed that PGE_2, and its stable analog, Nocloprost, failed to affect basal pancreatic secretion but inhibited secretagogue-induced amylase release from the rat pancreatic acini. When compared on molar basis, the inhibitory effect of Nocloprost was about 1000 times stronger than that of PGE_2, indicating that this new analog is more potent that native PGE_2 probably due to its greater stability in the incubation medium and greater resistance to chemical degradation. Among the others eicosanoids, PGI_2, and $PGF_{1\alpha}$ also showed the inhibitory effect on amylase secretion similar to that of PGE_2.

Although the inhibitory action on PG on exocrine pancreas seems to be well supported by previous studies (12) and by the present report, the cellular mechanism of this inhibition still remains unclear. Some investigators proposed that PGE_2 acts on enzyme secretion via specific receptor in pancreatic acinar cells, probably by interaction on inositol-1,4,5-trisphosphate formation (12). Recently PGE_2 was found to produce an inhibition of pancreatic amylase release stimulated by secretin a secretagogue that is known to increase the accumulation of cAMP in the cytosol of acinar cell (21). These observations could be interpreted that PGE_2 acts as an unspecific, local inhibitor of pancreatic secretion induced by secretagogues activating via different intracellular mechanisms such as cAMP formation and calcium ions in cytosol.

The concept of the involvement of PGE_2 as local inhibitor of exocrine pancreas apparently disagrees with recent finding in conscious dogs showing that the blockade with indomethacin of PG formation virtually abolished the pancreatic volume flow and bicarbonate secretion induced by secretin but did not affect enzyme protein secretion by secretin or CCK (10). It was proposed in that study that intact PG biosynthesis is necessary for the action of secretin on exocrine pancreatic secretion. Since indomethacin in this and previous studies (11) did not exert any direct action on pancreatic secretory cells it is likely that the failure of secretagogues such as secretin to stimulate exocrine pancreas in indomethacin-treated animals could be attributed to the changes in the pancreatic blood flow. Indeed, Homma and Malik (19, 22) reported that the pretreatment of animals with cyclooxygenase inhibitors such as indomethacin resulted in a decrease in the blood flow to the pancreas.

In the present study, PGE_2 and its stable analog, Nocloprost, inhibited the amylase release from isolated rat pancreatic acini stimulated by various

concentrations of caerulein or urecholine. Caerulein and urecholine are known to stimulate pancreatic enzyme secretion through the activation of membrane receptors and calcium ions as a second messengers (23). Since the dose response curves to these secretagogues were not shifted to the right by PGE_2 or Nocloprost, we suggest that these PG do not interact with specific membrane receptors for these hormones on pancreatic acinar cells.

Bauduin et al. (2) reported that PGs are released spontaneously in nanogram concentrations from the resting pancreas and that the stimulation with caerulein, carbamylcholine or secretin, produced further increase in this PG biosynthesis. These concentrations of PG may not be sufficient to affect the release of amylase under physiological conditions.

LTC_4, another product of arachidonate metabolism via lipoxygenase pathway (24), was found in the study to stimulate dose-dependently amylase release, when added to the isolated rat pancreatic acini. Our previous report showed, that the administration of LTC_4 to the dogs equipped with chronic pancreatic fistulas produced an inhibition of the pancreatic exocrine secretion (3) but this could be explained by a marked reduction of the blood flow to the pancreas. It is well known, that such restriction of the pancreatic circulation could produce the attenuation of the enzyme release observed in these experiments (3, 25). Thus, the inhibition of the pancreatic exocrine secretion produced by LTC_4 *in vivo* could be attributed to its effect on pancreatic blood flow, whereas LTC_4 administered to the acini is able to stimulate directly pancreatic amylase release.

Recent reports demonstrated that the effects of LTC_4 in several kinds of tissues were reversed by FPL-55712, which acts as a specific agonist of LTC_4 receptor (26–28). Since FPL-55712 was able to reverse the stimulation of pancreatic enzyme secretion produced by LTC_4, we suggest that the secretory effect of LTC_4 on pancreatic acini depends on the interaction of this leukotriene with its specific receptor on pancreatic cell membranes. Binding studies are necessary to support this hypothesis.

It remains to establish whether LTC_4 is involved in the control of pancreatic secretion. Pancreas was reported to generate relatively large amounts of LTC_4 that was about 2–3 times larger than that produced by gastrodudenal mucosa (24, 26). In our present study A-63162, the potent and selective inhibitor of endogenous leukotriene formation (29, 30) failed to affect LTC_4-induced amylase release, as well as, basal or secretagogue-stimulated enzyme secretion from isolated the acini. It means that under normal conditions endogenous LTs produced locally in the pancreas play little role in the control of exocrine pancreatic secretion.

Our present study demonstrated that PAF increased dose-dependently amylase release from the rat isolated pancreatic acini and this effect was completely reversed by the addition of TCV-309, the highly potent antagonist

of PAF receptore (31). This observation indicates, that PAF stimulates pancreatic enzyme secretion acting directly on the acinar cells. The results are in agreement with previous report showing that PAF stimulated enzyme secretion from the pancreas in an acetylocholine-like fashion (13). Soling and Feat (5) reported, that isolated pancreatic lobules are able to synthesize and release PAF, when stimulated by pancreatic secretagogues such as CCK and secretin. This observation suggests the possible physiological role for PAF in the modulation of enzyme secretion from the acinar cells stimulated with pancreatic secretagogues. However, in our experiments we have not found any effect of TCV-309, the antagonist of PAF rceptor, on amylase dose-response curves for caerulein or urecholine. This discrepancy could be explained that in our study employed somewhat different experimental model than that used by Soling and Fest (5), and the amount of PAF released from the acini in our experiments was not sufficient to affect pancreatic secretion. The results of the present study clearly demonstrate that inhibition of pancreatic enzyme release by PGs and stimulation of this secretion produced by LTC_4 or PAF depends on their direct action on pancreatic acinar cells. However, the role of these substances in the regulation of the pancreatic exocrins secretion under normal conditions seems to be rather negligible.

Recent reports showed that PG, LT and PAF have been implicated in the pathogenesis of acute pancreatitis but their exact role in the development of pancreatic injury is poorly understood (32–38). Experimental acute pancreatitis is accompanied by the marked increase of PG, LT and PAF in the pancreas. Our present results suggest that LTC_4 and PAF, causing local stimulation of pancreatic enzymes secretion and a marked reduction in the pancreatic blood flow could contribute to acute tissue inflammation, whereas PG could protect the pancreas against acute damage by inhibiting the release of pancreatic enzymes and maintaining the tissue blood flow.

REFERENCES

1. Wallace JL, Whittle BJR. Gastrointestinal damage induced by platelet-activating factor. Inhibition by the corticoid, dexamethason. *Dig Dis Sci* 1988; 33: 225–232.
2. Bauduin H, Galand N, Boyenaems JM. In vitro stimulation of prostaglandin synthesis in the rat pancreas by carbamylcholine, caerulein and secretin. *Prostaglandins* 1981; 22: 35–51.
3. Konturek SJ, Pawlik WW, Czarnobilski K, et al. Effect of leukotriene C_4 on pancreatic secretion and circulations in dogs. *Am J Physiol* 1988; 254: G849–G855.
4. Kuroda T, Sodeyama H, Hanazaki K, et al. Involvement of endogenous prostaglandins in pancreatic endocrine and exocrine secretion in dog pancreas. *Pancreas* 1989; 4(16): 702–707.
5. Soling HD, Fest W. Synthesis of 1-0-alkyl-2-acetyl-sn-glycero-3-physophocholine (platelet-activating factor) in exocrine glands and its control by secretagogues. *J Biol Chem* 1986; 261: 13916–13922.

6. Konturek SJ, Kwiecien N, Świerczek J, Oleksy J. Effect of methylated PGE_2 analogs given orally on pancreatic response to secretin in man. *Am J Dig Dis* 1977; 22: 16–19.
7. Marshall PJ, Dixon JF, Hokin LE. Direct demonstration of the formation of PGE_2 due to phosphoinositol breakdown associated with stimulation of enzyme secretion in the pancreas. *J Biol Chem* 1981; 256: 844–847.
8. Konturek SJ, Tasler J, Jaworek J. Prostacyclin inhibits pancreatic secretion. *Am J Physiol* 1980; 238: G531–G536.
9. Iwatsuki K, Chiba S. Effects of prostacyclin and prostaglandin E_2 on the secretion of pancreatic juice in the dog. *Clin Exp Pharmacol Physiol* 1982; 9 (5): 495–498.
10. Beauchamp RD, MacLellan DG, Upp JR Jr, Nealon WH, Townsend CM Jr, Thompson JC. The role of endogenous prostaglandins in hormone-stimulated pancreatic exocrine secretion. *Gastroenterology* 1992; 102: 272–279.
11. Chauvelot L, Heisler S, Huot J, Gagnon D. Prostaglandins and enzyme secretion from dispersed rat pancraetic acinar cells. *Life Sci* 1979; 25: 913–920.
12. Mossner J, Secknus R, Spiekermann H, et al. Prostaglandin E_2 inhibits secretagogue-induced enzyme secretion from rat pancreatic acini. *Am J Physiol* 1991; 260: G711–G719.
13. Soling HD, Eibl H, Fest W. Acetylocholine-like effects of 1-0-alkyl-2-acetyl--sn-glycero-3-phosphocholine (platelet-activating factor) and its analogs in exocrine secretory glands. *Eur J Biochem* 1984; 144: 65–72.
14. Amsterdam A, Solomon TE, Jamieson JD. Sequential dissociation of exocrine pancreas into lobules, acini and individual cells. *Methods Cell Biol* 1978; 20: 362–376.
15. Bernfeld P. Amylases alpha and beta. Methods in Enzymology, New York, Academic Press, 1955, 139–148.
16. Marshall PJ, Dixon JF, Hokin LE. Prostaglandin E_2 derived from phosphatidylinositol breakdown in the exocrine pancreas facilitates secretion by an action on the ducts. *J Pharmacol Exr Thep* 1982; 221: 645–649.
17. Sauterau D, Chovet M, Chariot J, Tsocas A, Rose C. Central and peripheral effect of prostaglandin E_2 and enoprostil on exocrine pancreatic secretion in rats. *Pancreas* 1989; 4: 210–218.
18. Angelini G, Rose B, Covi M, et al. Effect of graded doses of PGE_2 on pancreatic exocrine secretion of bicarbonate, chymotrypsin and cyclic nucleotides during i.v. infusion of secretin in man. *Hepatogastroenterology* 1981; 28: 213.
19. Homma T, Malik KV. Effect of secretin and caerulein in canine pancreas. Relation to prostaglandins. *Am J Physiol* 1991; 244: G660–G667.
20. Lee LB. *Prostaglandins,* New York, Elsevier 1982.
21. Ogami Y, Kimura T, Nawata H. Role of prostaglandin E_2 in stimulus-secretion coupling in rat exocrine pancreas. *Pancreas* 1990; 5: 598–605.
22. Homma T, Malik KU. Effect of prostaglandins on pancreatic circulation in anasthetized dogs. *J Pharmacol Exp Ther* 1982; 222: 613–628.
23. Gardner JD, Jensen RT. Secretagogue receptor on pancreatic acinar cells. *Physiology of Gastrointesinal Tract,* L. R. Johnson (ed.), Raven Press, New York 1987, pp. 1109–1127.
24. Dreiling KW, Hoppe V, Pescar BM. Leukotriene synthesis by human gastrointestinal tissues. *Biochim Biophys Acta* 1986; 878: 184–193.
25. Whittle BJ, Oren-Wolman N, Guth P. Gastric vasconstrictor actions of leukotriene C, PGF_2 and thromboxane mimic V-46619 on rat submucosal microcirculation *in vivo*. *Am J Physiol* 1985; 248: G580–G586.
26. Konturek SJ, Brzozowski T, Drozdowicz D, Garlicki J, Beck G. Role of leukotrienes and platelet activating factor in acute gastric mucosal lesions in rats. *Eur J Pharmacol* 1989; 164: 285–292.

27. Basuray R, De Jonge C, Zaneveld LJ. Evidence for a role of cysteinyl leukotrienes in mouse and human sperm function. *J Androl* 1990; 11(1): 47–51.
28. Cristol JP, Provencal B, Borgeat P, Sirois P. Specific leukotriene D_4 receptors on guinea-pig alveolar macrophages. *Prostaglandins* 1988; 35(5): 747–756.
29. Yamamoto A, Shikada K, Tanaka S. Arachidonate lipoxygenase inhibitors in guinea-pig isolated trachea. Effects on contractions to antigen and various agonists. *Prostaglandins* 1990; 40: 615–625.
30. Hui KP, Lotvall J, Chung KF, Barnes PJ. Attenuation of inhaled allergen-induced airway microvascular leakage and airflow obstruction in guinea pigs by 5-lopoxygenase inhibitor (A-63162), *Am Rev Respir Dis* 1991; 143 (5 Pt 1): 1015–1019.
31. Takatani M, Maezaki N, Imura Y, Terashita Z, Nishikawa K, Tsushima S. Platelet activating factor (PAF) antagonists: development of a highly potent PAF antagonist, TCV-309. *Adv Prostaglandin Thromboxane Leukotriene Res* 1991; 21B: 943–946.
32. Robert A, Lum JT, Lancaster C, Olafson A, Kolbasa K, Nezamis J. Prevention of prostaglandins of caerulein-induced pancreatitis in rats. *Laboratory Investigation* 1989; 60: 677–691.
33. Van Ooijen B, Kort WJ, Tinge C, Wilson P. Significance of thromboxane A_2 and prostaglandin I_2 in acute necrotizing pancreatitis in rats. *Dig Dis Sci* 1990; 35: 1078–1084.
34. Vollmar B, Waldner H, Schmand J et al. Release of arachidonic acid metabolites during acute pancreatitis in pigs. *Scand J Gastroenterol* 1989; 24: 1253–1264.
35. Zhou W, Chao W, Levine BA, Olson MS. Evidence for platelet-activating factor as a late-phase mediator of chronic pancreatitis in the rat. *Am J Pathol* 1990; 137: 1501–1508.
36. Ais G, Lopez-Farre A, Gomez-Garre DN et al. Role of platelet-activating factor in hemodynamic derangements in an acute rodent pancreatitis model. *Gastroenterology* 1992; 102: 181–187.
37. Konturek SJ, Dembiński A, Konturek PJ et al. Role of platelet activating factor in pathogenesis of acute pancreatitis in rats. *Gut* 1992; 33: 1268–1274.
38. Fujimura K, Kubota Y, Ogura M, et al. Role of endogenous platelet-activating factor in caerulein-induced acute pancreatitis in rats. Protective effects of PAF-antagonist. *J Gastroenterol Hepatol* 1992; 33: 1268–1274.

Received: August 31, 1993
Accepted October 1, 1993

Author's address: S. J. Konturek, Institute of Physiology, University School of Medicine, 31-531 Cracow, ul. Grzegórzecka 16, Poland.