Biochemical Pharmacology, Vol. 46, No. 4, pp. 567–573, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

PHOTODYNAMIC DRUG ACTION ON ISOLATED RAT PANCREATIC ACINI

MOBILIZATION OF ARACHIDONIC ACID AND PROSTAGLANDIN PRODUCTION

MARIAM AL-LAITH, E. KEITH MATTHEWS* and ZONG JIE CUI
Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, U.K.

(*Received* 2 *April* 1993; *accepted* 28 *May* 1993)

Abstract—Chloro-aluminium phthalocyanine sulphonate (SALPC) when photon-activated generates singlet oxygen, elicits amylase release and causes plasma membrane permeabilization of pancreatic acinar cells (Matthews and Cui, *Biochem Pharmacol* **39**: 1444–1457, 1990). Amylase release precedes membrane permeabilization suggesting that the initial release of amylase may be due to direct stimulation by singlet oxygen of secretagogue receptors or their coupled guanine nucleotide binding proteins (G-proteins) and effector systems including phospholipase A_2 (PLA_2). The aim of the experiments reported here was to establish the extent to which PLA_2 activation, arachidonic acid mobilization, and prostaglandin production are involved in the photon-induced action of SALPC on dispersed, perifused acini isolated from the rat pancreas. The mobilization of arachidonic acid by a major secretory stimulant of pancreatic exocrine cells, cholecystokinin octapeptide, was also assessed: it produced a time- and concentration-dependent (10^{-10}–10^{-6} M) stimulation of arachidonic acid output from acini prelabelled with [1-^{14}C]arachidonic acid. In contrast, the kinetics of arachidonic acid mobilization with photon-activated SALPC 1 μM, 4500 or 18,400 lux light intensity ($\lambda > 570$ mm), was biphasic, an intensity-dependent stimulation being preceded by a more immediate initial inhibition of output. Light activation of SALPC and singlet oxygen generation may evoke the stimulatory phase of arachidonic acid release by an action on G-proteins, or by PLA_2 activated directly, or via calcium influx, because NaF 20 mM, mellitin 2 mg/mL and the calcium ionophore A23187 1 μM caused a 2.9-, 33- and 5-fold increase, respectively, in arachidonic acid output. However, not only was the arachidonate stimulation delayed in response to SALPC but in other experiments designed to gain more insight into the turnover of arachidonic acid and its metabolites, the photodynamic release of amylase preceded maximum prostaglandin E_2 (PGE_2) output and amylase release was completely unaffected when PGE_2 production was blocked by the cyclo-oxygenase inhibitor, indomethacin 10 μM. It is therefore likely that the rapid initial photodynamic release of amylase from pancreatic acini induced by SALPC is mediated by activation of the signal transduction pathway involving the release of intracellular calcium; arachidonic acid mobilization and prostanoid production may then be linked to the longer-term, cytolytic action of SALPC, especially in tumour cells.

Membrane-located photodynamic agents, upon photon activation at the appropriate wavelength, can cause contraction of smooth muscle cells [1], permeabilization of thymocytes [2], stimulation of secretion of pancreatic acinar cells [3] and inhibition of constitutive secretion of pancreatic carcinoma AR4-2J cells [4]. Chloro-aluminium phthalocyanine sulphonate (SALPC†) when photon-activated generates singlet oxygen and has been shown to elicit amylase release and membrane permeabilization in perifused freshly isolated rat pancreatic acini, but amylase release was found to precede membrane permeabilization by several minutes [5]. This "acute" initial release of amylase may therefore be due to a more immediate photodynamic stimulation of membrane secretagogue receptors or their coupled guanine nucleotide binding proteins (G-proteins) and effector systems, including phospholipase A_2 (PLA_2; EC 3.1.1.4), rather than occurring simply as a consequence of membrane permeabilization. We report here the results of experiments to establish the extent to which PLA_2 activation, arachidonic acid mobilization and prostaglandin production are involved in photon-induced SALPC action on pancreatic acini. In addition, the mobilization of arachidonic acid by a major secretory stimulant of pancreatic exocrine cells, cholecystokinin octapeptide (CCK-8), has been tested for comparison since arachidonic acid release may be implicated in the effects of high concentrations of cholecystokinin on acinar cells.

* Corresponding author. Tel. (0223) 334032; FAX (0223) 334040.

† Abbreviations: SALPC, chloro-aluminium phthalocyanine sulphonate; PLA_2, phospholipase A_2; PLC, phospholipase C; CCK-8, cholecystokinin octapeptide; PGE_2, prostaglandin E_2; G-protein, guanine nucleotide binding protein.

MATERIALS AND METHODS

Isolation of tissue. Pancreatic acini from male Sprague–Dawley rats (250–450 g) were isolated as described previously [5]. A quarter of the acini from

one rat pancreas was used; after isolation the tissue was washed three times with buffered saline of the following composition (mM): NaCl 118, KCl 4.7, $MgCl_2$ 1.16, $CaCl_2$ 2.0, NaH_2PO_4 1.16, glucose 14, HEPES 25; the pH was adjusted to 7.3 with NaOH 1 N and the solution oxygenated.

Labelling of acini with arachidonic acid. The method of labelling with [1-^{14}C]arachidonic acid was that of Marshall *et al.* [6]. Briefly, washed acini were resuspended in 2 mL of buffer solution and incubated in a shaking water bath (30 cycles/min, 37°) with bethanechol 1 mM, and [1-^{14}C]arachidonic acid 0.5 μCi (sp. act. 55.7 mCi/mmol) for 30 min. After such treatment most of the radioactivity was incorporated by the cells (93 ± 0.3%; N = 3). The labelled acini were then washed three times with buffer solution containing 0.1 mM atropine and 1% bovine serum albumin (fatty acid free) before incubation in fresh buffer solution (2 mL) in a shaking water bath (30 cycles/min, 37°) for a further 30 min. The prelabelled acini (2 mL) were then divided into four aliquots, each of which was mixed with 25 mg Biogel beads (P_2) and loaded into a four-chamber unit for perifusion at 37°. Each chamber was constructed from a 2-mL plastic hypodermic syringe and had a volume of 1 mL; the flow rate through the chamber was 0.5 mL/min. The acini were perifused for at least 50 min before any stimulation was applied. Light (>570 nm) was delivered from above the chamber unit at a set illuminance for a period of 10 min as described previously [5]. Two-minute fractions of perifusate were collected from each of the four chambers and the released radiolabel in each channel was measured by liquid scintillation spectrometry. All dpm values measured in the same channel were normalized to the mean value of the three fractions collected before stimulation (i.e. the mean of the fractions at the 53rd, 55th and 57th min was taken as 1.0).

Measurement of amylase release and prostaglandin E_2 (PGE_2) production. In other experiments not involving radiolabel, the PGE_2 content of the perifusate was measured by a peroxidase-conjugated enzyme immunoassay (Amersham, RPN 222) which involved comparison at constant assay volume with standard PGE_2 solutions: results are therefore expressed as PGE_2 content per sample per assay well. Measurements of amylase output by an amylose-azure absorbance assay [5] were carried out in parallel with PGE_2 determinations in the same experiments. In these experiments perifusate samples were collected at 4-min intervals and the amylase output normalized to the mean basal output determined in the two samples (collected at the 53rd and 57th min) preceding stimulation of the acini.

Exposure of pancreatic acini to SALPC. The acini were exposed to SALPC 1 μM for 10 min (i.e. from the 34th to the 44th min of perifusion) before irradiation 15 min later. The SALPC used in these experiments has a molecular sulphonate/phthalocyanine ratio of approximately 3.

Student's *t*-test was used for testing the significance of difference between means and $P < 0.05$ was taken as significant. Mean values ± SEM are plotted unless otherwise stated. SALPC was a gift from Ciba-Geigy (Basel, Switzerland); CCK-8 (26-33) amide (sulphated) was from Cambridge Research Biochemicals Ltd; Biogel beads (P_2) were from Biorad (Watford, U.K.). All other chemicals were of the best grade from the Sigma Chemical Co. (Poole, U.K.).

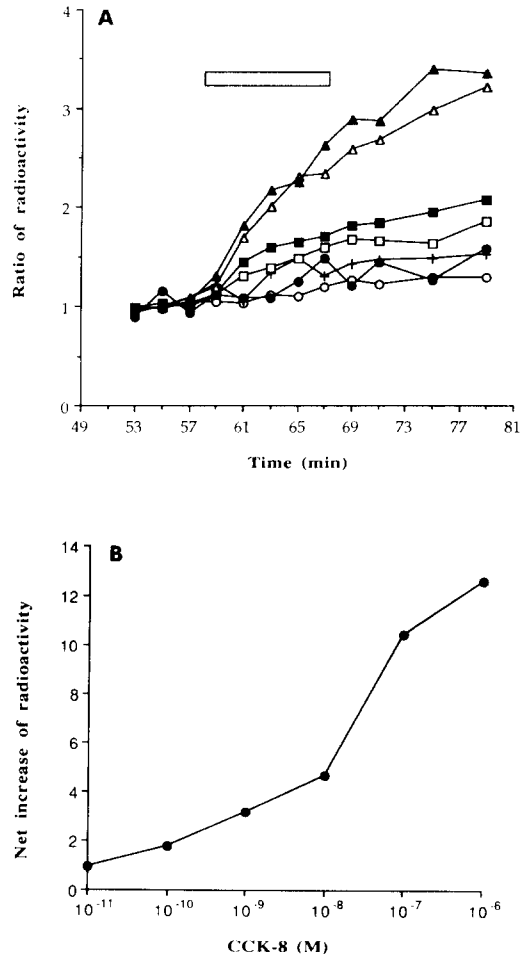

Fig. 1. Effect of CCK-8 on arachidonic acid mobilization from isolated pancreatic acini. (A) Time-dependent release of arachidonic acid on exposure for 10 min (open bar) to CCK-8 10^{-11} M (●), 10^{-10} M (+), 10^{-9} M (□), 10^{-8} M (■), 10^{-7} M (△) and 10^{-6} M (▲); control, without CCK-8 (○) (N = 3–11; for clarity means only are plotted; SEM range ± 0.01–0.36). (B) Dose–response relationship: net increase of arachidonic acid release for each CCK-8 dose in (A).

RESULTS

Dose- and time-dependent stimulation of arachidonic acid release by CCK-8

CCK is a major secretagogue in pancreatic acinar cells acting primarily on CCK_A receptors [7] to evoke exocytotic amylase release via the agonist–phospholipase C (PLC) effector pathway. This entails diacylglycerol generation, inositol 1,4,5-triphosphate production and release of calcium from intracellular stores [8]. Other interacting pathways

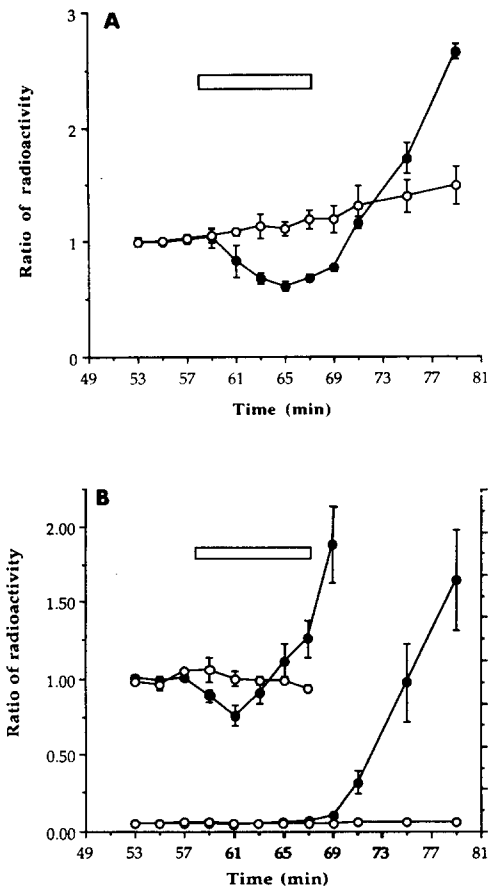

Fig. 2. Photodynamic action of SALPC on arachidonic acid mobilization from pancreatic acini. Acini were exposed to SALPC 1 μM from the 34th to the 44th min of perifusion and irradiated for 10 min at the time indicated by the horizontal bar. (A) Irradiation at 4500 lux (N = 6), and (B) irradiation at 18,400 lux (N = 6); inset: arachidonic acid release from the 53rd to the 69th min shown on larger scale (left ordinate). Parallel control experiments without SALPC treatment (○).

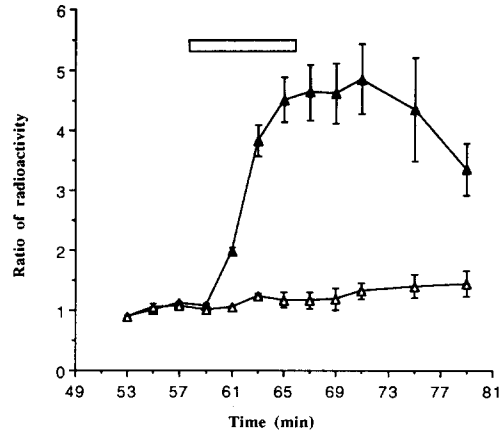

Fig. 3. Effect of the ionophore A23187 on arachidonic acid release from pancreatic acini. Acini were exposed to A23187 1 μM (▲) for 10 min indicated by the horizontal bar; control experiments in the absence of A23187, vehicle only 0.1% DMSO (△) (N = 4).

may be involved [9] and recently it has been suggested in a number of different cell types that arachidonic acid and its metabolites can act as second messengers [10–13]. CCK-8 was therefore tested for its ability to mobilize arachidonic acid from perfused rat pancreatic acini. Figure 1 shows that CCK-8 stimulates arachidonic acid release in both a time- and concentration-dependent manner. The minimal effective concentration was 10^{-10} M with 10^{-6} M giving a near maximal response.

Photodynamic action of SALPC on arachidonic acid mobilization

Previous experiments have shown that membrane-bound SALPC can elicit a rapid release of amylase from perifused acini immediately upon photon activation under conditions in which most of the SALPC is likely to be bound to the plasma membrane [5]. In contrast, the kinetics of arachidonic acid release with the same stimulation (SALPC 1 μM, 4500 lux) is delayed and distinctly biphasic (Fig. 2A). Arachidonic acid release was first suppressed, with peak suppression at the 65th min (ratio of radioactivity 0.61 ± 0.04, N = 6, $P < 0.05$). The inhibition of basal release was followed by marked stimulation (ratio of radioactivity at the 79th min 2.66 ± 0.15, N = 4, $P < 0.05$) and the balance between inhibition and stimulation was such that at the 73rd min, the rate of release was similar in both test and control acini (Fig. 2A).

With the same concentration of SALPC (1 μM) and greater light intensity (18,400 lux), the inhibition of basal release still persisted (ratio of radioactivity at the 61st min 0.76 ± 0.07, N = 6, $P < 0.05$) (Fig. 2B, inset). However, the onset of stimulation was more rapid (at the 67th min, $P < 0.05$; Fig. 2B, inset), and the stimulation was of a much greater magnitude (the ratio of radioactivity at the 79th min was 29.4 ± 7.7, N = 6; Fig. 2B).

Effects of A23187, mellitin and sodium fluoride on arachidonic acid mobilization

Arachidonic acid is an integral component of pancreatic plasma membrane phospholipid from which it is released via deacylation by activation of the Ca^{2+}-dependent enzyme PLA_2 [14]. Calcium influx following membrane permeabilization by photodynamic drug action may therefore activate PLA_2 and account for the stimulatory phase of arachidonic acid release, an effect which should be reproduced by the calcium ionophore A23187. It can be seen from Fig. 3 that A23187 1 μM evoked a rapid, almost 5-fold, increase in the release of arachidonic acid while vehicle alone (DMSO 0.1%) had no effect. Alternatively, PLA_2 may be stimulated more immediately by photon-activation of SALPC and the generation of singlet oxygen. We have therefore employed the direct PLA_2 activator mellitin [15] as a reference compound for comparison, and

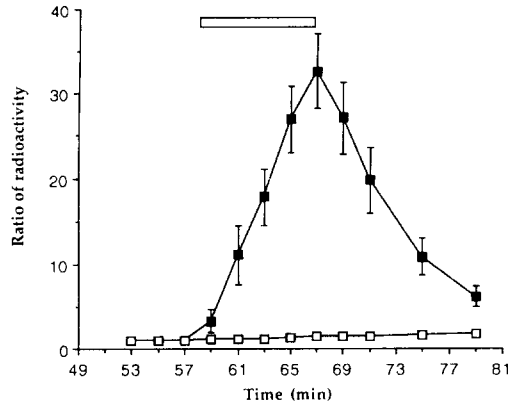

Fig. 4. Effect of mellitin on arachidonic acid release from pancreatic acini. Acini were exposed to mellitin 2 mg/mL (■) for 10 min, indicated by the horizontal bar; control experiments in the absence of mellitin (□) (N = 4).

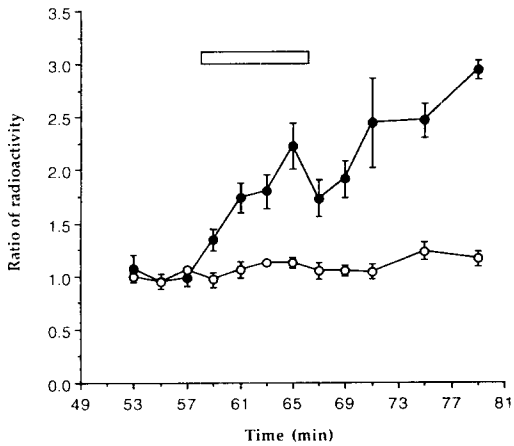

Fig. 5. Effect of sodium fluoride on arachidonic acid release from pancreatic acini. Acini were exposed to NaF 20 mM (●) for 10 min, indicated by the horizontal bar; control experiments in the absence of NaF (○) (N = 4).

Fig. 4 shows that mellitin 2 mg/mL produced a profound but reversible release of arachidonic acid with a peak 33-fold increase in output. In pancreatic cells arachidonic acid can also be released by sequential stimulation of PLC and diacylglycerol lipase [16]; activation of PLC in the exocrine pancreas also involves membrane G-proteins [17]. It is therefore of particular interest to note that NaF which directly activates G-proteins [18] and stimulates a secretory response in isolated rat pancreatic acini [19] produced a time-dependent 2.9-fold increase in arachidonic acid output (Fig. 5).

Photodynamic action of SALPC on amylase release and PGE_2 production

Photodynamic drug action leads to eicosanoid production in a number of tissues [20] and pancreatic

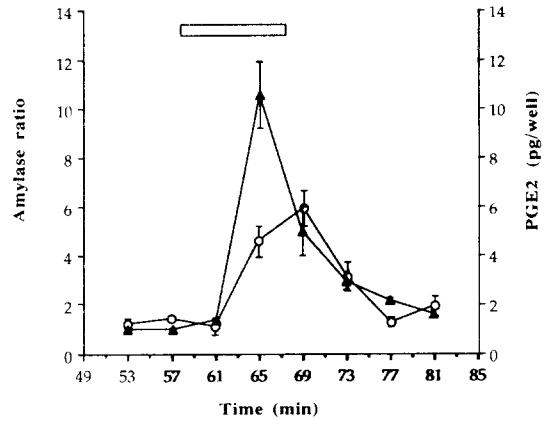

Fig. 6. Photodynamic action on SALPC on amylase release and PGE_2 production by pancreatic acini. Acini were exposed to SALPC 1 μM from the 34th to the 44th min of perifusion and irradiated at 18,400 lux for 10 min at the time indicated by the horizontal bar. Measurements, in the same experiment, of amylase release (▲) and PGE_2 production (○) (N = 7).

acini are known to be capable of synthesizing and releasing PGE_2 from arachidonic acid via the cyclo-oxygenase pathway [21]. In order to gain more insight into the turnover of arachidonic acid and its metabolites, PGE_2 release from pancreatic acini was measured during the photodynamic activation of SALPC. In these experiments it was also possible to monitor in parallel the release of amylase to define further the time course of SALPC action. Comparative effects of the direct stimulator of PLA_2, mellitin, were also studied. Measurements of PGE_2 and amylase in the same experiments (Fig. 6) reveal that when membrane-bound SALPC is activated by light, peak amylase release ($10.6 \pm 1.3 \times$ basal; $P < 0.001$, $N = 7$) precedes peak PGE_2 production ($5.2 \pm 0.9 \times$ basal; $P < 0.001$, $N = 7$). The cyclo-oxygenase inhibitor indomethacin 10 μM blocked almost completely the photodynamic release of PGE_2 (Fig. 7B) or that evoked by mellitin 2 μg/mL (Fig. 8B). In contrast, in the same experiments indomethacin had no effect on the release of amylase caused either by photodynamic action (Fig. 7A) or by mellitin (Fig. 8A). It can also be seen that although mellitin, a potent activator of PLA_2 and arachidonic acid mobilization (Fig. 4), caused PGE_2 production it had very little effect on amylase release when compared to the photodynamic action of SALPC (i.e. Figs 7B and 8B; and Figs 7A and 8A). These experiments therefore confirm that prostaglandin production is not obligatory for amylase release [21].

DISCUSSION

CCK receptor activation initiates the release of hydrolase enzymes from pancreatic acinar cells. However, the signal transduction pathway from the membrane, i.e. receptor occupation → G-protein activation → IP_3 generation → $[Ca^{2+}]_i$ mobilization

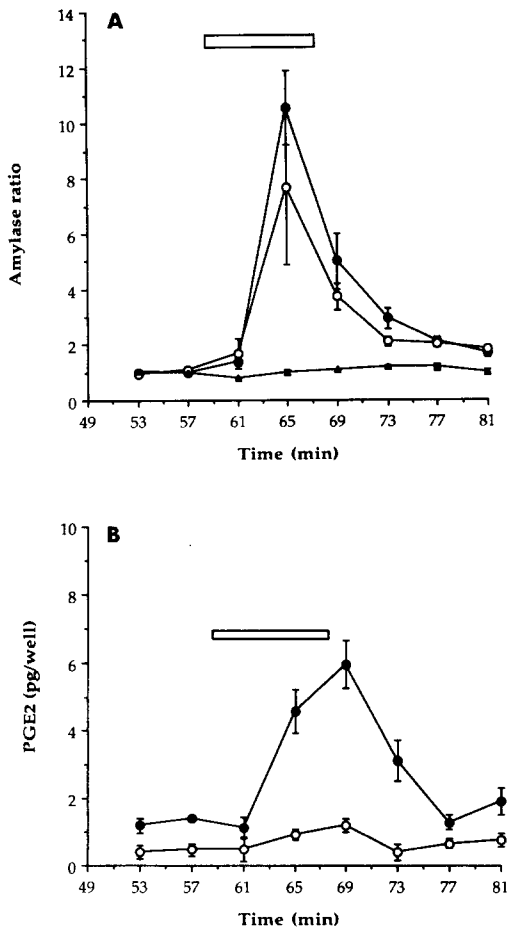

Fig. 7. Photodynamic action of SALPC on amylase release and PGE_2 production by pancreatic acini: effect of indomethacin. Acini were exposed to SALPC 1 μM from the 34th to the 44th min of perifusion and irradiated at 18,400 lux for 10 min at the time indicated by the horizontal bar. (A) Amylase release in the presence (○) or absence (●) of indomethacin 10 μM; control experiments without SALPC treatment (▲). (B) PGE_2 production in the presence (○) or absence (●) of indomethacin 10 μM (N = 3–7).

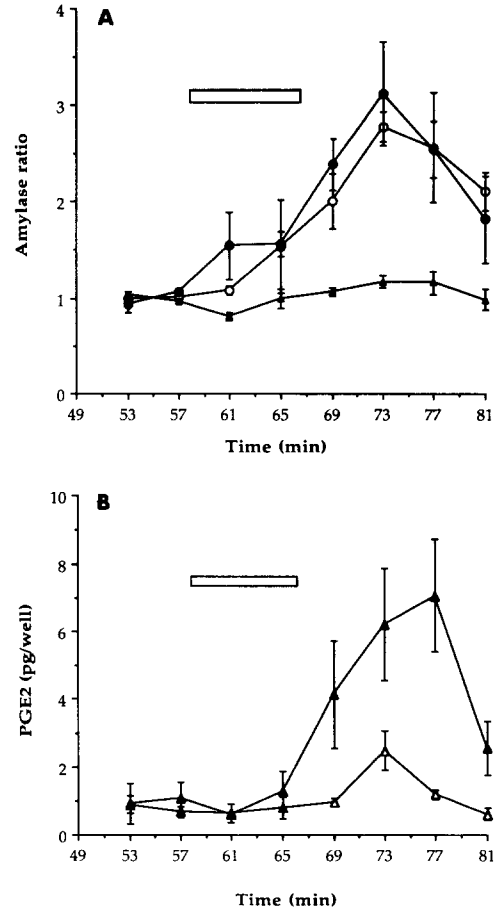

Fig. 8. Action of mellitin on amylase release and PGE_2 production by pancreatic acini: effect of indomethacin. Acini were exposed to mellitin 2 mg/mL for 10 min, at the time indicated by the horizontal bar. (A) Amylase release in the presence (○) or absence (●) of indomethacin 10 μM; control experiments without mellitin treatment (▲). (B) PGE_2 production in the presence (△) or absence (▲) of indomethacin 10 μM (N = 3–4).

→ enzyme release, has a complex stoichiometry [7] because the CCK–amylase dose–response curve is bell-shaped with higher concentrations of CCK ($>10^{-10}$ M) causing less amylase release than lower concentrations [9]. It is not clear how closely related this profile of dose-dependent amylase release is to two different kinetic states of the CCK receptor or even to the existence of two distinct receptor subspecies, i.e. CCK_A and CCK_B [7]. At higher agonist concentrations the generation of cyclic GMP [9] or activation of protein kinase C acting negatively on the production of IP_3 and diacylglycerol may also be involved [22]. However our present results suggest an additional possibility since a progressive release of arachidonic acid in response to CCK occurred only at concentrations of agonist $> 10^{-10}$ M. At these high CCK concentrations it is likely that the IP_3-induced increase in $[Ca^{2+}]_i$ is sufficient to activate PLA_2 and so release arachidonic acid which in turn inhibits IP_3-dependent Ca^{2+} mobilization and shuts off amylase secretion. Direct evidence for such an effect of arachidonic acid in inhibiting the muscarinic receptor-linked Ca^{2+}-mobilizing signal has been found recently in whole-cell dialysis of pancreatic acinar cells [22].

It is evident from the kinetic experiments that SALPC had a biphasic photodynamic effect on the release of labelled arachidonic acid from pancreatic exocrine cells which was both time and light intensity dependent. There are, as pointed out already, two major routes for arachidonic acid liberation from pancreatic cell membranes. One is via stimulation of PLA_2 which will cleave the ester bond at the 2 position of glycerophospholipids. Arachidonic acid can also be released by sequential stimulation of PL (phosphoinosidase) C and diacylglycerol lipase [16]. Of these enzymes, pancreatic PLA_2 can be directly

activated by mellitin to release arachidonic acid [15], an action confirmed in our experiments. A similar activation, possibly via singlet oxygen generation, may also account for the photodynamic release of arachidonic acid. Alternatively, the influx of extracellular calcium accompanying photodynamic permeabilization of the exocrine cell membrane may trigger activation of PLA_2, an effect which was mimicked by the calcium ionophore, A23187. Photodynamic activation of the receptor signal transduction pathway outlined above will also result in an increase in $[Ca^{2+}]_i$ by mobilization of stored calcium and this may become sufficient to activate PLA_2. Furthermore, not only does this signalling pathway involve stimulation of PLC and liberation of diacylglycerol but transduction is initiated by receptor activation of G-proteins [17] which can be stimulated directly with NaF [19]. After short periods of exposure to highly negatively charged photosensitizers such as the sulphonated phthalocyanines it is likely that most of the photosensitizer uptake will be confined to the plasma membrane [5]. Photon activation of SALPC therefore may trigger, as does NaF, the membrane G-protein intracellular signalling pathway and release intracellular calcium. Activation of this pathway would not only account for the rapid photodynamic release of amylase by IP_3 generation and calcium release but also initiate the turnover of substrates via the second route by which arachidonic acid is released. However, to judge by the relative turnover of arachidonic acid and labelling of different phospholipid pools in the pancreas [15], the contribution of arachidonic acid mobilization by this sequential two-stage pathway is likely to be much smaller than that involving PLA_2 activation. The fact also that the release of arachidonic acid induced by NaF in the present experiments was modest even though F^- 20 mM is beyond the concentration producing maximum amylase release from pancreatic acini [19] gives additional support to this interpretation.

A further distinction between the output of arachidonic acid and photodynamic amylase release is seen in the earliest phase of photodynamic action because in the first few minutes of SALPC photon activation there is a marked release of amylase but arachidonic acid release is actually inhibited during this time. This initial decrease in arachidonic acid release may indicate a photodynamic inhibition of the enzymes responsible for basal arachidonic acid release or alternatively photo-oxidation may prompt the re-acylation of phospholipid and a temporarily decreased release of arachidonic acid because membrane phospholipid de-acylation and re-acylation are known to be rapid processes [14]. Finally, the measurements of PGE_2 and amylase in the same experiments showed clearly that when membrane-bound SALPC was activated by light, peak amylase release preceded peak PGE_2 production. Furthermore, indomethacin was able to block PGE_2 production but this had no effect upon the photodynamic release of amylase by SALPC.

In conclusion therefore, light activation of SALPC and singlet oxygen generation may elicit arachidonic acid mobilization via an action on G-proteins or via PLA_2, activated either directly or via calcium influx. However, (i) arachidonic acid mobilization is delayed, (ii) the photodynamic release of amylase precedes maximum PGE_2 output and (iii) amylase release is completely unaffected by an indomethacin block of PGE_2 production. This evidence now makes it much more likely that the initial photodynamic release of amylase from normal pancreatic cells induced by SALPC is mediated by rapid activation of the transduction signalling pathway involving the release of intracellular calcium. Future experiments will be directed more specifically towards establishing the extent to which this pathway participates in photodynamic action. The possibility does still exist however that arachidonic acid mobilization and eicosanoid production are linked to the longer-term cytolytic action of SALPC, especially in tumour cells, since in some cell lines [23, 24] photodynamic activation of PLA_2 may be a prime event for the induction of cellular apoptosis.

Acknowledgements—This work was supported by the Cancer Research Campaign, and Z. J. Cui by the British Council and St John's College, Cambridge.

REFERENCES

1. Matthews EK and Mesler DE, Photodynamic effects of erythrosine on the smooth muscle cells of guinea-pig taenia coli. *Br J Pharmacol* **83**: 555–566, 1984.
2. Yonuschot G, Matthews EK, Corps AN and Metcalfe JC, Permeabilization of thymocytes by photon activation of erythrosin. *FEBS Lett* **213**: 401–405, 1987.
3. Matthews EK and Cui ZJ, Photodynamic action of rose bengal on isolated rat pancreatic acini: stimulation of amylase release. *FEBS Lett* **256**: 29–32, 1989.
4. Matthews EK and Cui ZJ, Photodynamic action of sulphonated aluminium phthalocyanine (SALPC) on AR4-2J cells, a carcinoma cell-line of rat exocrine pancreas. *Br J Cancer* **61**: 695–770, 1990.
5. Matthews EK and Cui ZJ, Photodynamic action of sulphonated aluminium phthalocyanine (SALPC) on isolated rat pancreatic acini. *Biochem Pharmacol* **39**: 1445–1457, 1990.
6. Marshall PJ, Boatman DE and Hokin LE, Direct demonstration of the formation of prostaglandin E_2 due to phosphatidylinositol breakdown associated with stimulation of enzyme secretion in the pancreas. *J Biol Chem* **256**: 844–847, 1981.
7. Jensen RT, Wark SA, Rowley WH, Sato S and Gardner JD, Interaction of CCK with pancreatic acinar cells. *Trends Pharmacol Sci* **10**: 418–423, 1989.
8. Matozaki T and Williams JA, Multiple sources of 1,2-diacylglycerol in isolated rat pancreas acini stimulated by cholecystokinin. Involvement of phosphatidylinositol bisphosphate and phosphatidylcholine hydrolysis. *J Biol Chem* **264**: 14729–14734, 1989.
9. Rogers J, Hughes RG and Matthews EK, Cyclic GMP inhibits protein kinase c-mediated secretion in rat pancreatic acini. *J Biol Chem* **263**: 3713–3719, 1988.
10. Kim D and Clapham DE, Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. *Science* **244**: 1174–1176, 1989.
11. Ordway RW, Walsh JV and Singer JJ, Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. *Science* **244**: 1176–1179, 1989.
12. Kurachi Y, Ito H, Sugimoto T, Shimizu T, Miki I and Ui M, Arachidonic acid metabolites as intracellular

modulators of the G-protein gated cardiac K^+-channel. *Nature* **337**: 555–557, 1989.
13. Axelrod J, Burch RM and Jelsema CL, Receptor-mediated activation of phospholipase A_2 via GTP-binding proteins: arachidonic acid and its metabolites as second messengers. *Trends Neurosci* **11**: 117–123, 1988.
14. Halenda SP and Rubin RP, Phospholipid turnover in isolated rat pancreatic acini. *Biochem J* **208**: 713–721, 1982.
15. Heisler S, Phospholipase A_2 activation by mellitin causes amylase release from exocrine pancreas. *Can J Physiol Pharmacol* **67**: 411–416, 1989.
16. Dixon JF and Hokin LE, Secretagogue-stimulated phosphatidylinositol breakdown in the exocrine pancreas liberates arachidonic acid, stearic acid and glycerol by sequential actions of phospholipase C and diglyceride lipase. *J Biol Chem* **259**: 14418–14425, 1984.
17. Merritt JE, Taylor CW, Rubin RP and Putney JW, Evidence suggesting that a novel guanine nucleotide regulatory protein couples receptors to phospholipase C in exocrine pancreas. *Biochem J* **236**: 337–343, 1986.
18. Cockroft S and Taylor JA, Fluoroaluminates mimic guanosine 5′ [r-thio] triphosphate in activating the polyphosphoinositide phosphodiesterate of hepatocyte membrane. *Biochem J* **241**: 409–414, 1987.
19. Habara Y, Satoh Y, Saito T and Kanno T, A G-protein activator, NaF, induces $[Ca^{2+}]_o$-dependent $[Ca^{2+}]_c$ oscillation and secretory response in rat pancreatic acini. *Biomed Res* **11**: 389–398, 1990.
20. Henderson BW and Donovan JM, Release of prostaglandin E_2 from cells by photodynamic treatment *in vitro*. *Cancer Res* **49**: 6896–6900, 1989.
21. Baudin H, Galard N and Boeynaems JM, *In vitro* stimulation of prostaglandin synthesis in the rat pancreas by carbamylcholine, caerulein and secretin. *Prostaglandins* **22**: 35–51, 1981.
22. Maruyama Y, Inhibitory effects of arachidonic acid on muscarinic current response in single pancreatic acinar cells of rats. *J Physiol* **430**: 471–482, 1990.
23. Agarwal ML, Clay ME, Harvey EJ, Evans HH, Antunez AR and Oleinick NL, Photodynamic therapy induces rapid cell death by apoptosis in L5178Y mouse lymphoma cells. *Cancer Res* **51**: 5993–5996, 1991.
24. Oleinick NL, Agarwal ML, Antunez AR, Aulfinger H and He J, Signals induced by photodynamic therapy during cell death by apoptosis. *Lasers Med Sci* **7**: 263, 1992.