

# Ca<sup>2+</sup>-Independent Phospholipase A<sub>2</sub> Contributes to the Insulinotropic Action of Cholecystokinin-8 in Rat Islets

## Dissociation From the Mechanism of Carbachol

Erik Simonsson, Sven Karlsson, and Bo Ahrén

Insulin secretion induced by cholecystokinin-8 (CCK-8) was recently suggested to involve phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activation. In this study, we examined whether CCK-8 stimulates the Ca<sup>2+</sup>-independent form of PLA<sub>2</sub> in isolated rat islets, in comparison with stimulation by the PLA<sub>2</sub>-activating cholinergic agonist carbachol. We found that CCK-8 (100 nmol/l; 5.6 mmol/l glucose) induces lysophosphatidylcholine accumulation from [<sup>3</sup>H]palmitate-prelabeled islets (170 ± 39%; *P* = 0.003) as well as arachidonic acid (AA) efflux from [<sup>3</sup>H]AA-prelabeled islets (190 ± 13%; *P* < 0.001), and that *p*-amylcinnamoylantranilic acid (ACA) (50 μmol/l)-mediated PLA<sub>2</sub> inhibition reduces CCK-8-induced AA efflux (52 ± 11%; *P* = 0.001) and insulin secretion (67 ± 16%; *P* < 0.001). Neither the Ca<sup>2+</sup> channel antagonist verapamil (100 μmol/l) nor the Ca<sup>2+</sup>ATPase inhibitor thapsigargin (1 μmol/l) affected CCK-8-induced AA efflux and insulin secretion. Furthermore, despite removal of extracellular Ca<sup>2+</sup>, CCK-8 still increased AA efflux (48 ± 14%; *P* = 0.006) and insulin secretion (105 ± 46%; *P* = 0.025). In contrast, carbachol (100 μmol/l)-stimulated AA efflux was reduced by verapamil by 36 ± 6% (*P* < 0.001) and abolished by removal of extracellular Ca<sup>2+</sup>. Overnight protein kinase C (PKC) downregulation by 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) (500 nmol/l) reduced CCK-8-induced AA efflux (45 ± 12%; *P* = 0.003) and insulin secretion (40 ± 16%; *P* = 0.020). No additive action regarding either AA formation or insulin secretion was seen by combining TPA overnight and ACA, which implies the involvement of an additional PLA<sub>2</sub>- and PKC-independent signaling mechanism. The results show that CCK-8, in contrast to carbachol, activates Ca<sup>2+</sup>-independent PLA<sub>2</sub> in islets and that the PLA<sub>2</sub>-activating capacity of CCK-8 is partly PKC dependent. Hence, Ca<sup>2+</sup>-independent PLA<sub>2</sub> seems important for the insulinotropic effect of CCK-8, but not for that of carbachol. *Diabetes* 47:1436–1443, 1998

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AA, arachidonic acid; ACA, *p*-amylcinnamoylantranilic acid; CCK-8, cholecystokinin-8; PKC, protein kinase C; PLA<sub>2</sub>, phospholipase A<sub>2</sub>; PLC, phospholipase C; RHC 80267, 1,6-bis(cyclohexylideneamino)oxycarbonylamino-hexane; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.

Besides circulating nutrients, insulin secretion is affected by a variety of hormones and neuropeptides (1,2). The role of these peptides in islet physiology as well as their putative involvement in the regulation of insulin release in states of type 2 diabetes is largely unknown. Cholecystokinin is a gastrointestinal hormone released during meal intake (3), and also a pancreatic neuropeptide (4). Its COOH-terminal octapeptide (cholecystokinin-8 [CCK-8]) has been shown to activate specific CCK receptors on the β-cell (5) and to stimulate insulin secretion (6). CCK-8 is released into the circulation upon food intake and has been suggested to participate physiologically in the regulation of postprandial insulin secretion (7). The postprandial release of CCK-8 has been shown to be reduced in patients with type 2 diabetes, and the concomitant rise in blood glucose has been shown to be abolished by CCK-8 infusion (8). A putative role for CCK-8 in type 2 diabetes has been further underlined by studies in different animal models, which show a coupling between type 2 diabetes predisposition and insensitivity to CCK-8 stimulation (9) and an enhanced compensatory β-cell responsiveness to CCK-8 during type 2 diabetes development (10). Because CCK-8 thus seems to be a physiological regulator of insulin secretion with a possible involvement in the pathophysiology of type 2 diabetes, its mechanisms of action in the β-cell are important to delineate.

In islets, CCK-8 is known to stimulate phospholipase C (PLC) (11,12) to release Ca<sup>2+</sup> from intracellular Ca<sup>2+</sup> stores (13) and to activate protein kinase C (PKC) (14). However, we presented results implying that this pathway is not the sole signaling mechanism underlying CCK-8-stimulated insulin secretion, because PKC downregulation only partially reduces CCK-8-stimulated insulin secretion (14). Instead, we suggested that phospholipase A<sub>2</sub> (PLA<sub>2</sub>) is also of importance for CCK-8-stimulated insulin secretion, because *p*-amylcinnamoylantranilic acid (ACA), which is a specific PLA<sub>2</sub> inhibitor (15), diminished CCK-8-stimulated insulin secretion from isolated rat islets (16). A role for PLA<sub>2</sub> in CCK-8-stimulated insulin secretion is further supported by the finding that the peptide promoted efflux of [<sup>3</sup>H]arachidonic acid (AA) from prelabeled islets, which reflects PLA<sub>2</sub> activity (17), concomitantly with its stimulation of insulin secretion (16). Therefore, analogous to the studies on CCK-8-induced amylase secretion in rat pancreatic acini (18), activation of PLA<sub>2</sub>

and subsequent formation of AA might also be of importance for the insulinotropic effect of CCK-8.

PLA<sub>2</sub> activation with formation of AA has previously been proposed as an important intracellular signaling pathway that mediates a variety of regulatory influences in several different cell systems (19,20). PLA<sub>2</sub> has been shown to be present within both human and rat islets (21–23), where it is assumed to exist in a Ca<sup>2+</sup>-dependent (24) and a Ca<sup>2+</sup>-independent ATP-stimulatable (25) form, both participating in the glucose-dependent insulin secreting process (26,27). Ca<sup>2+</sup>-independent ATP-stimulatable PLA<sub>2</sub> has been proposed as an alternative way for glucose metabolism to generate insulin secretion when only small increments in ATP production are possible (27). It is not known, however, to what extent this PLA<sub>2</sub> form is sensitive to CCK-8 stimulation and whether it differs from stimulation by the cholinergic agonist carbachol, which, like CCK-8, is known to activate PLA<sub>2</sub> in islets (17). Neither is it known whether there is an interrelationship between CCK-8-stimulated PLA<sub>2</sub> activation and the signaling pathway involving PKC, which is of importance for CCK-8-stimulated insulin release (7).

Therefore, in the present study, we have examined the ability of CCK-8 to stimulate Ca<sup>2+</sup>-independent PLA<sub>2</sub> in isolated rat islets compared with the ability of carbachol. To investigate the contribution of PLA<sub>2</sub>, we examined AA efflux, lysophosphatidylcholine accumulation, and insulin secretion from prelabeled islets in the presence and absence of the PLA<sub>2</sub> inhibitor ACA (15) and the diglyceride lipase inhibitor RHC 80267 (1,6-biscyclohexylideneaminoxy-carbonylamino-hexane) (17). The dependence on extracellular Ca<sup>2+</sup> influx was studied by using the Ca<sup>2+</sup> channel inhibitor verapamil (17) and by removal of extracellular Ca<sup>2+</sup>, whereas the dependence on intracellular Ca<sup>2+</sup> stores was investigated by the use of the Ca<sup>2+</sup>-ATPase inhibitor thapsigargin (28). Furthermore, the possible interrelationship between CCK-8-induced PLA<sub>2</sub> activation and PKC was examined by studying islets subjected to PKC downregulation induced by an overnight incubation with the phorbol ester 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) (29).

## RESEARCH DESIGN AND METHODS

**Animals.** The experiments were performed on pancreatic islets isolated from male Sprague-Dawley rats (Møllegaard A/S, Li. Skensved, Denmark), weighing 200–300 g, with free access to a standard pellet diet and tap water.

**Isolation and incubation of islets.** Islets were isolated by the collagenase digestion technique (30). In brief, the common bile duct was cannulated and ligated at the papilla Vateri. The pancreas was filled through the cannula with 10 ml of cold Hanks' balanced salt solution (Sigma, St. Louis, MO), supplemented with 1.0 mg/ml collagenase P (activity: 1.52 U/mg; Boehringer Mannheim, Mannheim, Germany). The pancreas was then removed and incubated for 24 min at 37°C. After being rinsed in Hanks' solution, selected islets were hand-picked under a stereomicroscope and cultured overnight in 10 ml of RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2.05 mmol/l L-glutamine, 2.5 µg/ml amphotericin B (all GIBCO BRL, Paisley, Scotland), 100 IU/ml penicillin, and 100 µg/ml streptomycin (both Biological Industries, Beit Haemek, Israel) at 37°C (pH 7.4) in a humid atmosphere of 95% air and 5% CO<sub>2</sub>. After one night's incubation, the islets were rinsed in a modified HEPES medium (pH 7.4) consisting of 125 mmol/l NaCl, 5.9 mmol/l KCl, 1.2 mmol/l MgCl<sub>2</sub>, 1.28 mmol/l CaCl<sub>2</sub>, and 25 mmol/l HEPES (Boehringer Mannheim), supplemented with 0.1% human serum albumin (Behringwerke, Marburg, Germany) and 3.3 mmol/l glucose (Fluka Chemie AG, Buchs, Switzerland). The islets were then pre-incubated for 30 or 60 min in the modified HEPES medium at 3.3 mmol/l glucose in an atmosphere of 5% CO<sub>2</sub> at 37°C (pH 7.4). Thereafter, groups of islets were transferred into separate chambers for 30 or 60 min of incubation in air equilibrated with 5% CO<sub>2</sub> at 37°C (pH 7.4) in the HEPES medium supplemented with 0.1% human serum albumin, with or without addition of synthetic sulfated CCK-8 (the COOH-terminal octapeptide of cholecystokinin; CCK<sub>26–33</sub>; 100 nmol/l; Sigma), carbachol (100

µmol/l; BDH Chemicals, Poole, England, U.K.), and different inhibitors. The glucose concentration in the medium was 5.6 mmol/l, which previously has been shown to promote a maximal insulin-releasing effect of CCK-8 (16). The HEPES medium was continuously gassed in an atmosphere of 95% air and 5% CO<sub>2</sub>.

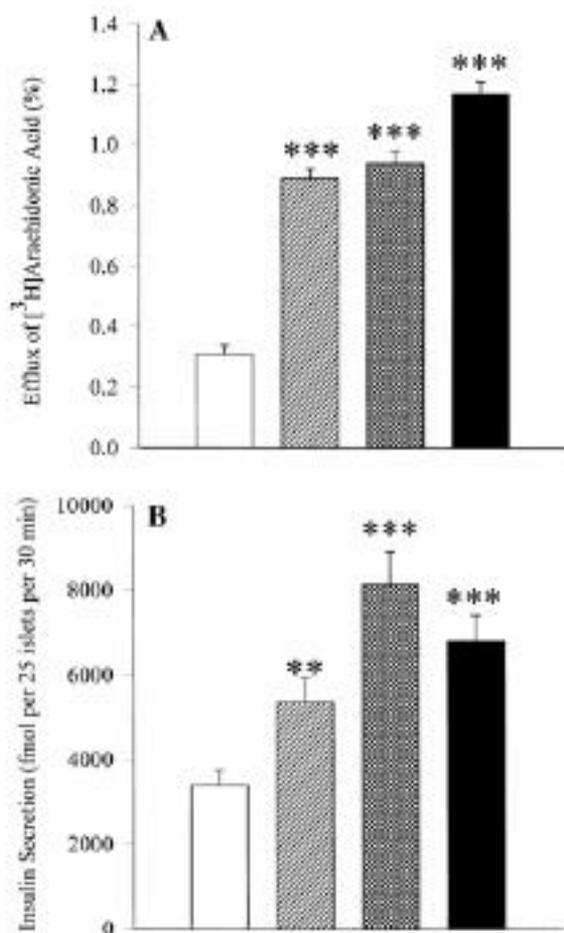
**Induction of PKC downregulation.** Isolated islets were incubated overnight at 37°C (pH 7.4) in an atmosphere of 5% CO<sub>2</sub> in 10 ml of RPMI 1640 (complete as above) in the presence of the phorbol ester TPA (500 nmol/l; Sigma). Prolonged exposure to TPA has previously been shown to downregulate PKC activity in pancreatic islets (28). The control islets were incubated overnight with the phorbol ester 4 $\alpha$ -phorbol 12,13-didecanoate (Sigma; 500 nmol/l), which is without activity regarding PKC (29).

**Incubation of [<sup>3</sup>H]AA-labeled islets.** Islets, isolated as above, were incubated overnight in 10 ml of RPMI 1640 (complete as above) supplemented with 4 µCi of [<sup>3</sup>H]AA (specific activity: 100 Ci/mmol; Du Pont-NEN, Boston, MA) at 37°C (pH 7.4) in an atmosphere of 95% air and 5% CO<sub>2</sub>. After the overnight incubation in [<sup>3</sup>H]AA, the islets were pre-incubated for 30 min and then transferred in groups of 25 into separate chambers, where they were incubated for another 30 min in 1 ml of HEPES medium supplemented with 0.1% human serum albumin and 5.6 mmol/l glucose at 37°C (pH 7.4) in air equilibrated with 5% CO<sub>2</sub>, with or without the addition of CCK-8 (100 nmol/l), carbachol (100 µmol/l), ACA (50 µmol/l; Calbiochem, La Jolla, CA), RHC 80267 (1,6-biscyclohexylideneaminoxy-carbonylamino-hexane; 35 µmol/l; Calbiochem), thapsigargin (1 µmol/l; Sigma), and verapamil (100 µmol/l; Sigma). The studies performed after removal of extracellular Ca<sup>2+</sup> were executed as described above, with the exception that the incubation medium, both during the pre-incubation and the experimental incubation, was composed without CaCl<sub>2</sub> and supplemented with 0.1 mmol/l of the calcium chelator EGTA (Sigma). After the incubation, 500 µl of the medium surrounding the islets was taken from each chamber, and the radioactivity was determined by liquid scintillation counting using the scintillation cocktail Optiphase (Wallac Oy, Turku, Finland). The 25 islets were retrieved as well, and their radioactivity was determined. Then, the radioactivity in the medium removed was expressed as a percentage of the total islet radioactivity. Another 25 µl of the medium was removed and frozen at –18°C until it was analyzed for its content of insulin.

**Extraction of AA.** AA was extracted as previously described by Hou et al. (31). After the overnight incubation in [<sup>3</sup>H]AA, the islets were preincubated for 30 min. Thereafter, groups of 50 islets were incubated for another 30 min in 1 ml of HEPES medium supplemented with 0.1% human serum albumin and 5.6 mmol/l glucose (37°C; pH 7.4; 95% air/5% CO<sub>2</sub>), with or without addition of CCK-8 (100 nmol/l). After incubation, 750 µl of the medium of two separate chambers was retrieved and transferred to polypropylene tubes, thus containing 1.5 ml of the medium each. The tubes were frozen and stored at –18°C until thin-layer chromatography analysis of AA was performed. Before the extraction, 25 µl of non-labeled AA (0.5 mg/ml; Sigma) was added to each tube to improve recovery. Then, 1.5 ml of methanol/chloroform/HCl (200:200:1 by volume) were added to the 1.5 ml medium. The tubes were vortex-mixed for 1 min, when the aqueous phase was re-extracted twice with 1.5 ml CHCl<sub>3</sub>. The samples were centrifuged for 5 min at 4°C and 1,500 rpm after each extraction step. The lower organic phase of each extraction was transferred to a new polypropylene tube. Thereafter, the organic phase was concentrated under a steam of nitrogen and reconstituted in 55 µl of chloroform.

**One-dimensional thin-layer chromatography analysis of AA.** A sample (25 µl) from each tube was applied to 10 × 20 cm silica-gel thin-layer chromatography plates (Merck, Darmstadt, Germany). Another 25-µl sample was removed for detection of total radioactivity. The plates were developed with petroleum spirit 40–60/diethyl ether/acetic acid (60:45:1 by volume). AA was identified by an unlabeled AA standard and detected by I<sub>2</sub> vapor. The AA R<sub>f</sub> value was 0.040. The curettag of the AA spot was examined for its content of radioactivity, which was determined by liquid scintillation counting. Radioactivity in each peak is expressed as a percentage of the total counts of the sample.

**Extraction of lysophospholipids.** Lysophospholipids were extracted as previously described by Konrad et al. (17). Islets, isolated as above, were incubated overnight in 10 ml of RPMI 1640 (complete as above) supplemented with 40 µCi of [<sup>3</sup>H]palmitic acid (specific activity: 51 Ci/mmol; Du Pont-NEN) at 37°C (pH 7.4) in an atmosphere of 95% air and 5% CO<sub>2</sub>. After the overnight incubation in [<sup>3</sup>H]palmitic acid, the islets were pre-incubated for 30 min. Thereafter, groups of 50 islets were incubated for another 30 min in 1 ml of HEPES medium supplemented with 0.1% human serum albumin and 5.6 mmol/l glucose (37°C; pH 7.4; 95% air/5% CO<sub>2</sub>), with or without addition of CCK-8 (100 nmol/l). After incubation, 50 islets and 250 µl of the medium of four separate chambers were retrieved and transferred to polypropylene tubes, thus containing 200 islets and 1 ml medium each. Two milliliters of ice-cold chloroform/methanol (1:2 by volume) supplemented with 0.25% of the antioxidant butylated hydroxytoluene was added. The tubes were then immediately chilled for 15 min in a dry ice/ethanol bath and stored at –18°C until the thin-layer chromatography of lysophospholipid analysis was performed. Before the thin-layer chromatography, 10 µl of a phospholipid standard mixture (Sigma) containing lysophosphatidylcholine (0.3 mg/ml), phosphatidylcholine (1.5



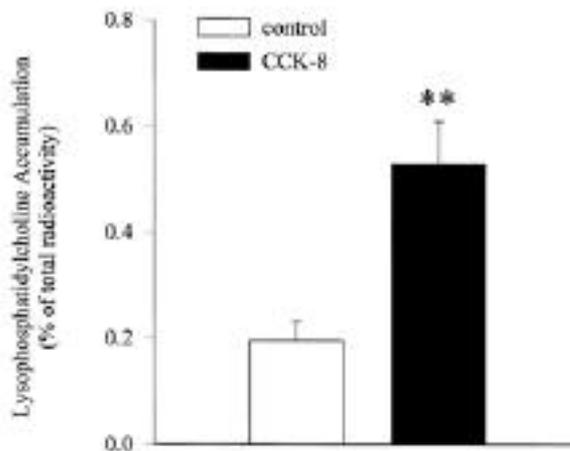
**FIG. 1.** Fractional efflux of [<sup>3</sup>H]AA expressed as the percentage release of total incorporated radioactivity (**A**) and concomitant insulin secretion (**B**) from prelabeled isolated islets incubated for 30 min in the presence of glucose (5.6 mmol/l), with or without CCK-8 (100 nmol/l) and carbachol (100  $\mu$ mol/l).  $n = 21$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  versus control. □, control; ▨, CCK-8; ▩, carbachol; ■, CCK-8 and carbachol.

mg/ml), phosphatidylethanolamine (1.2 mg/ml), and phosphatidylinositol (0.9 mg/ml) were added to each tube to aid in recovery, followed by 1 ml chloroform. The tubes were then vortex-mixed for 1 min, sonicated for 30 min, vortex-mixed for another 1 min, and centrifuged for 15 min at 4°C and 800g. Then, the lower organic phase was transferred to a polypropylene tube. The upper aqueous phase was re-extracted twice with 1 ml chloroform, whereupon the extracts were combined with the previously transferred organic phase. After that, the organic phase was washed with 1 ml of water, concentrated twice under a steam of nitrogen, and reconstituted in 25  $\mu$ l of chloroform containing the above-mentioned standard lipids, to visualize the spots on the two-dimensional thin-layer chromatography.

**Two-dimensional thin-layer chromatography analysis of lysophospholipids.** A sample (10  $\mu$ l) from each tube was applied to 10  $\times$  10 cm silica-gel thin-layer chromatography plates (Merck). Another 10  $\mu$ l sample was removed for detection of total radioactivity. Then, the plates were developed in the first dimension with chloroform/methanol/28% NH<sub>4</sub>OH (130:70:11 by volume). After being carefully dried, the plates were developed in the second dimension with chloroform/methanol/formic acid/water (55:28.5:1 by volume). Radioactivity in each peak is expressed as a percentage of the total counts of the sample. The identity of the peaks was assigned by comparison with the unlabeled phospholipid standards. The lysophosphatidylcholine  $R_f$  value was 0.04 in the first dimension and 0.07 in the second dimension.

**Analysis of insulin.** After incubation, 25  $\mu$ l of the medium surrounding the islets were removed and frozen at -18°C for subsequent analysis of insulin. The analysis was performed radioimmunochemically, using a guinea pig anti-rat insulin antibody, mono [<sup>125</sup>I]-labeled human insulin as a tracer and rat insulin as standard (Linco Research, St. Charles, MO). For the separation of free and bound radioactivity, the double antibody technique was used.

**Statistical analysis.** All values are presented as means  $\pm$  SE. Student's  $t$  test for unpaired data was used for statistical evaluation. A  $P$  value of  $P < 0.05$  was considered significant.



**FIG. 2.** Lysophosphatidylcholine accumulation expressed as the percentage ratio of total radioactivity from isolated islets prelabeled with [<sup>3</sup>H]palmitidic acid and incubated for 30 min in the presence of glucose (5.6 mmol/l), with or without CCK-8 (100 nmol/l).  $n = 4-6$ , \*\* $P < 0.01$  versus control.

## RESULTS

**Effect of CCK-8 and carbachol on [<sup>3</sup>H]AA efflux, lysophosphatidylcholine accumulation, and insulin secretion.** To establish the involvement of PLA<sub>2</sub> in CCK-8-stimulated and carbachol-stimulated insulin secretion, we examined the efflux of radioactivity and the concomitant insulin secretion during a 30-min incubation period from islets prelabeled with [<sup>3</sup>H]AA (Fig. 1). We found that CCK-8 (100 nmol/l) increased efflux of [<sup>3</sup>H]AA by  $190 \pm 13\%$  ( $P < 0.001$ ;  $n = 21$ ) and insulin secretion by  $58 \pm 20\%$  ( $P = 0.006$ ;  $n = 21$ ). To confirm that the detected radioactivity was indeed [<sup>3</sup>H]AA, its nature was characterized by thin-layer chromatography. We found that  $60 \pm 9\%$  of the radioactivity released from the islets was detected at the AA band on the thin-layer chromatography plate. We also found that the amount of AA compared with controls increased by  $309 \pm 49\%$  after CCK-8 stimulation ( $P < 0.001$ ;  $n = 5$ ). To further establish the involvement of PLA<sub>2</sub>, we also examined the effect of CCK-8 on lysophosphatidylcholine accumulation from islets prelabeled with [<sup>3</sup>H]palmitidic acid (Fig. 2). Thereby, we found that CCK-8 (100 nmol/l) increased lysophosphatidylcholine accumulation by  $170 \pm 39\%$  ( $P = 0.003$ ;  $n = 4-6$ ).

Furthermore, carbachol (100  $\mu$ mol/l) enhanced efflux of [<sup>3</sup>H]AA by  $203 \pm 16\%$  ( $P < 0.001$ ;  $n = 21$ ) and insulin secretion by  $140 \pm 21\%$  ( $P < 0.001$ ;  $n = 21$ ). CCK-8 and carbachol in combination increased [<sup>3</sup>H]AA efflux by  $277 \pm 15\%$  ( $P < 0.001$ ;  $n = 21$ ) and insulin secretion by  $101 \pm 21\%$  ( $P < 0.001$ ;  $n = 21$ ). The effect on [<sup>3</sup>H]AA efflux due to the combination of carbachol and CCK-8 was additive when compared with the effect of either agonist alone. Thus, [<sup>3</sup>H]AA efflux after CCK-8 plus carbachol was  $24 \pm 6\%$  higher than the effect of CCK-8 alone ( $P < 0.001$ ;  $n = 21$ ) and  $31 \pm 6\%$  higher than the effect of carbachol alone ( $P < 0.001$ ;  $n = 21$ ). In contrast, regarding insulin secretion, no additional effect was seen by combining CCK-8 and carbachol (NS;  $n = 21$ ). Thus, both CCK-8 and carbachol are able to activate PLA<sub>2</sub> in rat islets.

**Effect of Ca<sup>2+</sup> channel inhibition on CCK-8-induced and carbachol-induced [<sup>3</sup>H]AA efflux and insulin secretion.** To study whether PLA<sub>2</sub> activation due to CCK-8 and carbachol was dependent on influx of extracellular Ca<sup>2+</sup>, we studied the

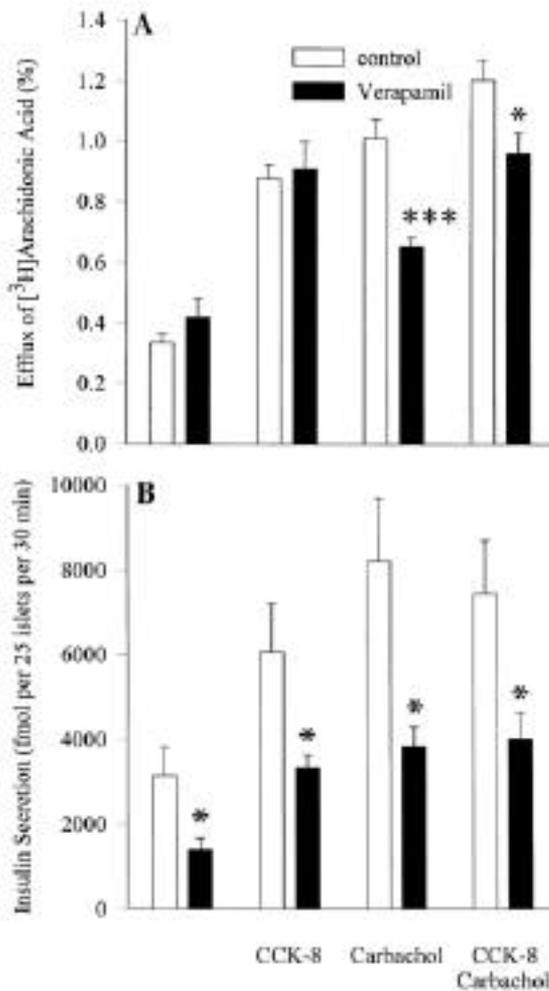


FIG. 3. Fractional efflux of [ $^3\text{H}$ ]AA expressed as the percentage release of total incorporated radioactivity (A) and concomitant insulin secretion (B) from pre-labeled isolated islets incubated for 30 min in the presence of glucose (5.6 mmol/l), with or without CCK-8 (100 nmol/l), carbachol (100  $\mu\text{mol/l}$ ), and verapamil (100  $\mu\text{mol/l}$ ).  $n = 9$ , \* $P < 0.05$ , \*\*\* $P < 0.001$  versus respective controls.

efflux of [ $^3\text{H}$ ]AA and the concomitant insulin secretion from pre-labeled islets incubated for 30 min together with the  $\text{Ca}^{2+}$  channel inhibitor verapamil (100  $\mu\text{mol/l}$ ; Fig. 3). It was found that the efflux of [ $^3\text{H}$ ]AA induced by CCK-8 (100 nmol/l) remained unchanged (NS;  $n = 9$ ). In contrast, the efflux induced by the cholinergic agonist carbachol (100  $\mu\text{mol/l}$ ) was, in the presence of 5.6 mmol/l glucose, reduced by  $36 \pm 7\%$  by verapamil ( $P < 0.001$ ;  $n = 9$ ). This was equivalent to a reduction by  $66 \pm 12\%$  of the net carbachol-stimulated efflux (i.e., after subtracting efflux values of control islets incubated with glucose alone). CCK-8-mediated insulin secretion was reduced by  $45 \pm 20\%$  ( $P = 0.036$ ;  $n = 9$ ), and carbachol-mediated insulin secretion was reduced by  $53 \pm 19\%$  ( $P = 0.013$ ;  $n = 9$ ) by verapamil. When CCK-8 and carbachol were combined, efflux of [ $^3\text{H}$ ]AA was diminished by  $20 \pm 8\%$  ( $P = 0.021$ ;  $n = 9$ ) and insulin secretion was diminished by  $54 \pm 19\%$  ( $P = 0.027$ ;  $n = 9$ ). Thus,  $\text{Ca}^{2+}$  channel blockade by verapamil reduces carbachol- but not CCK-8-induced  $\text{PLA}_2$  activation. **Effect of  $\text{Ca}^{2+}$  omission on CCK-8- and carbachol-induced [ $^3\text{H}$ ]AA efflux and insulin secretion.** To further investigate if  $\text{PLA}_2$  activation due to CCK-8 and carbachol

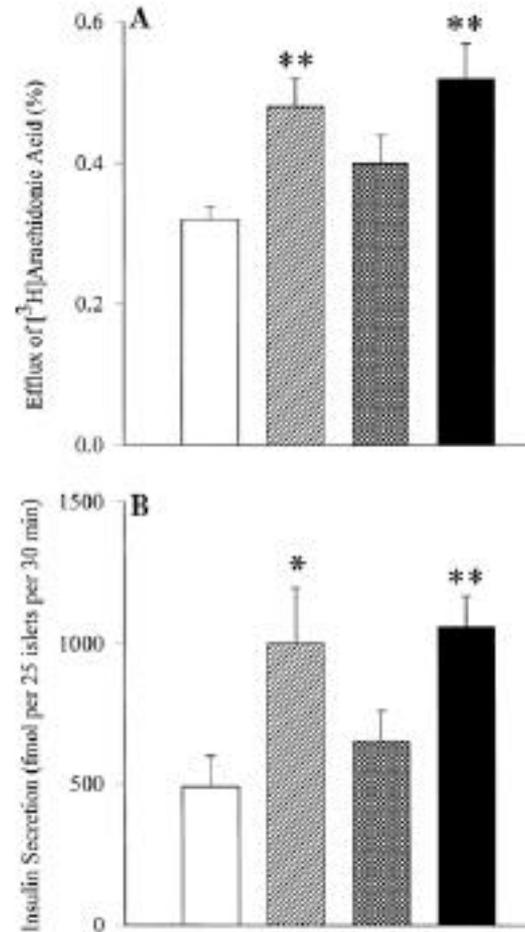


FIG. 4. Fractional efflux of [ $^3\text{H}$ ]AA expressed as the percentage release of total incorporated radioactivity (A) and concomitant insulin secretion (B) from pre-labeled isolated islets incubated for 30 min in a calcium-deficient medium in the presence of glucose (5.6 mmol/l), with or without CCK-8 (100 nmol/l) and carbachol (100  $\mu\text{mol/l}$ ).  $n = 6$ , \* $P < 0.05$ , \*\* $P < 0.01$  versus respective controls. □, control; ▨, CCK-8; ▩, carbachol; ■, CCK-8 and carbachol.

was dependent on uptake of extracellular  $\text{Ca}^{2+}$ , we studied the efflux of [ $^3\text{H}$ ]AA and the concomitant insulin secretion from pre-labeled islets incubated for 30 min in a medium where  $\text{Ca}^{2+}$  was removed (Fig. 4). Although CCK-8-mediated [ $^3\text{H}$ ]AA efflux and insulin secretion under these conditions were reduced by  $43 \pm 7\%$  ( $P < 0.001$ ;  $n = 6$ ) and  $78 \pm 14\%$  ( $P < 0.001$ ;  $n = 6$ ), respectively, CCK-8 still increased [ $^3\text{H}$ ]AA efflux by  $48 \pm 14\%$  ( $P = 0.006$ ;  $n = 6$ ) and insulin secretion by  $105 \pm 46\%$  ( $P = 0.025$ ;  $n = 6$ ) compared with controls. In contrast, in the absence of extracellular  $\text{Ca}^{2+}$ , carbachol was unable to alter either [ $^3\text{H}$ ]AA efflux or insulin secretion (NS;  $n = 6$ ). When combining CCK-8 and carbachol in the absence of extracellular  $\text{Ca}^{2+}$ , no additional effect was seen when compared with the effect of the separate agonists alone, either for [ $^3\text{H}$ ]AA efflux (NS;  $n = 6$ ) or insulin secretion (NS;  $n = 6$ ). Thus, omission of extracellular  $\text{Ca}^{2+}$  inhibits carbachol- but not CCK-8-induced  $\text{PLA}_2$  activation.

**Effect of intracellular  $\text{Ca}^{2+}$  store depletion on CCK-8-induced and carbachol-induced [ $^3\text{H}$ ]AA efflux and insulin secretion.** To examine whether  $\text{PLA}_2$  activation due to CCK-8 and carbachol was dependent on release of  $\text{Ca}^{2+}$

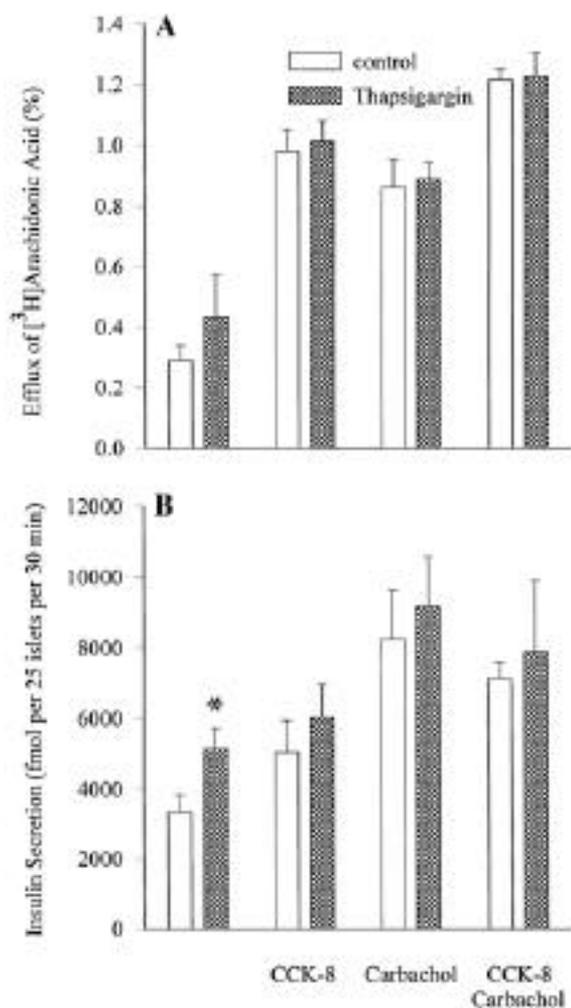


FIG. 5. Fractional efflux of [<sup>3</sup>H]AA expressed as the percentage release of total incorporated radioactivity (A) and concomitant insulin secretion (B) from prelabeled isolated islets incubated for 30 min in the presence of glucose (5.6 mmol/l), with or without CCK-8 (100 nmol/l), carbachol (100 μmol/l), and thapsigargin (1 μmol/l). *n* = 6, \**P* < 0.05.

from intracellular Ca<sup>2+</sup> stores, we studied the efflux of [<sup>3</sup>H]AA and the concomitant insulin secretion from prelabeled islets incubated together with the Ca<sup>2+</sup>-ATPase inhibitor thapsigargin (1 μmol/l), which induces depletion of intracellular Ca<sup>2+</sup> stores (Fig. 5). However, during a 30-min incubation period, efflux of [<sup>3</sup>H]AA and concomitant insulin secretion because of the two secretagogues, alone or in combination, remained unaltered compared with controls (NS; *n* = 6). Thus, depletion of intracellular Ca<sup>2+</sup> stores does not affect CCK-8- or carbachol-induced PLA<sub>2</sub> activation.

**Effect of diglyceride lipase inhibition and PLA<sub>2</sub> inhibition on CCK-8-induced [<sup>3</sup>H]AA efflux in the presence and absence of extracellular Ca<sup>2+</sup>.** To delineate to what extent CCK-8-stimulated AA generation is dependent on PLA<sub>2</sub> activation versus diglyceride lipase activation, we measured the efflux of [<sup>3</sup>H]AA and from prelabeled islets during a 30-min incubation period in the presence of the diglyceride lipase inhibitor RHC 80267 (35 μmol/l) and the PLA<sub>2</sub> inhibitor ACA (50 μmol/l; Fig. 6A). After subtracting values of control islets incubated with 5.6 mmol/l glucose alone from the values of islets

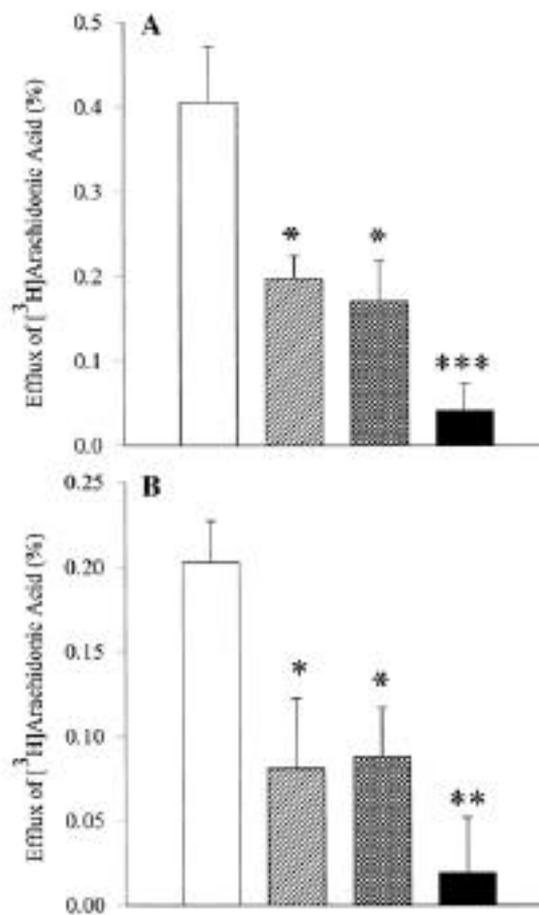
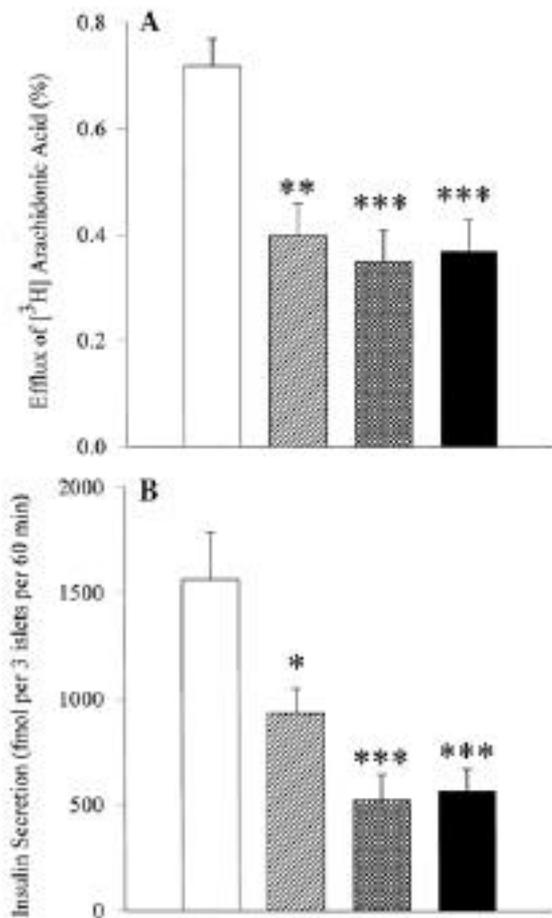


FIG. 6. Fractional efflux of [<sup>3</sup>H]AA expressed as the percentage release of total incorporated radioactivity from prelabeled isolated islets incubated for 30 min in the presence of glucose (5.6 mmol/l), with or without CCK-8 (100 nmol/l), RHC 80267 (35 μmol/l), and ACA (50 μmol/l), in the presence (A) or absence (B) of extracellular Ca<sup>2+</sup>. The calculated net effects of CCK-8 are shown after subtracting values of control islets incubated with 5.6 mmol/l glucose alone from the values of islets incubated with glucose and CCK-8 together. *n* = 6, \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 versus controls. □, CCK-8; ▨, CCK-8 + RHC 80267; ▩, CCK-8 + ACA; ■, CCK-8 + RHC 80267 + ACA.

incubated with glucose and CCK-8 together, we found that RHC 80267 diminished the net CCK-8-induced efflux of [<sup>3</sup>H]AA by 51 ± 17% (*P* = 0.014; *n* = 6). Furthermore, ACA reduced the net CCK-8-stimulated efflux of [<sup>3</sup>H]AA by 58 ± 20% (*P* = 0.016; *n* = 6). When combining RHC 80267 and ACA, the net CCK-8-induced efflux of [<sup>3</sup>H]AA was decreased by 90 ± 18% (*P* = 0.001; *n* = 6). To study the role of extracellular Ca<sup>2+</sup> during the involvement of PLA<sub>2</sub> and diglyceride lipase, we also performed the experiments with ACA and RHC 80267 in a Ca<sup>2+</sup>-deficient environment (Fig. 6B). Then, after subtracting controls incubated with 5.6 mmol/l glucose alone, we found that RHC 80267 (35 μmol/l) diminished the net CCK-8-induced efflux of [<sup>3</sup>H]AA by 58 ± 25% (*P* = 0.041; *n* = 6). Furthermore, ACA (50 μmol/l) reduced the net CCK-8-stimulated efflux of [<sup>3</sup>H]AA by 55 ± 20% (*P* = 0.020; *n* = 6). When RHC 80267 and ACA were combined, the net CCK-8-induced efflux of [<sup>3</sup>H]AA was decreased by 90 ± 21% (*P* = 0.002; *n* = 6). Thus, CCK-8-induced AA generation and insulin secretion are partly dependent on diglyceride lipase activation and partly dependent on PLA<sub>2</sub> activation in both the presence and absence of extracellular Ca<sup>2+</sup>.



**FIG. 7.** Fractional efflux of [<sup>3</sup>H]AA expressed as the percentage release of total incorporated radioactivity from prelabeled isolated islets incubated for 30 min (**A**) and insulin release from prelabeled islets incubated for 60 min (**B**) in the presence of glucose (5.6 mmol/l) and CCK-8 (100 nmol/l), with or without the PLA<sub>2</sub> inhibitor ACA (50 μmol/l), in islets subjected or not subjected to PKC downregulation by an overnight incubation with TPA. The calculated net effects of CCK-8 are shown after subtracting values of control islets incubated with 5.6 mmol/l glucose alone from the values of islets incubated with glucose and CCK-8 together. For **A**, *n* = 6; for **B**, *n* = 13–16. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 versus controls. □, CCK-8; ▨, CCK-8 + TPA overnight; ▩, CCK-8 + ACA; ■, CCK-8 + TPA overnight + ACA.

**Effect of PKC downregulation and PLA<sub>2</sub> inhibition on CCK-8-induced [<sup>3</sup>H]AA efflux and insulin secretion.** To examine the PKC dependence of CCK-8-stimulated PLA<sub>2</sub> activation, we studied the efflux of [<sup>3</sup>H]AA from prelabeled islets during a 30-min incubation period (Fig. 7A) and the insulin secretion from unlabeled islets during a 60-min incubation period (Fig. 7B), after inducing PKC downregulation through an overnight incubation with the phorbol ester TPA (500 nmol/l). After subtracting values of control islets incubated with 5.6 mmol/l glucose alone from the values of islets incubated with glucose and CCK-8 together, we found that overnight incubation with TPA reduced the net CCK-8-induced efflux of [<sup>3</sup>H]AA by 45 ± 12% (*P* = 0.003; *n* = 6) and the insulin secretion by 40 ± 16% (*P* = 0.020; *n* = 14–16). Furthermore, the PLA<sub>2</sub> inhibitor ACA (50 μmol/l) diminished the net CCK-8-stimulated efflux of [<sup>3</sup>H]AA by 52 ± 11% (*P* = 0.001; *n* = 6) and the insulin secretion by 67 ± 16% (*P* < 0.001; *n* = 13–16). When overnight incubation with TPA and ACA

were combined, no further inhibition of the net effects of CCK-8 was seen regarding either [<sup>3</sup>H]AA efflux (NS; total diminution by 49 ± 11%; *P* = 0.001; *n* = 6) or insulin secretion (NS; total reduction by 64 ± 16%; *P* = 0.001; *n* = 16). Thus, CCK-8-induced PLA<sub>2</sub> activation is partly dependent on PKC.

## DISCUSSION

The results of this study imply that both CCK-8 and carbachol activate PLA<sub>2</sub> in rat islets. Our results further indicate that PLA<sub>2</sub> activation due to CCK-8 stimulation is independent of both influx of extracellular Ca<sup>2+</sup> and liberation of intracellular Ca<sup>2+</sup>. This was in contrast to the PLA<sub>2</sub>-stimulating effect of carbachol, which was dependent on extracellular Ca<sup>2+</sup> influx. Moreover, the results suggest that the CCK-8-induced PLA<sub>2</sub> activation is partly dependent on PKC stimulation, even though a considerable share of the CCK-8-mediated PLA<sub>2</sub> activation seems to occur independently of PKC.

Earlier studies have shown that PLA<sub>2</sub> can be divided into two different subtypes: the secretory (extracellular, low molecular mass) form and the cytosolic (intracellular, high molecular mass) form (32,33). The cytosolic PLA<sub>2</sub>, present in human and rat islets (21–23), hydrolyses plasma membrane phospholipids after having been translocated to the plasma membrane upon cellular activation through a process involving the binding of Ca<sup>2+</sup> (34). However, during recent years, it has been apparent that PLA<sub>2</sub> can also be activated independently of Ca<sup>2+</sup>, i.e., during Ca<sup>2+</sup> channel blockade or after removal of extracellular Ca<sup>2+</sup> (35,36). In addition, the Ca<sup>2+</sup>-independent PLA<sub>2</sub> activity has been described to exist in substantial amounts within rat and human islets and has been shown to be activated by glucose metabolism-generated ATP (25). Its importance is further underscored by findings that when the Ca<sup>2+</sup>-independent PLA<sub>2</sub> is pharmacologically inhibited, the glucose-induced stimulation of insulin secretion is severely suppressed (27). In fact, it has been suggested that this ATP-stimulatable Ca<sup>2+</sup>-independent PLA<sub>2</sub> is required for optimal glucose responsiveness, because it has been shown that clonal insulinoma cell lines, which express ATP-stimulatable Ca<sup>2+</sup>-independent PLA<sub>2</sub> activity, exhibit glucose responsiveness, whereas other insulinoma cell lines, which do not express ATP-stimulatable Ca<sup>2+</sup>-independent PLA<sub>2</sub> activity, are in general unresponsive to glucose (37).

Whether CCK-8 stimulates a Ca<sup>2+</sup>-dependent or a Ca<sup>2+</sup>-independent form of islet PLA<sub>2</sub>, or perhaps both, as well as whether the mechanism of CCK-8 in this regard is different from that of the cholinergic agonist carbachol, is unknown and was therefore examined. The study was methodologically based on three different approaches, all known to reflect the involvement of PLA<sub>2</sub>. First, we delineated the efflux of [<sup>3</sup>H]AA from prelabeled islets, which is a technique shown by Konrad et al. (17) to indirectly determine PLA<sub>2</sub> activity. Second, we detected lysophosphatidylcholine accumulation, which also reflects PLA<sub>2</sub> activity (17). Third, we induced PLA<sub>2</sub> inhibition by the PLA<sub>2</sub> inhibitor ACA at a concentration that previously has been shown to inhibit glucose-induced AA accumulation in rat islets without disturbing glucose metabolism or affecting PLC activity (15).

The results of all three approaches show that CCK-8 stimulates PLA<sub>2</sub>. However, the results also imply that the AA generation induced by CCK-8 does not solely reflect PLA<sub>2</sub> activity, but also the sequential actions of PLC and diglyceride

lipase, which is a well-known pathway mediating AA generation in islets (38). In fact, the results indicate that the  $\text{PLA}_2$  pathway and the PLC/diglyceride lipase pathway are almost equally responsible for the CCK-8-mediated AA formation, and that the effects of these two pathways are additive. However, cautious interpretations should be made when quantifying the role of the two separate pathways, because the inhibitors used have been suspected to be not entirely specific. Thus, it was recently shown that both RHC 80267 and ACA, concomitantly with their inhibitory action on insulin secretion, increased  $^{86}\text{Rb}^+$  efflux from prelabeled mouse islets, although in this respect the effect of ACA was weak (39). This indicates that besides their inhibitory action on AA accumulation (15,17), RHC 80267 and, to a smaller extent, ACA, might open ATP-sensitive  $\text{K}^+$ -channels. Therefore, our results in this regard need to be further confirmed. The equal contribution of the  $\text{PLA}_2$  and the PLC/diglyceride lipase pathways in our study was evident regardless of whether or not extracellular  $\text{Ca}^{2+}$  was present. This is interpreted to indicate that CCK-8 is able to exploit  $\text{Ca}^{2+}$ -independent actions of both  $\text{PLA}_2$  and PLC/diglyceride lipase to accomplish AA generation. The other two  $\text{PLA}_2$  subclasses besides the  $\text{Ca}^{2+}$ -independent form that are shown to be present in islets—the cytosolic form and the secretory form (21–23)—are known to require  $\text{Ca}^{2+}$  for their activation (33). Therefore, although a direct demonstration of ACA-induced inhibition of the  $\text{Ca}^{2+}$ -independent form of  $\text{PLA}_2$  was not performed, our finding that ACA inhibits AA formation under  $\text{Ca}^{2+}$ -deficient conditions suggests that ACA inhibits the  $\text{Ca}^{2+}$ -independent form of  $\text{PLA}_2$ .

During  $\text{Ca}^{2+}$  channel blockade by verapamil, used at a concentration assumed to inhibit the entry of extracellular  $\text{Ca}^{2+}$  (17), CCK-8-induced efflux of  $^3\text{H}$ AA remained unchanged. In contrast, the efflux induced by carbachol was markedly reduced. Both agonists were used at dose levels that stimulate insulin secretion in rat islets (40,41). The findings are in accordance with our results obtained by removal of extracellular  $\text{Ca}^{2+}$ , implying that CCK-8, but not carbachol, induces activation of  $\text{Ca}^{2+}$ -independent  $\text{PLA}_2$ . Although the total amount of secreted insulin was considerably lower than in the presence of  $\text{Ca}^{2+}$ , CCK-8 was also able to stimulate insulin secretion in the absence of extracellular  $\text{Ca}^{2+}$ , whereas carbachol did not promote insulin release under these conditions. Thus, it seems as if the two potent AA generators CCK-8 and carbachol have a major dissimilarity in their modes of activating  $\text{PLA}_2$ . Such a hypothesis is further supported by our finding that the AA-generating effect of CCK-8 and carbachol in combination was significantly higher when compared with the effect of either of the two agents alone and by the fact that this additive effect was abolished when extracellular  $\text{Ca}^{2+}$  was omitted.

Because CCK-8 is known to liberate  $\text{Ca}^{2+}$  from intracellular stores in islets (13), we investigated if the  $\text{PLA}_2$  activation induced by CCK-8 was dependent on release of  $\text{Ca}^{2+}$  from intracellular stores, by incubating islets together with thapsigargin. Thapsigargin at the concentration used inhibits the  $\text{Ca}^{2+}$ -ATPase pump activity of the endoplasmic reticulum, which impairs the reabsorption of  $\text{Ca}^{2+}$  and depletes the  $\text{Ca}^{2+}$  content of the endoplasmic reticulum (28). However, in spite of the presence of thapsigargin, the efflux of  $^3\text{H}$ AA and insulin secretion due to stimulation by CCK-8 or carbachol were unaffected, indicating that release of  $\text{Ca}^{2+}$  from intra-

cellular stores is of no major importance for the activation of  $\text{PLA}_2$  due to these two secretagogues. Thus, the ability of CCK-8 to activate  $\text{PLA}_2$  seems independent of both extra- and intracellular  $\text{Ca}^{2+}$ , whereas the action of carbachol seems dependent on extracellular  $\text{Ca}^{2+}$ .

$\text{Ca}^{2+}$ -independent  $\text{PLA}_2$  activity has previously been shown to be directly stimulated by ATP (25). In fact,  $\text{Ca}^{2+}$ -independent  $\text{PLA}_2$  has been proposed as an alternative way for glucose metabolism to generate insulin secretion when only small increments in ATP production are possible (27). Our results suggest that CCK-8, in this particular respect, might resemble ATP. Therefore, stimulation by CCK-8 would offer the  $\beta$ -cell an alternative possibility to activate  $\text{Ca}^{2+}$ -independent  $\text{PLA}_2$  and accomplish  $\text{Ca}^{2+}$  influx and exocytosis under conditions of severely reduced ATP production, as, for example, under conditions of low glucose sensitivity such as during development of type 2 diabetes. CCK-8-mediated activation of  $\text{Ca}^{2+}$ -independent  $\text{PLA}_2$  might therefore be an alternative rather than an additional mode to induce insulin secretion. Such a role is in line with our results that the combined effect of CCK-8 plus carbachol is additive regarding AA generation but not regarding insulin secretion. If this action of CCK-8 is preserved and perhaps exaggerated in type 2 diabetes, it could be a target for development of new drugs specifically activating  $\beta$ -cell CCK receptors. A previous clinical study indicating an important role for the insulinotropic effect of CCK-8 during type 2 diabetes might support such a theory (8).

The cellular events underlying CCK-8-induced  $\text{PLA}_2$  activation have not been characterized. One possible constituent could be PKC, which previously has been proposed as a  $\text{PLA}_2$  activator (32,42) and to be involved in CCK-8-induced insulin secretion (14). In the present study, we found that PKC downregulation, induced by an overnight incubation with TPA, reduced both CCK-8-induced efflux of  $^3\text{H}$ AA and insulin secretion, which suggests that PKC is important for the CCK-8-induced activation of  $\text{PLA}_2$ . However, a substantial part of the stimulating mechanism seems to be independent of PKC. Of further interest is the fact that when PKC downregulation and  $\text{PLA}_2$  inhibition are combined, no additive reduction was seen regarding either the  $^3\text{H}$ AA efflux or the insulin secretion induced by CCK-8 when compared with the reduction caused by  $\text{PLA}_2$  inhibition alone. This suggests that at least one additional  $\text{PLA}_2$ - and PKC-independent signaling pathway is involved in the AA-generating and insulinotropic effects of CCK-8.

In conclusion, the results of this study show that CCK-8-induced  $\text{PLA}_2$  activation in islets also occurs after removal of extracellular  $\text{Ca}^{2+}$  and is insensitive to inhibition of plasma membrane  $\text{Ca}^{2+}$  channels as well as to depletion of intracellular  $\text{Ca}^{2+}$  stores, suggesting that CCK-8 has the ability to activate a  $\text{Ca}^{2+}$ -independent  $\text{PLA}_2$ . This indicates a dissimilarity between CCK-8 and the cholinergic agonist carbachol, which is unable to stimulate  $\text{PLA}_2$  in the absence of extracellular  $\text{Ca}^{2+}$ . Furthermore, the results imply that the  $\text{PLA}_2$ -activating capacity of CCK-8 is partly dependent on PKC, even though a considerable share of the stimulating mechanism seems to be independent of PKC and, moreover, that CCK-8 is able to induce  $\text{PLA}_2$  activation and insulin secretion despite inhibition of both PKC and  $\text{PLA}_2$ . These results suggest the involvement of at least one additional signaling pathway for CCK-8 that is independent of both  $\text{PLA}_2$  and PKC.

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## REFERENCES

- Creutzfeldt W, Ebert R: New developments in the incretin concept. *Diabetologia* 28:565–573, 1985
- Ahrén B, Taborsky GJ Jr, Porte D Jr: Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. *Diabetologia* 29:827–836, 1986
- Liddle RA, Goldfine ID, Rosen MS, Taplitz RA, Williams JA: Cholecystokinin bioactivity in human plasma: molecular forms, responses to feeding and relationship to gallbladder contraction. *J Clin Invest* 75:1144–1152, 1985
- Rehfeld JF, Larsson LI, Goltermann NR, Schwartz TW, Holst JJ, Jensen SL, Morley JS: Neural regulation of pancreatic hormone secretion by the C-terminal tetrapeptide of CCK. *Nature* 284:33–38, 1980
- Verspohl EJ, Ammon HPT, Williams JA, Goldfine ID: Evidence that cholecystokinin interacts with specific receptors and regulates insulin release in isolated rat islets of Langerhans. *Diabetes* 35:38–43, 1986
- Zawalich WS, Cote SB, Diaz VA: Influence of cholecystokinin on insulin output from isolated perfused pancreatic islets. *Endocrinology* 119:616–621, 1986
- Karlsson S, Ahrén B: Cholecystokinin and the regulation of insulin secretion. *Scand J Gastroenterol* 27:161–165, 1992
- Rushakoff RA, Goldfine ID, Beccaria LJ, Mathur A, Brand RJ, Liddle RA: Reduced postprandial cholecystokinin (CCK) secretion in patients with non-insulin-dependent diabetes mellitus: evidence for a role for CCK in regulating postprandial hyperglycemia. *J Clin Endocrinol Metab* 76:489–493, 1993
- Tachibana I, Akiyama T, Kanagawa K, Shiohara H, Furumi K, Watanabe M, Otsuki M: Defect in pancreatic exocrine and endocrine response to CCK in genetically diabetic OLETF rats. *Am J Physiol* 270:G730–G737, 1996
- Chen NG, Romsos D: Enhanced sensitivity of pancreatic islets from preobese 2-week-old ob/ob mice to neurohormonal stimulation of insulin secretion. *Endocrinology* 136:505–511, 1995
- Zawalich WS, Takuwa N, Takuwa Y, Diaz VA, Rasmussen H: Interactions of cholecystokinin and glucose in rat pancreatic islets. *Diabetes* 36:426–433, 1987
- Malm D, Gjaever A, Vonen B, Florholmen J: Cholecystokinin and somatostatin modulate the glucose-induced insulin secretion by different mechanisms in pancreatic islets: a study on phospholipase C activity and calcium requirement. *Scand J Clin Lab Invest* 53:671–676, 1993
- Fridolf T, Karlsson S, Ahrén B: Effects of CCK-8 on the cytoplasmic free calcium concentration in isolated rat islet cells. *Biochem Biophys Res Commun* 184:878–882, 1992
- Karlsson S, Ahrén B: Cholecystokinin-stimulated insulin secretion and protein kinase C in rat pancreatic islets. *Acta Physiol Scand* 142:397–403, 1991
- Konrad RJ, Jolly C, Major C, Wolf BA: Inhibition of phospholipase A<sub>2</sub> and insulin secretion in pancreatic islets. *Biochim Biophys Acta* 1135:215–220, 1992
- Simonsson E, Karlsson S, Ahrén B: Involvement of phospholipase A<sub>2</sub> and arachidonic acid in cholecystokinin-8-induced insulin secretion. *Regul Pept* 65:101–107, 1996
- Konrad RJ, Jolly YC, Major C, Wolf BA: Carbachol stimulation of phospholipase A<sub>2</sub> and insulin secretion in pancreatic islets. *Biochem J* 287:283–290, 1992
- Tsunoda Y, Owyang C: Differential involvement of phospholipase A<sub>2</sub>/arachidonic acid and phospholipase C/phosphoinositol pathways during cholecystokinin receptor activated Ca<sup>2+</sup> oscillations in pancreatic acini. *Biochem Biophys Res Commun* 194:1194–1202, 1993
- Jones PM, Persaud SJ: Arachidonic acid as a second messenger in glucose-induced insulin secretion from pancreatic beta-cells. *J Endocrinol* 137:7–14, 1993
- Naor Z: At the cutting edge: is arachidonic acid a second messenger in signal transduction? *Mol Cell Endocrinol* 80:C181–C186, 1991
- Loweth AC, Scarpello JHB, Morgan NG: Phospholipase A<sub>2</sub> expression in human and rodent insulin-secreting cells. *Mol Cell Endocrinol* 112:177–183, 1995
- Parker KJ, Jones PM, Hunton CH, Persaud SJ, Taylor CG, Howell SL: Identification and localisation of a type IV cytosolic phospholipase A<sub>2</sub> in rat pancreatic beta-cells. *J Mol Endocrinol* 17:31–43, 1996
- Chen M, Yang Z-D, Naji A, Wolf BA: Identification of calcium-dependent phospholipase A<sub>2</sub> isoforms in human and rat pancreatic islets and insulin secreting β-cell lines. *Endocrinology* 137:2901–2909, 1996
- Laychock SG: Phospholipase A<sub>2</sub> activity in pancreatic islets is calcium-dependent and stimulated by glucose. *Cell Calcium* 3:43–54, 1982
- Gross RW, Ramanadham S, Kruszka KK, Han X, Turk J: Rat and human pancreatic islet cells contain a calcium ion independent phospholipase A<sub>2</sub> activity selective for hydrolysis of arachidonate which is stimulated by adenosine triphosphate and is specifically localized to islet beta-cells. *Biochemistry* 32:327–336, 1993
- Yamamoto S, Nakadate T, Nakaki T, Ishii K, Kato R: Prevention of glucose-induced insulin secretion by lipoxygenase inhibitor. *Eur J Pharmacol* 78:225–227, 1982
- Ramanadham S, Gross RW, Han X, Turk J: Inhibition of arachidonate release suppresses both insulin secretion and the rise in β-cell cytosolic Ca<sup>2+</sup> concentration. *Biochemistry* 32:337–346, 1993
- Aizawa T, Yada T, Asanuma N, Sato Y, Ishihara F, Hamakawa N, Yaekura K, Hashizume K: Effects of thapsigargin, an intracellular Ca<sup>2+</sup> pump inhibitor, on insulin release by rat pancreatic β-cell. *Life Sci* 57:1375–1381, 1995
- Hii CST, Jones PM, Persaud SJ, Howell SL: A re-assessment of the role of protein kinase C in glucose-stimulated insulin secretion. *Biochem J* 246:489–493, 1987
- Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16:35–39, 1967
- Hou W, Arita Y, Morisset J: Dual pathways for carbamylcholine-stimulated arachidonic acid release in rat pancreatic acini. *Endocrine* 5:67–74, 1996
- Glaser KB, Mobilio D, Chang JY, Senko N: Phospholipase A<sub>2</sub> enzymes: regulation and inhibition. *Trends Pharmacol Sci* 14:92–98, 1993
- Dennis EA: Diversity of group types, regulation, and function of phospholipase A<sub>2</sub>. *J Biol Chem* 269:13057–13060, 1994
- Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, Milona N, Knopf JL: A novel arachidonic acid-selective cytosolic PLA<sub>2</sub> contains a Ca<sup>2+</sup>-dependent translocation domain with homology to PKC and GAP. *Cell* 65:1043–1051, 1991
- Wolf BA, Pasquale SM, Turk J: Free fatty acid accumulation in secretagogue-stimulated pancreatic islets and effects of arachidonate on depolarization-induced insulin secretion. *Biochemistry* 30:6372–6379, 1991
- Turk J, Mueller M, Bohrer A, Ramanadham S: Arachidonic acid metabolism in isolated pancreatic islets. VI. Carbohydrate insulin secretagogues must be metabolized to induce eicosanoid release. *Biochim Biophys Acta* 1125:280–291, 1992
- Ramanadham S, Wolf MJ, Li B, Bohrer A, Turk J: Glucose-responsivity and expression of an ATP-stimulatable, Ca<sup>2+</sup>-independent phospholipase A<sub>2</sub> enzyme in clonal insulinoma cell lines. *Biochim Biophys Acta* 1344:153–164, 1997
- Konrad RJ, Major CD, Wolf BA: Diacylglycerol hydrolysis to arachidonic acid is necessary for insulin secretion from isolated pancreatic islets: sequential actions of diacylglycerol and monoacylglycerol lipases. *Biochemistry* 33:13284–13294, 1994
- Tams P, Capito K: Inhibition of glucose-induced insulin secretion by the diacylglycerol lipase inhibitor RHC 80267 and the phospholipase A<sub>2</sub> inhibitor ACA through stimulation of K<sup>+</sup> permeability without diminution by exogenous arachidonic acid. *Biochem Pharm* 53:1077–1086, 1997
- Karlsson S, Ahrén B: Dependence on extracellular Ca<sup>2+</sup> for the effects of cholecystokinin-8 in rat pancreatic islets. *Diabetes Res* 16:139–143, 1991
- Persaud SJ, Jones PM, Sugden D, Howell SL: The role of protein kinase C in cholinergic stimulation of insulin secretion from rat islets of Langerhans. *Biochem J* 264:753–758, 1993
- Nemenoff RA, Winitz S, Qian NX, Van Putten V, Johnson GL, Heasley LE: Phosphorylation and activation of a high molecular weight form of phospholipase A<sub>2</sub> by p42 microtubule-associated protein 2 kinase and protein kinase C. *J Biol Chem* 268:1960–1964, 1993