0026-895X/88/040543-06$02.00/0

MOLECULAR PHARMACOLOGY, 34:543–548

Relationship between Δ-9-Tetrahydrocannabinol-Induced Arachidonic Acid Release and Secretagogue-Evoked Phosphoinositide Breakdown and Ca^{2+} Mobilization of Exocrine Pancreas

ARCHANA CHAUDHRY, RAIFORD H. THOMPSON, RONALD P. RUBIN, and SUZANNE G. LAYCHOCK

Department of Pharmacology, Medical College of Virginia, Richmond, Virginia 23298-0001

Received April 28, 1988; Accepted July 15, 1988

SUMMARY

We have previously shown that addition of exogenous arachidonic acid to pancreatic acinar cells inhibits the incorporation of *myo*-[^{3}H]inositol into membrane phosphoinositides and causes a reduction in the steady state levels of [^{32}P]phosphatidylinositol-4,5-bisphosphate (PtdIns4,5P_2). In the present study, Δ-9-tetrahydrocannabinol (THC) was utilized to raise endogenous levels of arachidonic acid. In acinar cells simultaneously prelabeled with [^{3}H]arachidonic acid and [^{32}P]P_i, THC (1–20 μM) produced a concentration-dependent increase in free [^{3}H]arachidonic acid release and a reduction in the steady state levels of [^{32}P]PtdIns4,5P_2. THC (1–20 μM) also caused a concentration-dependent inhibition of *myo*-[^{3}H]inositol trisphosphate accumulation, cytoplasmic Ca^{2+} level, and amylase secretion elicited by 0.1 μM caerulein. The findings that THC (20 μM) was unable to inhibit either the rise in $[Ca^{2+}]_i$ elicited by ionomycin, or the secretory response to phorbol myristic acid or ionomycin, indicate that THC exerts a selective inhibitory effect on the phosphoinositide messenger system. These results support the postulate that endogenous arachidonic acid serves as a negative feedback regulator of phosphoinositide turnover in exocrine pancreas.

The primary response of many tissues, including exocrine pancreas, to Ca^{2+}-mobilizing agonists is phospholipase C-mediated hydrolysis of PtdIns4,5P_2 leading to the formation of the putative second messengers $InsP_3$ and diacylglycerol (1, 2). In many secretory systems, including the exocrine pancreas, Ca^{2+}-mobilizing agonists also liberate unesterified arachidonic acid either through activation of phospholipase A_2, the sequential activation of phospholipase C and diacylglycerol lipase, or phosphatidate-specific phospholipase A_2 (3–6). A role for free arachidonic acid in the cellular function of exocrine pancreas was suggested by our recent study, which showed that exogenous arachidonic acid causes a reduction in the steady state levels of [^{32}P]PtdIns4,5P_2 (6). The decrease in the PtdIns4,5P_2 pool is not due to PLC-stimulated PtdIns4,5P_2 hydrolysis, inasmuch as arachidonic acid does not promote the accumulation of [^{3}H]$InsP_3$ but inhibits the stimulated incorporation of radiolabeled inositol into inositol phospholipids (6). These findings suggest that in the exocrine pancreas arachidonic acid, generated by Ca^{2+}-mobilizing agonists, serves as a negative feedback regulator of phosphoinositide turnover by inhibiting the resynthesis of the polyphosphoinositide pool utilized by Ca^{2+}-mobilizing agonists.

The present study was designed to investigate the putative role of endogenously released arachidonate on phosphoinositide turnover and consequently on agonist-mediated Ca^{2+} mobilization and amylase secretion. We utilized THC as a tool to increase endogenous levels of unesterified arachidonate in the exocrine pancreas. THC promotes the release of arachidonic acid from cells by activating phospholipase A_2 and by inhibiting acyl-CoA acyltransferase, which blocks the reincorporation of fatty acids into phospholipids (7–10). Our results show that the liberation of arachidonic acid induced by THC in exocrine pancreas is associated with a decrease in [^{32}P]PtdIns4,5P_2 levels and the inhibition of caerulein-stimulated [^{3}H]$InsP_3$ formation, Ca^{2+} mobilization, and amylase secretion. These findings support a modulatory role for arachidonic acid in the phosphoinositide messenger system in exocrine pancreas.

Experimental Procedures

Materials. Collagenase (0.27 units/mg) derived from *Clostridium histolyticum* was obtained from Boehringer Mannheim (Indianapolis,

This work was supported by Research Grant AM-28029 from the National Institutes of Health.

ABBREVIATIONS: PtdIns4,5P_2, phosphatidylinositol 4,5-bisphosphate; PMA, phorbol myristic acid; PtdIns4P, phosphatidylinositol 4-phosphate; PtdIns, phosphatidylinositol; $InsP_3$, *myo*-inositol trisphosphate; THC, Δ-9-tetrahydrocannabinol; BSA, bovine serum albumin; DMSO, dimethylsulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; fura-2, 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methylphenoxyl-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; fura-2/AM, the pentaacetoxy methyl ester derivative of fura-2.

IN). *myo*-[^{3}H]Inositol (15 Ci/mM) was obtained from American Radiolabeled Chemicals (St. Louis, MO). [^{3}H]Arachidonic acid (190 Ci/mmol) was obtained from New England Nuclear Research Products (Boston, MA). [^{32}P]P_i was purchased from ICN Radiochemicals (Irvine, CA). Essential amino acids (50× concentrate) were purchased from GIBCO Laboratories (Grand Island, NY). HEPES, BSA (fraction V), butylated hydroxytoluene, and soybean trypsin inhibitor were obtained from Sigma Chemical Company (St. Louis, MO). Dowex resin (AG 1-X8), formate form, was purchased from Bio-Rad Laboratories (Richmond, CA). Fura-2/AM was obtained from Molecular Probes Inc. (Eugene, OR). THC was obtained from the National Institute on Drug Abuse. Caerulein (Peninsula Laboratories, Belmont, CA), ionomycin (Calbiochem, La Jolla, CA), PMA (L. C. Services, Woburn, MA), and THC were stored frozen as stock solutions in DMSO. The final concentration of DMSO, which was never more than 2% (v/v), had no effect on any of the parameters studied.

Preparation of rat pancreatic acini and acinar cells. The basic medium used was a HEPES-buffered Krebs Henseleit medium (HKH buffer) with the following composition (mM): NaCl, 98; KCl, 4.7; KH_2PO_4, 1.2; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 2.5; HEPES, 10; and dextrose, 11. Unless otherwise stated, the Ca^{2+} and Mg^{2+} concentrations were 1.3 and 1.2 mM, respectively. The medium also contained soybean trypsin inhibitor (0.1 mg/ml for acinar cells and 0.2 mg/ml for acini) and essential amino acids and was maintained at pH 7.4 under an atmosphere of O_2/CO_2 (19:1).

Male Sprague-Dawley rats were sacrificed by decapitation and the pancreata were removed and placed in HKH buffer containing low Ca^{2+} and Mg^{2+} (0.1 mM) and 0.4% BSA. The pancreata were dissected free of fat and lymph nodes and minced with scissors. Pancreatic acinar cells were prepared as described previously (11). Acini were prepared by a modification of previously described methods (4, 12). Minced pancreatic tissue was incubated for 10 min at 37°, with rapid shaking, in 10 ml of medium containing low Ca^{2+} and Mg^{2+} (0.1 mM), crude collagenase (0.1 units/ml), and BSA (0.4%). The medium was replaced with fresh medium containing collagenase and incubated for an additional 20 min. Acini were dispersed by vigorous shaking and a 10-ml aliquot of the basic medium containing 4% BSA was added. The acini were then removed by means of a Pasteur pipette, centrifuged for 5 min at 50 × *g*, and washed three times by layering the acini on top of 10 ml of medium containing 4% BSA and centrifuging at 50 × *g* for 5 min. After resuspension in medium containing 1% BSA, acini were incubated at 37° and shaken for 10 min at 300 cycles/min before use.

Amylase secretion. Acini were used to measure amylase release because this preparation elicits a greater secretory response than isolated cells. Acini (0.2–0.5 mg/ml) were incubated with 1–20 μM THC in medium containing 0.1% BSA at 37° under O_2 with shaking. Some acini were exposed to 1% DMSO alone. After a 5-min preincubation period with THC or DMSO, acini were exposed to either caerulein, PMA, ionomycin, or DMSO. A 500-μl aliquot of the acinar suspension was withdrawn at various times and added to 200 μl of silicone oil (Nyosil) in microcentrifuge tubes, which were centrifuged for 10 sec at 15,600 × *g*. Amylase activity in the medium was determined by the method of Bernfeld (13). The total (acinar plus medium) amylase content was determined by lysis of a portion of cells with 0.2% Triton X-100. Amylase secretion is expressed as a percentage of total content after subtraction of the amylase present in the medium at zero time.

Measurement of [^{3}H]arachidonic acid release and [^{32}P]PtdIns4,5P_2 levels. In these experiments, the phosphate concentration was reduced to 10 μM. Cells (50 mg/ml) were simultaneously incubated in BSA-free medium containing [^{3}H]arachidonic acid (20 μCi/ml) and [^{32}P]P_i (10–25 μCi/ml). Carbachol (10 μM) was also included in the medium, because it has been shown to enhance the incorporation of radiolabeled arachidonic acid into pancreatic membrane phospholipids (4). After 15 min, 100 μM atropine was added to terminate the action of carbachol and after another 30 min, an equal volume of fresh medium was added to the cells. After a total incubation period of 90 min in medium containing [^{3}H]arachidonic acid and [^{32}P]P_i, which labels the [^{32}P]polyphosphoinositides to steady state levels (14), the cells were isolated by layering the cells on top of 10 ml of medium containing 4% BSA and centrifuging at 50 × *g* for 5 min. The cells were washed once more by layering on top of 4% BSA and then resuspended at a final concentration of 0.2–0.5 mg of protein/ml in medium containing 0.1% BSA plus [^{32}P]P_i (10–25 μCi/ml) and incubated for an additional 25 min. The cell suspensions were then exposed for various times to either THC or melittin. The reaction was terminated by removing 500-μl aliquots of the suspension and extracting the lipid components with 2.25 ml of chloroform/methanol (1:2, v/v) containing 20 μl 6 N HCl plus butylated hydroxytoluene (0.1 mg/ml). Analysis of [^{32}P]PtdIns4,5P_2 was carried out as described previously (15), whereas [^{3}H]arachidonic acid was isolated on silica gel 60 thin layer chromatography plates (E. M. Science; Media, PA) which were developed in a solvent system of diethyl ether/petroleum ether/acetic acid (50:50:1, v/v).

Measurement of [^{3}H]InsP_3. Acinar cell phosphoinositides were labeled with *myo*-[^{3}H]inositol as described previously (6), and then the labeled cells (0.2–0.5 mg of protein/ml) were resuspended in HKH buffer containing 0.1% BSA. A 2-ml aliquot of the cell suspension was preincubated for 5 min with 1–20 μM THC or 1% DMSO. After the preincubation period, some cells were exposed for an additional 5 min to 0.1 μM caerulein, whereas others were exposed to 1% DMSO. The reaction was terminated by adding 500 μl of the cell suspension to 1 ml of cold 4.5% perchloric acid. The perchloric acid extract was centrifuged at 200 × *g* for 5 min, and the supernatant was adjusted to pH 8–9 with 0.5 M KOH, 9 mM $Na_2B_4O_7$, and 1.9 mM EDTA. The [^{3}H]InsP_3 fraction [which is a mixture of Ins(1,4,5)P_3, Ins(1,3,4)P_3, and Ins(1,3,4,5)P_4] was separated from other inositol phosphates by anion exchange chromatography as described previously (16).

Measurement of $[Ca^{2+}]_i$. Acinar cells (10 mg/ml) were loaded with fura-2 by incubation for 30 min at room temperature under O_2 with rapid shaking in medium containing 0.2% BSA and 5 μM fura-2/AM. The cells were subsequently washed by layering them on top of 10 ml of medium containing 4% BSA and then centrifuging at 50 × *g* for 5 min. The cells were resuspended in medium containing 0.1% BSA and kept at room temperature before use. A 1-ml aliquot of the cell suspension was rapidly centrifuged at 15,600 × *g* and resuspended in 2 ml of the same medium. The samples were continuously stirred and maintained at 28° and their fluorescence was recorded using a Spex fluorolog dual excitation wavelength spectrofluorometer (Spex Industries, Edison, NJ). The emission wavelength was set at 505 nm and $[Ca^{2+}]_i$ was calculated from the ratio of the fluorescent intensity at the two excitation wavelengths, 340 and 380 nm, as described previously (17). Manganese could not be used to measure autofluorescence because it formed a white precipitate in our buffer. Therefore, the autofluorescence of unloaded cells was subtracted from the fluorescence at both excitation wavelengths before calculation of $[Ca^{2+}]_i$.

Statistical methods. Student's *t* test was used to determine statistical significance between two sample means. A one-way analysis of variance, using Dunnett's test, was used to compare multiple treatment groups. *p* values of less than 0.05 were considered significant.

Results

Effects of THC on [^{3}H]arachidonic acid release and [^{32}P]PtdIns4,5P_2 levels. In acinar cells prelabeled with [^{3}H]arachidonic acid, 20 μM THC caused a time-dependent increase in the accumulation of unesterified. [^{3}H]arachidonic acid (Fig. 1). After 5 and 30 min of exposure to THC, the levels of unesterified arachidonic acid increased to 163% and 210%, respectively, of control levels (100%). The enhancement by THC of arachidonate release was also concentration-dependent (Fig. 2).

In acinar cells simultaneously labeled with [^{32}P]P_i, THC caused a time- and concentration-dependent decrease in [^{32}P]PtdIns4,5P_2 levels. In fact, THC decreased the steady state

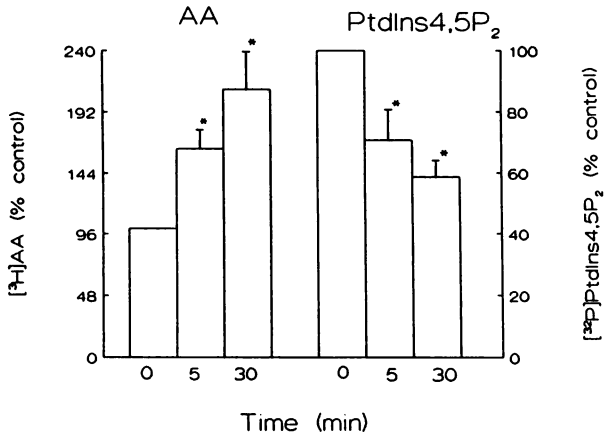

Fig. 1. Time-dependent effects of THC on [^{3}H]arachidonic acid (*AA*) release and [^{32}P]PtdIns4,5P_2 levels in pancreatic acinar cells. Cells prelabeled with [^{3}H]arachidonic acid and [^{32}P]P_i were exposed to 20 μM THC for the times indicated. The levels of [^{3}H]arachidonic acid and [^{32}P]PtdIns4,5P_2 are expressed as a percentage of the corresponding control value. Each *vertical bar* represents the mean value (± standard error) from three to eight different experiments. *Significantly different from corresponding control values as determined by one-way analysis of variance using Dunnett's test with $p < 0.05$.

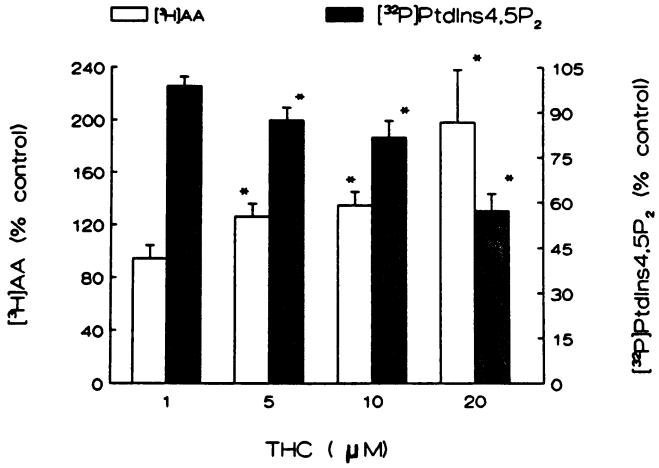

Fig. 2. Concentration-dependent effects of THC on [^{3}H]arachidonic acid (*AA*) release and [^{32}]PtdIns4,5P_2 levels in acinar cells. Cells prelabeled with [^{3}H]arachidonic acid and [^{32}P]P_i were exposed for 30 min to the concentrations of THC indicated. [^{3}H]Arachidonic acid release and [^{32}P]PtdIns4,5P_2 levels are expressed as a percentage of the control values, which averaged 3386 and 1491 cpm for [^{3}H]arachidonic acid and [^{32}P]PtdIns4,5P_2, respectively. Each *vertical bar* represents the mean of data (± standard error) from five or six different experiments. *Significantly different from control as determined by analysis of variance using Dunnett's test with $p < 0.05$.

levels of [^{32}P]PtdIns4,5P_2 over the same time course and range of concentrations that enhanced [^{3}H]arachidonic acid release (Figs. 1 and 2). The reduction in [^{32}P]PtdIns4,5P_2 levels at 5 μM (13%) and 20 μM THC (43%) corresponded to the increase in [^{3}H]arachidonic acid release observed at the threshold concentration of 5 μM (26%) and maximal concentration of 20 μM THC (100%). Concentrations of THC higher than 20 μM began to cause cell lysis.

In contrast to the consistent and marked effects of THC on [^{32}P]PtdIns4,5P_2 levels, variable and less striking effects on [^{32}P]PtdIns4P levels were observed. Thus, [^{32}P]PtdIns4P levels after a 30-min exposure to 20 μM THC were 92 ± 10% of control levels (six experiments).

Effect of THC on [^{3}H]InsP_3 levels. We next examined whether the reduction in PtdIns4,5P_2 levels caused by THC leads to an inhibition of phospholipase C-mediated [^{3}H]InsP_3 accumulation. THC alone in concentrations that enhanced [^{3}H]arachidonate release did not significantly alter [^{3}H]InsP_3 levels (Table 1). In contrast, THC inhibited caerulein-stimulated [^{3}H]InsP_3 accumulation in a concentration-dependent manner (Fig. 3). The inhibition by THC was significant at 5 μM (30%) and maximal at 20 μM (88%). Thus, similar concentrations of THC enhance [^{3}H]arachidonic acid release, suppress [^{32}P]PtdIns4,5P_2 levels, and inhibit caerulein-stimulated [^{3}H]InsP_3 accumulation. These results indicate that THC depletes the polyphosphoinositide pool utilized by caerulein.

As with caerulein, the stimulation of [^{3}H]InsP_3 accumulation elicited by carbachol was depressed by THC. Thus, after a 5-min pretreatment with 20 μM THC, the enhanced accumulation of [^{3}H]InsP_3 induced by a 5-min exposure to 10 μM carbachol was reduced to 52 ± 5% of carbachol-stimulated control values (three experiments).

Inhibition of caerulein-evoked increases in $[Ca^{2+}]_i$ by THC. The effects of THC on cytoplasmic Ca^{2+} levels ($[Ca^{2+}]_i$) elicited by Ca^{2+}-mobilizing agonists were also examined. The inhibitory effect of THC on the caerulein-stimulated rise in $[Ca^{2+}]_i$ was concentration dependent (Fig. 4). Significant inhib-

TABLE 1

Effects of THC, melittin, and caerulein on [^{3}H]InsP_3 levels in pancreatic acinar cells

Cells prelabeled with *myo*-[^{3}H]inositol were exposed for 5 min to 20 μM THC, 1 μg/ml melittin, or 0.1 μM caerulein and the accumulation of [^{3}H]InsP_3 was determined. Each value is the mean ± standard error of three independent experiments.

Treatment	[^{3}H]InsP_3
	cpm/mg of protein
None	843 ± 84
THC	761 ± 71
Melittin	2228 ± 345*
Caerulein	2334 ± 572*

*$p < 0.05$ compared with paired control samples.

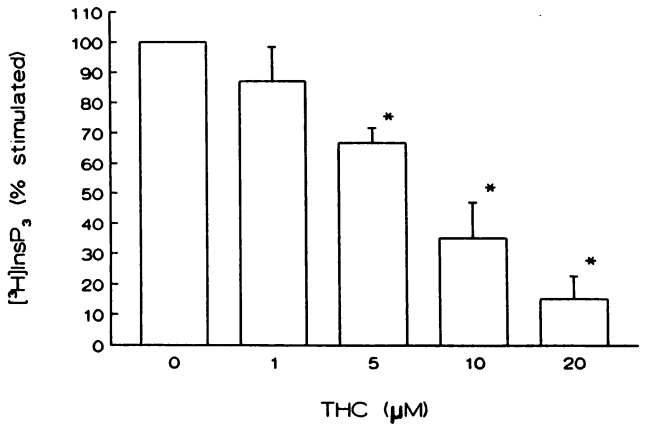

Fig. 3. Inhibition by THC of caerulein-stimulated [^{3}H]InsP_3 accumulation. Acinar cells prelabeled with *myo*-[^{3}H]inositol were pretreated for 5 min with various doses of THC before being exposed to 0.1 μM caerulein for an additional 5 min. [^{3}H]InsP_3 was separated from other inositol phosphates and quantitated. Caerulein-stimulated [^{3}H]InsP_3 formation after THC pretreatment is shown as a percentage of the [^{3}H]InsP_3 accumulation elicited by caerulein alone (1491 ± 537 cpm/mg of protein). Each *vertical bar* represents the mean value (± standard error) from three independent experiments. *Significantly different from control as determined by one-way analysis of variance using Dunnett's test with $p < 0.05$.

itory effects were observed with THC in the concentration range of 5–20 μM, which correspond to the inhibitory concentrations of THC on [^{3}H]InsP_3 accumulation (see Fig. 3). A concentration of THC that markedly inhibited the caerulein-induced rise in cytoplasmic Ca^{2+} failed to depress the elevation in $[Ca^{2+}]_i$ produced by ionomycin (Table 2). This finding indicates that the inhibitory effect of THC on caerulein-induced Ca^{2+} mobilization is not due to some action of the drug that is unrelated to its inhibitory effects on phosphoinositide metabolism. Table 2 also shows that THC does not alter basal $[Ca^{2+}]_i$.

Inhibition of caerulein-stimulated amylase secretion by THC. THC alone did not alter amylase secretion, although 20 μM THC caused a small but nonsignificant decrease in secretion (data not shown). However, the preincubation of acini with THC resulted in a concentration-related inhibition of caerulein-stimulated amylase secretion during a 30-min incubation (Fig. 5). The inhibition was significant at 5 μM THC and maximal at 20 μM THC. The concentration response of the inhibition of secretion by THC was comparable to the concentration-response relationships of PtdIns4,5P_2 levels (Fig. 2), [^{3}H]InsP_3 formation (Fig. 3), and Ca^{2+} mobilization (Fig. 4).

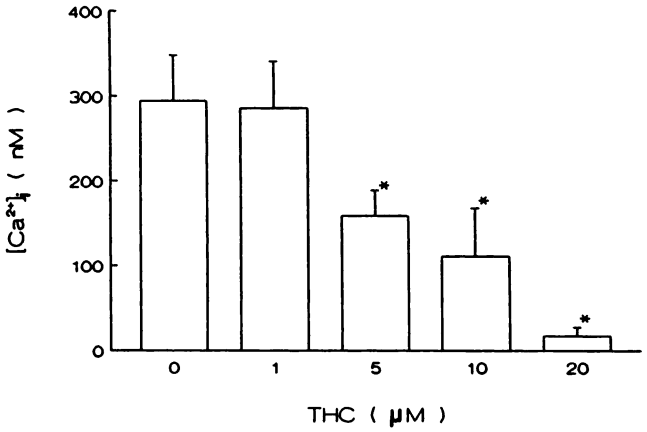

Fig. 4. Inhibitory effects of THC on caerulein-stimulated rise in $[Ca^{2+}]_i$. Fura-2-loaded cells were preincubated for 5 min with various doses of THC and exposed to 0.1 μM caerulein, and $[Ca^{2+}]_i$ was determined as described in Materials and Methods. The figure depicts initial peak $[Ca^{2+}]_i$ (with resting levels subtracted) measured 5–10 sec after the addition of caerulein. Each *vertical bar* represents the mean (± standard error) for five independent experiments. *Significantly different from samples treated with agonist alone as determined by paired Student's *t* test ($p < 0.05$).

TABLE 2

Comparative effects of THC on caerulein- and ionomycin-induced peak $[Ca^{2+}]_i$

Fura-2-loaded acinar cells were pretreated for 10 min with or without 20 μM THC and then exposed to 0.1 μM caerulein. After 3 min, when $[Ca^{2+}]_i$ had declined to 212 ± 27 nM, 1 μM ionomycin was added. $[Ca^{2+}]_i$ was determined by dual wavelength excitation spectrophotofluorometry. Each value is the mean (± standard error) of four independent experiments.

	Peak $[Ca^{2+}]_i$	
	−THC	+THC
	nM	
Control	123 ± 23	131 ± 14
Caerulein	430 ± 40	249 ± 23*
Ionomycin	541 ± 62	655 ± 93

* Significantly different from value of the corresponding control group as assessed by unpaired Student's *t* test, $p < 0.05$.

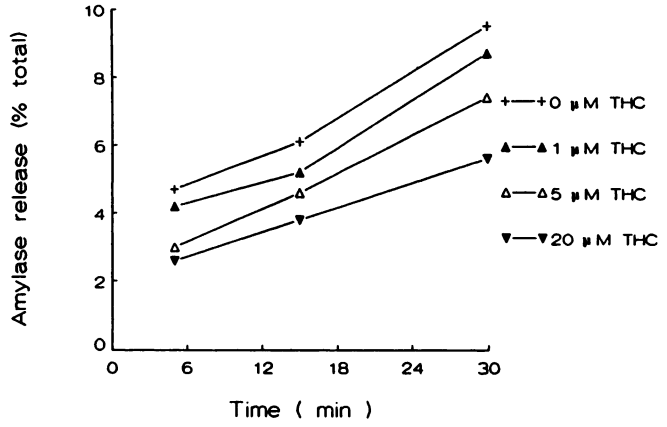

Fig. 5. The inhibitory effect of THC on caerulein-stimulated amylase secretion. Acini preincubated for 5 min with different doses of THC were exposed to 0.1 μM caerulein for the times indicated and amylase secretion was determined. Caerulein-stimulated amylase secretion was calculated by subtracting the corresponding value for basal secretion at each time point. Each *symbol* represents the mean value derived from three different experiments. The inhibitory effects of THC were significant at 5 and 20 μM, as assessed by analysis of variance. The standard error in each case did not vary by more than 20% of the mean value.

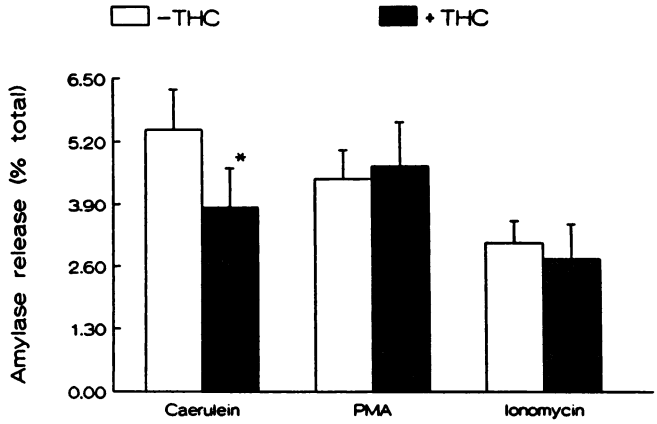

Fig. 6. The effect of THC on stimulated amylase secretion. Acini were preincubated for 5 min with and without 20 μM THC and then exposed to 0.01 μM caerulein, 1 μM PMA, or 0.5 μM ionomycin for 30 min. Net values for evoked secretion were determined after subtraction of basal secretion. Each *bar* represents the mean value (± standard error) from four independent experiments.

By contrast, THC at comparable concentrations failed to inhibit amylase secretion induced by either PMA or ionomycin (Fig. 6). Thus, the effects of THC on caerulein-induced amylase secretion were not due to some ill-defined action on the exocytotic process.

Effects of melittin on [^{3}H]arachidonate release and [^{32}P]PtdIns4,5P_2 levels. Melittin, a polypeptide isolated from bee venom, liberates free arachidonic acid in secretory cells, presumably by activating phospholipase A_2 (18–20). Incubation of acinar cells with melittin caused a concentration-dependent increase in the release of [^{3}H]arachidonic acid and a decrease in [^{32}P]PtdIns4,5P_2 levels (Fig. 7). However, the increase in arachidonic acid release was less marked than that produced by THC, even though melittin caused a greater reduction in [^{32}P]PtdIns4,5P_2 levels than THC (compare with Fig. 2). The magnified action of melittin to reduce [^{32}P]PtdIns4,5P_2 levels was reflected in its ability to stimulate the accumulation of [^{3}H]InsP_3 to levels comparable to those elicited by caerulein (Table 1). The activation of phospholipase C by

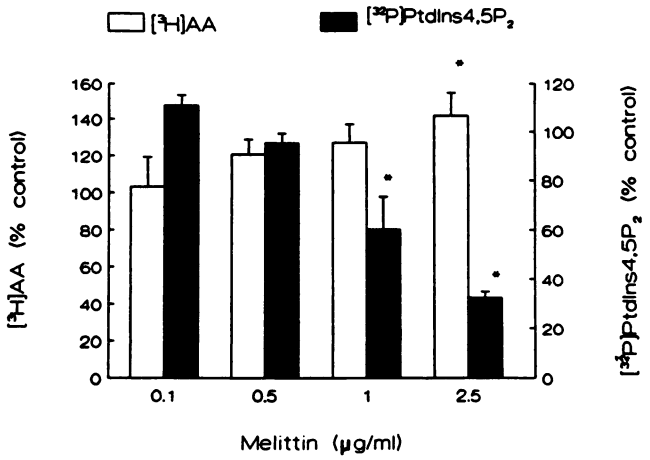

Fig. 7. The concentration-dependent effects of melittin on [^{3}H]arachidonic acid release and [^{32}P]PtdIns4,5,P_2 levels in acinar cells. Experimental conditions were identical to those described for Fig. 2, except that the cells were exposed for 30 min to the concentrations of melittin indicated.

melittin precluded any comparison of its effects on Ca^{2+} mobilization and amylase release with those obtained with THC.

Discussion

Our previous study, which used exogenous arachidonate, provided evidence for a unique negative feedback role for this fatty acid in phospholipase C-mediated phosphoinositide breakdown in exocrine pancreas (6). The ability of THC and melittin to promote unesterified arachidonic acid release in other cell systems (7–9, 18–20) led us to believe that these agents would afford us the opportunity to investigate the potential role of endogenously generated free arachidonic acid on phosphoinositide turnover, Ca^{2+} mobilization, and enzyme secretion in exocrine pancreas.

The present study has demonstrated that both THC and melittin stimulate the release of free [^{3}H]arachidonic acid in pancreatic acinar cells. Moreover, at concentrations effective for arachidonic acid release, both drugs also decreased the steady state levels of [^{32}P]PtdIns4,5P_2, thereby lending support for the hypothesis that endogenous arachidonic acid serves to inhibit polyphosphoinositide synthesis in exocrine pancreas. However, the greater decrease in [^{32}P]PtdIns4,5P_2 levels caused by melittin relative to its ability to enhance arachidonic acid release is explained by the finding that, unlike THC and exogenous arachidonate, melittin also stimulates the phospholipase C-mediated breakdown of PtdIns4,5P_2, leading to accumulation of [^{3}H]$InsP_3$ (Table 1). Hence, in subsequent studies, only the effects of THC were examined, because it increases endogenous arachidonate release without stimulating phospholipase C.

THC concentrations that enhance arachidonic acid release and decrease [^{32}P]PtdIns4,5P_2 levels (Fig. 2) also inhibit caerulein-stimulated [^{3}H]$InsP_3$ accumulation (Fig. 3). These observations support the contention that THC depletes the PtdIns4,5P_2 pool hydrolyzed by Ca^{2+}-mobilizing agonists. However, THC was found to inhibit caerulein-stimulated [^{3}H]$InsP_3$ accumulation to a greater extent (88%) than its maximal depletion of PtdIns4,5P_2 levels (43%). These findings suggest that there is a pool(s) of PtdIns4,5P_2 in acinar cells that is not utilized by caerulein and that is not depleted in response to THC. Previous studies conducted in other model systems have provided evidence for the existence of multiple cellular pools of PtdIns4,5P_2 (21–23).

The corresponding concentration-response relationships exhibited by THC on caerulein-stimulated [^{3}H]$InsP_3$ formation and Ca^{2+} mobilization support the findings of Streb *et al* (24), in which alterations in stimulated $InsP_3$ levels in permeabilized pancreatic acinar cells induced by various inhibitors were accompanied by parallel changes in Ca^{2+} release. In exocrine cells, the dose-response curves and time course for evoked $InsP_3$ formation and Ca^{2+} mobilization may coincide, although maximal Ca^{2+} mobilization can be obtained with less than maximal $InsP_3$ levels, implying the presence of receptor reserve (3). The finding that THC failed to inhibit the rise in $[Ca^{2+}]_i$ induced by ionomycin, which does not cause phospholipase C-mediated increases in $InsP_3$ levels (25), indicates that THC exerts a selective effect on the phospholipase C-phosphoinositide messenger system.

Although a maximal THC concentration almost completely abolished the caerulein-stimulated rise in $[Ca^{2+}]_i$, it only partially blocked evoked amylase release (approximately 50%). This result supports previous evidence that evoked amylase release can still occur at relatively low levels of cytoplasmic Ca^{2+} (26) and that other cellular messengers generated by receptor agonists, such as diacylglycerol, act synergistically with Ca^{2+} to modulate the secretory response (12, 26). Thus, even a markedly attenuated breakdown of phosphoinositides in response to a receptor agonist may still generate a sufficient elevation in $[Ca^{2+}]_i$ and diacylglycerol levels to promote secretion. Alternatively, the THC-induced decrease in PtdIns4,5P_2 levels may not necessarily lead to a marked depression of caerulein-stimulated diacylglycerol formation, because this putative activator of protein kinase C may be derived from PtdIns, as well as PtdIns4P and PtdIns4,5P_2 (27, 28). Extending this line of reasoning, the THC-induced decrease in [^{32}P]PtdIns4,5P_2 levels in pancreatic acinar cells was not accompanied by a comparable decrease in [^{32}P]PtdIns4P levels. In any event, THC is unable to inhibit amylase secretion elicited by PMA and ionomycin. Moreover, THC fails to inhibit caerulein-stimulated amylase release when added 2 min after the stimulus (data not shown), indicating a primary effect of the drug on an early step in the stimulus-secretion coupling pathway. These findings, taken together, suggest that the depression of caerulein-stimulated amylase secretion induced by THC is due to inhibition of agonist-induced $InsP_3$ formation and Ca^{2+} mobilization and not some ill-defined effect on the exocytotic process itself. In exocrine pancreas, the effects of carbachol and caerulein are expressed through distinct receptors and GTP-binding proteins (29). The ability of THC to attenuate [^{3}H]$InsP_3$ accumulation evoked by both carbachol and caerulein argues against the possibility that THC directly interacts with specific receptors or GTP-binding proteins to alter agonist binding or the functional coupling of receptors to phospholipase C. Still, THC may exert at least part of its inhibitory action on [^{3}H]$InsP_3$ accumulation by altering the binding of Ca^{2+}-mobilizing agonists to multiple receptor sites, perhaps by intercalating into the plasma membrane and/or altering membrane fluidity (10).

In comparison with the inhibitory effects of THC on stimulated amylase release, THC-induced arachidonate release in islets of Langerhans is associated with an enhancement of secretagogue-stimulated insulin secretion (9). These divergent

results can be interpreted to mean that arachidonic acid and/or its metabolites serve as either positive or negative modulators in secretory cells, depending upon the secretory cell under scrutiny. Ultimately, the basis of such divergencies must be understood if we are to gain a fundamental understanding of the interplay between various cellular messenger systems in secretory cells.

In conclusion, the present study reveals that the enhancement of endogenously released arachidonate leads to a decrease in PtdIns4,5P_2 levels and to caerulein-induced InsP_3 accumulation, $[Ca^{2+}]_i$ increase, and amylase secretion. These findings lend support to the hypothesis that in exocrine pancreas endogenously generated arachidonic acid serves as a negative feedback modulator of amylase secretion elicited by Ca^{2+}-mobilizing receptor agonists. One possible site of this putative regulatory action of arachidonic acid may involve PtdIns kinase, the enzyme system that regulates the synthesis of PtdIns4,5P_2. However, the elucidation of the biochemical mechanism accounting for this inhibition must await further experimentation.

Acknowledgments

We wish to thank Jerry S. McKinney for her expert assistance in carrying out the determinations of cytosolic Ca^{2+}.

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Send reprint requests to: Dr. Suzanne G. Laychock, Department of Pharmacology, Medical College of Virginia, Box 524, MCV Station, Richmond, VA 23298-0524.