0026-895X/90/060928-09$02.00/0

MOLECULAR PHARMACOLOGY, 37:928–936



# Fatty Acids and Cyclooxygenase and Lipoxygenase Pathway Inhibitors Modulate Inositol Phosphate Formation in Pancreatic Islets

SUZANNE G. LAYCHOCK

*Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298*

Received August 31, 1989; Accepted March 26, 1990

**SUMMARY**

Isolated rat pancreatic islets prelabeled with *myo*-[$^3$H]inositol respond to glucose and carbamylcholine with increased [$^3$H]inositol phosphate (InsP) production. Prostaglandin $E_2$ ($PGE_2$) inhibits the effects of glucose and carbamylcholine on [$^3$H]InsP production. Ionomycin reversed the effect of $PGE_2$ on glucose-stimulated [$^3$H]InsP production. The cyclooxygenase inhibitors indomethacin, ibuprofen, and eicosatetraynoic acid potentiated [$^3$H]InsP production in response to 5 and 10 mM glucose but not to 17 mM glucose. Indomethacin did not affect the carbamylcholine response. Unsaturated fatty acids, including arachidonic acid, linolenic acid, eicosapentaenoic acid, oleic acid, and eicosatetraynoic acid, increased [$^3$H]InsP production. Arachidonic acid potentiated [$^3$H]InsP accumulation in response to low concentrations of glucose. Indomethacin potentiated the response to arachidonic acid. $\Delta^9$-Tetrahydrocannabinol, which mobilizes endogenous fatty acids, also potentiated glucose-stimulated [$^3$H]InsP production. The lipoxygenase inhibitors BW755C and nordihydroguaiaretic acid inhibited [$^3$H]InsP production in response to glucose, carbamylcholine, and fatty acids. Thus, $PGE_2$ and endogenous cyclooxygenase products antagonize InsP production in islets, whereas fatty acids promote InsP accumulation.

The stimulation of pancreatic islets of Langerhans by glucose, muscarinic receptor agonists, and other insulin secretagogues induces activation of the phosphoinositide pathway, with the turnover and hydrolysis of phosphoinositides (1–3) and the accumulation of InsP (1, 4, 5). Phospholipase C-mediated InsP production appears to positively modulate insulin secretion (6), probably through the induction of $Ca^{2+}$ release from endoplasmic reticulum of islet cells (7, 8). The islet cell phospholipase C is modulated by a GTP-binding protein (9, 10) that is distinct from the protein modulating adenylate cyclase activity (10).

Phospholipase C activity can be activated by certain lipids (11, 12), especially unsaturated amphiphiles such as oleic acid, arachidonic acid, linoleic acid, and prostaglandins (12, 13). In intact cells, however, the relationship between unsaturated fatty acids and InsP generation is complex (14). In insulin-secreting cells, the fatty acid metabolite $PGE_2$ interacts with specific receptors and is inhibitory to insulin release (15–17) and phosphoinositide metabolism (18). $PGE_2$ affects an inhibitory GTP-binding protein that modulates the activity of islet cell phospholipase C (18). The pharmacological inhibition of arachidonic acid metabolism through the cyclooxygenase pathway in islets augments insulin release (19, 20), whereas the inhibition of lipoxygenase activity inhibits insulin secretion (21–23). The mechanisms responsible for the inhibitory effect of prostaglandins on insulin release include the inhibition of adenylate cyclase and glucose metabolism by $PGE_2$ (15, 24), whereas specific transduction mechanisms responsible for lipoxygenase product effects on secretion have not been identified. Whether endogenous fatty acids and fatty acid metabolites in islets affect phosphoinositide turnover and InsP production is not known. However, interaction between the phospholipase C-mediated lipid-metabolizing pathways and the arachidonic acid-metabolizing pathways may provide a novel mechanism for modulation of cell secretory activity. The interaction between these two pathways represents the basis of this study.

## Experimental Procedures

**Materials.** *myo*-[2-$^3$H(N)Inositol (10–20 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO). (5,8,11,14)-Eicosatetraenoic acid (arachidonic acid) was obtained from Serdary

Portions of this work were supported by National Institutes of Health Grant DK-25705 and National Institute on Drug Abuse Grant DA-05274.

**ABBREVIATIONS:** InsP, total *myo*-inositol mono-, bis-, and trisphosphates; BW755C, 3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline hydrochloride; ETYA, 5,8,11,14-eicosatetraynoic acid; NDGA, nordihydroguaiaretic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazinesulfonic acid; $InsP_3$, *myo*-inositol trisphosphate; $InsP_4$, *myo*-inositol tetrakisphosphate; KRB, Krebs Ringer bicarbonate; $PGE_2$, prostaglandin $E_2$; THC, $\Delta^9$-tetrahydrocannabinol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

Research Laboratories (Ontario, Canada); (9,12,15)-octadecatrienoic acid (linolenic acid), 9-octadecenoic acid (oleic acid), and (4,7,10,13,16,19)-docosahexaenoic acid were obtained from Nu Chek Prep (Elysian, MN); indomethacin was from Merck Sharp & Dohme Research Laboratories (Rahway, NJ); $PGE_2$ and ibuprofen were from Upjohn Co. (Kalamazoo, MI); (*cis*)(5,8,11,14,17)-eicosapentaenoic acid, NDGA, phorbol 12,13-dibutyrate, mannoheptulose, and bovine serum albumin (fraction V, fatty acid free) were from Sigma Chemical Co. (St. Louis, MO); ionomycin was from Calbiochem (La Jolla, CA); BW755C was a gift from Dr. P. J. McHale, the Wellcome Research Laboratories (Kent, England); and ETYA was from Cayman Chemical (Ann Arbor, MI). All other chemicals were reagent grade. THC was obtained from the National Institute on Drug Abuse. Collagenase (Type D) was from Boehringer-Mannheim (St. Louis, MO). Dowex 1-X8 formate form was from Bio-Rad Laboratories (Richmond, CA).

**Preparation and incubation of islets.** Islets from male Sprague-Dawley rats (225–250 g) were isolated following collagenase digestion of excised pancreata, as previously described (25). Batches of equal numbers of islets (an average of 50 islets/experimental sample) were incubated in KRB buffer (pH 7.4) equilibrated with $O_2/CO_2$ (19:1) and containing 16 mM HEPES, 0.01% bovine serum albumin, and 2.8 mM glucose (basal glucose concentration). All incubations were performed in a gyrorotatory water bath (180 rpm) at 37°, in an enclosed atmosphere of $O_2/CO_2$ (19:1).

Islets were prelabeled with *myo*-[$^3$H]inositol (3.8–5 $\mu$Ci) by incubation of the tissue in 0.1 ml of KRB buffer containing 7.3 mM glucose for 60 min. This brief period of stimulation allowed the tissue to incorporate *myo*-[$^3$H]inositol into pools of phosphoinositides that might be rapidly turning over in response to glucose. Additional KRB buffer was then added to yield a final volume of 0.3 ml and 4.3 mM glucose. The incubation was continued for an additional 60 min at the low glucose concentration, in order to allow the islets to assume a basal level of metabolic and secretory activity and to continue *myo*-[$^3$H]inositol incorporation into phospholipids. The unincorporated radiolabel was subsequently washed from the islets, as previously described (18). The islets were then suspended in KRB medium and incubated with various agents in a final volume of 0.3 ml, as described below.

Experimental protocols to determine the levels of [$^3$H]InsP in islets included LiCl (10 mM) and *myo*-inositol (1 mM) in order to inhibit the metabolism of InsP to inositol and to retard the further synthesis of radiolabeled phosphoinositides. Five minutes were allowed to elapse after the LiCl/*myo*-inositol addition, various agents were added as indicated in the text, and the experiment was continued for an additional 30 min. Experiments with cyclooxygenase and lipoxygenase inhibitors were designed such that the inhibitors were present with the islets for 10–15 min before the addition of fatty acids or LiCl/*myo*-inositol and subsequent glucose stimulation. This manner of addition was followed in order to allow adequate time for enzyme inhibition to occur before substrate or stimulus addition. Exogenous fatty acids or THC were added to the islets 15 min before the addition of LiCl/*myo*-inositol, except where indicated, in order to allow for the metabolism of arachidonic acid through the cyclooxygenase and lipoxygenase pathways. Because LiCl was added just before the glucose or carbamylcholine stimulus, the [$^3$H]InsP quantitated was not cumulative for the time of inhibitor or most fatty acid additions but rather was a measure of [$^3$H]InsP produced in response to glucose or carbamylcholine stimuli in combination with the other agents. Hydrophobic agents were dissolved in ethanol or dimethyl sulfoxide, with the final concentration of the vehicle in the islets less than 1%; paired control samples had vehicle added.

The reaction was stopped with acid extraction of the samples (26) followed by neutralization and isolation of the InsP by ion exchange chromatography, as described previously (27, 28). Radiolabeled *myo*-inositol 1-phosphate, *myo*-inositol 1,4-bisphosphate, *myo*-inositol 1,4,5-trisphosphate and *myo*-inositol 1,3,4-trisphosphate (together termed $InsP_3$), and $InsP_4$ (the tris- and tetrakisphosphate compounds coeluted) were quantitated, and the values were added together for a measure of total [$^3$H]InsP produced by islets, unless otherwise indicated.

**Statistical analysis.** Statistical analysis of data was by Student's *t* test or one-way analysis of variance; *p* values less than 0.05 were accepted as significant. Values are the mean ± standard error for independent experiments. When values are expressed as percentages, statistical analysis was performed on real data values.

## Results

**Effects of cyclooxygenase and lipoxygenase inhibitors on [$^3$H]InsP levels in isolated islets.** [$^3$H]InsP production

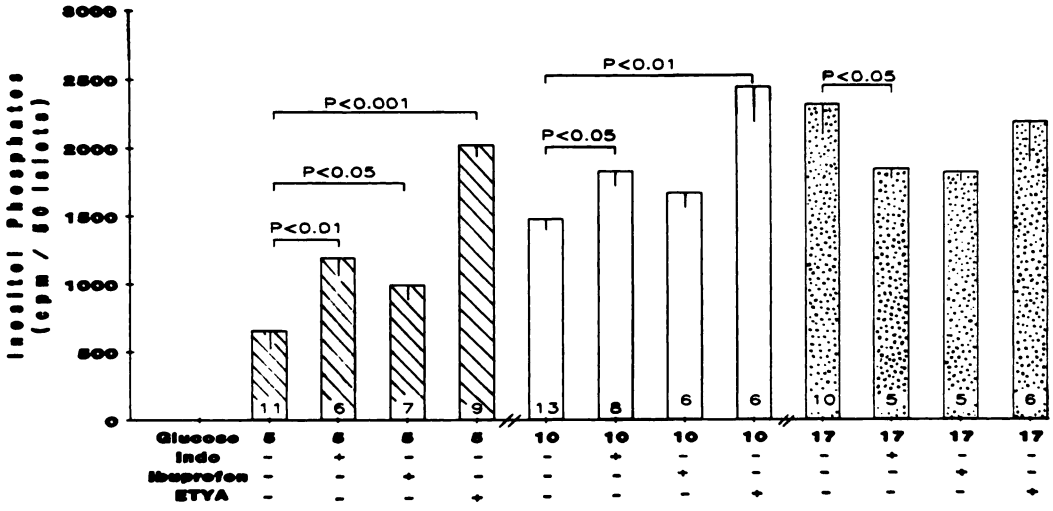


**Fig. 1.** Effects of glucose, indomethacin (*Indo*), ibuprofen, and ETYA on [$^3$H]InsP levels in isolated islets. Islets prelabeled with *myo*-[$^3$H]inositol were preincubated in KRB buffer for 15 min in the absence or presence of indomethacin (10 $\mu$M), ibuprofen (10 $\mu$M), or ETYA (20 $\mu$M). Then, LiCl (10 mM) and *myo*-inositol (1 mM) were added, 5 min later 5, 10, or 17 mM glucose (as indicated) was added to the islets, and the incubation was continued for 30 min. Total radiolabel in InsP was determined following anion exchange chromatography. Values are the mean ± standard error for the number of independent experiments indicated at the base of each *bar*. [$^3$H]InsP levels with 10 and 17 mM glucose were significantly ($p < 0.05$) greater than values with 5 mM glucose. *p* values were determined by one-way analysis of variance or Student's *t* test (paired) for effects of indomethacin with 17 mM glucose.

TABLE 1

**Effects of indomethacin, ibuprofen, and ETYA on [$^3$H]InsP$_3$/InsP$_4$ levels in islets**

Isolated islets prelabeled with *myo*-[$^3$H]inositol were preincubated in KRB buffer, in the absence (control) or presence of indomethacin (10 μM), ibuprofen (10 μM), or ETYA (20 μM), for 15 min. Five minutes after the addition of LiCl (10 mM) and *myo*-inositol (1 mM), various concentrations of glucose were added as indicated, and the incubation was continued for 30 min for the determination of [$^3$H]InsP$_3$/InsP$_4$ levels. Each value is the mean ± standard error for the number of independent experiments indicated in parentheses.

| Glucose | [$^3$H]InsP$_3$/InsP$_4$ levels | | | |
|---|---|---|---|---|
| | Control | Indomethacin | Ibuprofen | ETYA |
| *mM* | *cpm/50 islets* | | | |
| 5 | 81 ± 12 (7) | | 108 ± 11[a] (7) | 263 ± 16[b] (8) |
| 10 | 140 ± 20 (10) | 180 ± 25[b] (10) | | 274 ± 22[b] (6) |
| 17 | 224 ± 29 (6) | 155 ± 26 (6) | 207 ± 20 (5) | 232 ± 37 (6) |

[a] $p < 0.02$; [b] $p < 0.01$, compared with control values, as determined by Student's *t* test.

TABLE 2

**Effects of BW755C and NDGA on InsP accumulation in islets**

Islets prelabeled with *myo*-[$^3$H]inositol were incubated in KRB buffer for 15 min in the absence or presence of BW755C (250 μM) or NDGA (20 μM) and 2.8 mM glucose. Then, LiCl (10 mM) and *myo*-inositol (1 mM) were added, 5 min later 5, 10, or 17 mM glucose (as indicated) was added to the islets, and the incubation was continued for 30 min. Values are the mean ± standard error of cpm in total InsP or InsP$_3$/P$_4$ only for *n* experiments.

| Glucose | Treatment | [$^3$H]InsP | *n* | [$^3$H]InsP$_3$/P$_4$ | *n* |
|---|---|---|---|---|---|
| *mM* | | *cpm/50 islets* | | *cpm/50 islets* | |
| 5 | Control | 710 ± 63 | 5 | 84 ± 18 | 5 |
| 5 | BW755C | 413 ± 54[a] | 5 | 59 ± 17[a] | 5 |
| 5 | NDGA | 677 ± 123 | 5 | 72 ± 9 | 5 |
| 10 | Control | 1486 ± 238 | 4 | 165 ± 24 | 5 |
| 10 | BW755C | 686 ± 110[b] | 4 | 101 ± 17[b] | 5 |
| 10 | NDGA | 643 ± 150[b] | 3 | 76 ± 13[b] | 3 |
| 17 | Control | 2236 ± 126 | 4 | 242 ± 24 | 4 |
| 17 | BW755C | 1023 ± 220[a] | 4 | 124 ± 28[b] | 4 |
| 17 | NDGA | 887 ± 176[a] | 4 | 101 ± 5[b] | 4 |

[a] $p < 0.01$; [b] $p < 0.05$, compared with control values at similar glucose concentrations, as determined by Student's *t* test for paired values.

in isolated pancreatic islets increased in a concentration-dependent manner in response to glucose (Fig. 1). Inhibitors of cyclooxygenase activity affected glucose-stimulated [$^3$H]InsP production. [$^3$H]InsP production with a concentration of glucose at the threshold level for insulin secretion (5 mM) was potentiated by indomethacin (143 ± 14% of control) and ibuprofen (Fig. 1). Indomethacin also potentiated [$^3$H]InsP production with 10 mM glucose, although ibuprofen failed to significantly alter [$^3$H]InsP production at this concentration of glucose (Fig. 1). A higher concentration of ibuprofen (100 μM), which inhibits prostaglandin biosynthesis by 60% (29), did not further augment [$^3$H]InsP production at either glucose concentration (data not shown). At a maximally secretagogic concentration of glucose (17 mM), [$^3$H]InsP production with indomethacin or ibuprofen was reduced approximately 15% (Fig. 1).

ETYA, which inhibits arachidonic acid metabolism by both the cyclooxygenase and lipoxygenase pathways (30), potentiated [$^3$H]InsP production in the presence of 5 and 10 mM glucose to 315 ± 37 and 144 ± 14% of control, respectively (Fig. 1). The stimulatory effects of ETYA were markedly greater than those of either indomethacin or ibuprofen at low glucose concentrations. However, like indomethacin and ibuprofen, ETYA did not affect [$^3$H]InsP levels in the presence of 17 mM glucose (Fig. 1).

The production of islet [$^3$H]InsP$_3$/InsP$_4$ with glucose and the cyclooxygenase inhibitors reflected the changes in total [$^3$H]InsP levels. [$^3$H]InsP$_3$/InsP$_4$ levels with 5 mM glucose were increased in the presence of ibuprofen by 42 ± 14% above control islet values (Table 1). [$^3$H]InsP$_3$/InsP$_4$ levels with 10 mM glucose were increased in the presence of indomethacin by 23 ± 6% (Table 1). In the presence of ETYA, [$^3$H]InsP$_3$/InsP$_4$ levels were markedly increased with 5 or 50 mM glucose, by 238 ± 35% and 66 ± 14% above control values, respectively (Table 1). Ibuprofen, indomethacin, and ETYA failed to significantly alter [$^3$H]InsP$_3$/InsP$_4$ levels with 17 mM glucose (Table 1). In the presence of a basal nonsecretagogic concentration of glucose (2.8 mM), ETYA (20 μM) stimulated [$^3$H]InsP production by 119 ± 37% above control values ($p < 0.01$; three experiments) and [$^3$H]InsP$_3$/InsP$_4$ production (97 ± 14 cpm/50 islets) increased by 179 ± 35% ($p < 0.01$; three experiments).

When the experimental data with the cyclooxygenase inhibitors and ETYA were analyzed to determine the contribution of [$^3$H]InsP$_3$/InsP$_4$ to the total amount of radiolabel recovered in [$^3$H]InsP, no significant differences were found for any of the inhibitor-treated islet values, compared with control values (11 ± 1% of total). Thus, it did not appear that altered [$^3$H]

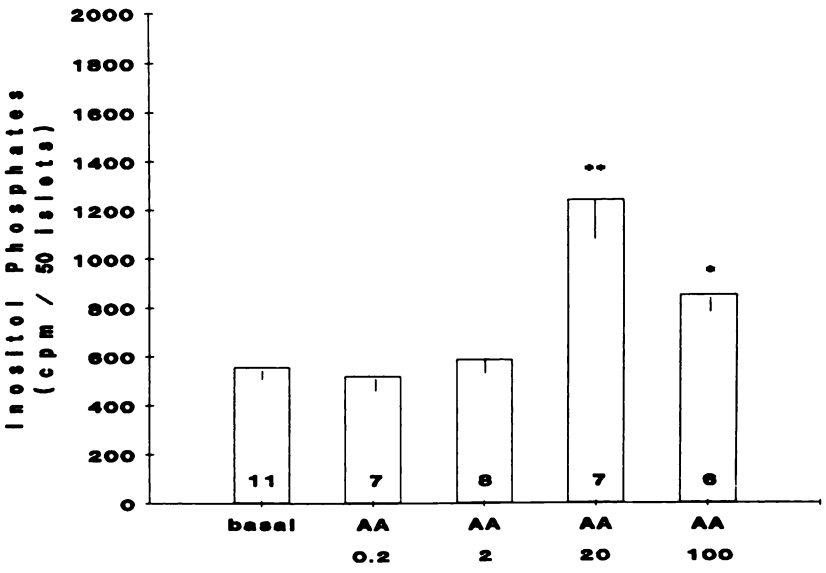


**Fig. 2.** Arachidonic acid (*AA*)-induced stimulation of [$^3$H]InsP production in islets. Islets prelabeled with *myo*-[$^3$H]inositol were preincubated in KRB buffer in the absence (*basal*) or presence of arachidonic acid, at the concentrations (μM) indicated, for 15 min. Then, LiCl (10 mM) and *myo*-inositol (1 mM) were added and the incubation was continued for 35 min. Values are the mean ± standard error for total [$^3$H]InsP levels. *$p < 0.05$; **$p < 0.001$, compared with basal values, as determined by one-way analysis of variance.

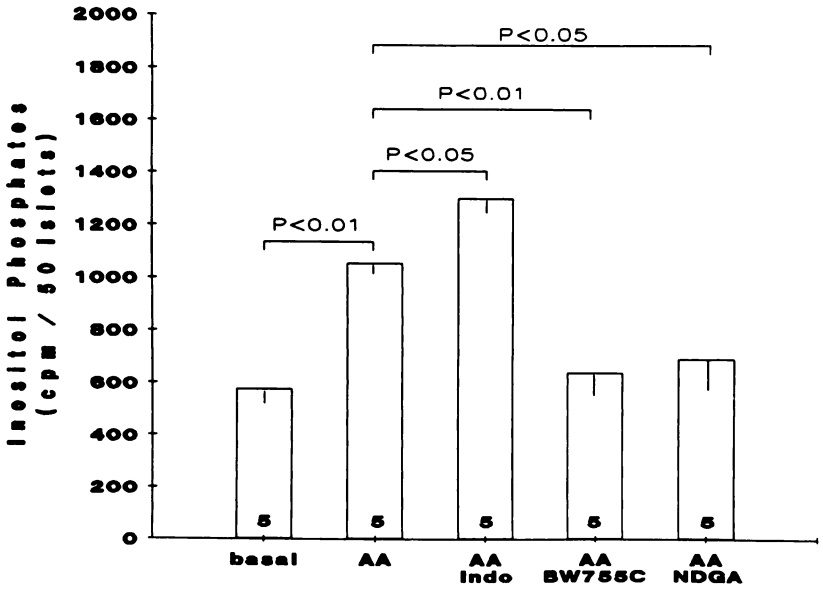


**Fig. 3.** Effects of cyclooxygenase and lipoxygenase inhibitors on arachidonic acid (*AA*)-stimulated [$^3$H]InsP production. Islets prelabeled with *myo*-[$^3$H]inositol were preincubated in KRB buffer in the absence (*basal*) or presence of indomethacin (*Indo*) (10 $\mu$M), BW755C (250 $\mu$M), or NDGA (20 $\mu$M) for 10 min, then AA (20 $\mu$M) was added, the incubation was continued for 15 min until LiCl (10 mM) and *myo*-inositol (1 mM) were added, and the incubation was continued for an additional 35 min. Values are the mean ± standard error for five independent experimental determinations. *p* values were determined by one-way analysis of variance.

TABLE 3

**Effect of THC on [$^3$H]InsP accumulation in islets**

Isolated islets prelabeled with *myo*-[$^3$H]inositol were preincubated in the presence or absence of THC, at the concentrations indicated, for 15 min. Five minutes after the addition of LiCl (10 mM) and *myo*-inositol (1 mM), glucose (10 mM) was added and the incubation was continued for 30 min. Indomethacin (10 $\mu$M) or BW755C (250 $\mu$M) was added for 10 min before THC in selected experiments. Values of percentages of control are the mean ± standard error for *n* independent determinations. Glucose (10 mM)-stimulated values (1166 ± 162 cpm/50 islets) were 147 ± 12% of basal values ($n$ = 16).

| Treatment | [$^3$H]InsP levels | *n* |
|---|---|---|
| | *% of control* | |
| Glucose | 100 | |
| Glucose + THC (0.2 $\mu$M) | 111 ± 11 | 12 |
| Glucose + THC (2 $\mu$M) | 140 ± 13[a] | 12 |
| Glucose + THC (20 $\mu$M) | 170 ± 20[a] | 10 |
| Glucose + THC (20 $\mu$M) + BW755C | 68 ± 10[b] | 7 |
| Glucose + THC (20 $\mu$M) + indomethacin | 105 ± 11[c] | 11 |

[a] $p < 0.01$, compared with glucose control values.
[b] $p < 0.05$ compared with glucose plus THC (20 $\mu$M) values, as determined by Student's *t* test for paired samples.
[c] Compared with glucose plus THC (20 $\mu$M) values.

InsP levels were the result of effects of the stimuli on InsP hydrolysis.

Other inhibitors of lipoxygenase product formation were evaluated for effects on [$^3$H]InsP production. BW755C and NDGA, at concentrations that inhibit insulin secretion (23), inhibited [$^3$H]InsP production in response to 5, 10, and 17 mM glucose (Table 2). Both inhibitors reduced [$^3$H]InsP production by approximately 60% at 10 and 17 mM glucose; at 5 mM glucose, only BW755C significantly reduced [$^3$H]InsP production, by 42 ± 6% (Table 2). A lower concentration of BW755C (25 $\mu$M) did not affect [$^3$H]InsP production (97 ± 11% of control) in response to 10 mM glucose; however, at 17 mM glucose, 25 $\mu$M BW755C reduced [$^3$H]InsP production by 19 ± 12% in paired samples ($p < 0.01$; six experiments). [$^3$H]$InsP_3$/$InsP_4$ production paralleled the changes in total [$^3$H]InsP in the experiments with BW755C and NDGA (Table 2).

**Effects of fatty acids on [$^3$H]InsP levels.** In order to determine whether arachidonic acid and/or its metabolites affected InsP production, islets were incubated with arachidonic acid and [$^3$H]InsP levels were quantitated under either basal or stimulatory glucose concentrations. Arachidonic acid stimulated basal [$^3$H]InsP production in a concentration-dependent manner (Fig. 2). The most stimulatory concentration of arachidonic acid tested was 20 $\mu$M, whereas a 5-fold higher concentration was somewhat less effective (Fig. 2). Arachidonic acid (20 $\mu$M) stimulated basal [$^3$H]InsP production to 181 ± 31% of control (Fig. 2) and also potentiated [$^3$H]InsP production in response to 5 mM glucose to 39 ± 14% ($p < 0.02$; eight experiments) above control values. At 17 mM glucose, arachidonic acid (20 $\mu$M) did not affect [$^3$H]InsP production. When islets were not preincubated with arachidonic acid but, rather, the arachidonic acid (20 $\mu$M) was added to islets after the LiCl/*myo*-inositol addition, arachidonic acid significantly ($p < 0.01$) enhanced [$^3$H]InsP levels (929 ± 177 cpm/50 islets) compared with basal values (556 ± 37 cpm/50 islets), following a 30-min incubation.

Treatment of islets with indomethacin potentiated the production of [$^3$H]InsP in response to arachidonic acid to 24 ± 7% above arachidonic acid-treated control values (Fig. 3). Indomethacin did not affect [$^3$H]InsP production in islets incubated with arachidonic acid and 10 or 17 mM glucose (data not shown). In contrast to cyclooxygenase inhibition, the addition of the lipoxygenase inhibitors BW755C and NDGA reduced [$^3$H]InsP production in response to arachidonic acid to basal levels (Fig. 3).

Evidence that endogenous fatty acids also modulate InsP production in islets was sought. THC, which enhances arachidonic acid mobilization in islets (23), was added to islets prelabeled with *myo*-[$^3$H]inositol. Pretreatment of islets with THC potentiated the [$^3$H]InsP response to glucose in a concentration-dependent manner (Table 3). The presence of BW755C antagonized the THC and glucose response (Table 3). The lack of an effect of indomethacin on the THC response in glucose-stimulated islets (Table 3) is similar to the results with this inhibitor in glucose-stimulated islets in the presence of arachidonic acid. THC (20 $\mu$M) did not significantly affect basal [$^3$H]InsP production (118 ± 18% of basal).

In order to determine whether the response to arachidonic acid (20:4) was specific, other unsaturated fatty acids were investigated for effects on InsP production. Linolenic acid (18:3), eicosapentaenoic acid (20:5), and oleic acid (18:1), but

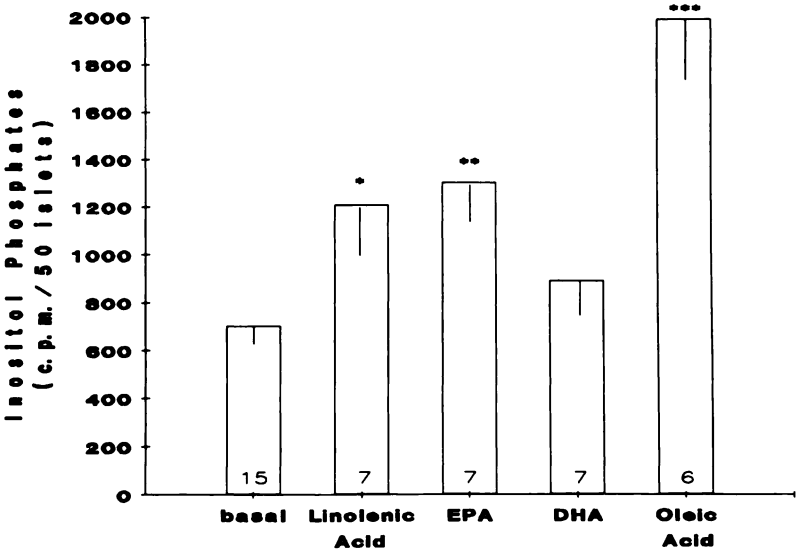


**Fig. 4.** Effects of unsaturated fatty acids on [$^3$H]InsP production in isolated islets. Islets prelabeled with *myo*-[$^3$H]inositol were incubated in KRB buffer in the absence (*basal*) or presence of linolenic acid (20 $\mu$M) eicosapentaenoic acid (*EPA*) (20 $\mu$M), docosahexaenoic acid (*DHA*) (20 $\mu$M), or oleic acid (20 $\mu$M) for 15 min. Then, LiCl (10 mM) and *myo*-inositol (1 mM) were added, and the incubation was continued for 35 min. Values are the mean ± standard error for the number of experiments indicated at the base of each *bar*. $^*p < 0.05$; $^{**}p < 0.02$; $^{***}p < 0.001$, compared with basal values, as determined by one-way analysis of variance.

TABLE 4

**Effect of ionomycin on [$^3$H]InsP accumulation**

Islets prelabeled with *myo*-[$^3$H]inositol were incubated in the absence or presence of $PGE_2$ (1 $\mu$M) for 25 min, and then LiCl (10 mM) and *myo*-inositol (1 mM) in the presence or absence of mannoheptulose (30 mM) were added. After 5 min, islets were incubated for 30 min longer in the presence or absence of ionomycin (1 $\mu$M) and 2.8 mM (basal) or 17 mM glucose, as indicated. Values are mean ± standard error for *n* experiments.

| Treatment | [$^3$H]InsP | *n* |
|---|---|---|
| | *cpm/50 islets* | |
| Group A | | |
| Basal | 447 ± 54 | 5 |
| Ionomycin | 612 ± 27[a] | 5 |
| Glucose (17 mM) | 1203 ± 98[a] | 6 |
| Glucose (17 mM) + $PGE_2$ | 938 ± 42[b] | 6 |
| Glucose (17 mM) + $PGE_2$ + ionomycin | 1269 ± 99[c] | 5 |
| Group B | | |
| Glucose (17 mM) | 1731 ± 210 | 6 |
| Glucose (17 mM) + mannoheptulose | 497 ± 97[b] | 5 |
| Glucose (17 mM) + mannoheptulose + ionomycin | 696 ± 96[d] | 5 |

[a] $p < 0.02$, compared with basal.
[b] $p < 0.05$, compared with glucose (17 mM).
[c] $p < 0.05$, compared with glucose (17 mM) plus $PGE_2$.
[d] $p < 0.05$, compared with glucose (17 mM) plus mannoheptulose, determined by Student's *t* test.

TABLE 5

**Effects of carbamylcholine and $PGE_2$ on [$^3$H]InsP levels in isolated islets**

Isolated islets prelabeled with *myo*-[$^3$H]inositol were preincubated in KRB buffer, in the absence or presence of $PGE_2$ as indicated, for 15 min. Five minutes after the addition of LiCl (10 mM) and *myo*-inositol (1 mM), carbamylcholine (10 $\mu$M) was added as indicated, and the incubation was continued for 30 min. Each value is the mean ± standard error for *n* independent determinations.

| Treatment | [$^3$H]InsP | *n* |
|---|---|---|
| | *cpm/50 islets* | |
| Basal | 505 ± 90 | 6 |
| Carbamylcholine | 1171 ± 111[a] | 6 |
| Carbamylcholine + $PGE_2$ (0.1 $\mu$M) | 988 ± 108[b] | 8 |
| Carbamylcholine + $PGE_2$ (1 $\mu$M) | 782 ± 90[c] | 7 |

[a] $p < 0.01$, compared with basal values; [b] $p < 0.02$; [c] $p < 0.05$, compared with carbamylcholine-stimulated control values, as determined by one-way analysis of variance and Student's *t* test.

not docosahexaenoic acid (22:6), stimulated [$^3$H]InsP production by more than 2-fold (Fig. 4). However, unlike the response to arachidonic acid, indomethacin did not potentiate the response to eicosapentaenoic acid or oleic acid (Fig. 5). In contrast, similar to the response with arachidonic acid, NDGA and BW755C inhibited [$^3$H]InsP production in the presence of eicosapentaenoic acid or oleic acid to 40 to 60% of control (Fig. 5).

In contrast to the responses to fatty acids, exogenous $PGE_2$ inhibited [$^3$H]InsP accumulation in response to glucose, as reported previously (18), by 26 ± 8% (Table 4). The inhibitory response to $PGE_2$ was reversed, however, by the addition of ionomycin, a $Ca^{2+}$ ionophore (Table 4). Ionomycin also augmented the accumulation of [$^3$H]InsP under basal conditions (Table 4), although the ionophore did not potentiate glucose (17 mM)-stimulated [$^3$H]InsP levels (data not shown).

Because $PGE_2$ inhibits glucose oxidation in islets (24), [$^3$H]InsP accumulation in the presence of another inhibitor of glucose metabolism, mannoheptulose, was investigated. Mannoheptulose completely inhibited the glucose-induced elevation of [$^3$H]InsP levels (Table 4). The addition of ionomycin augmented the levels of [$^3$H]InsP produced in the presence of glucose and mannoheptulose, although ionomycin did not restore [$^3$H]InsP levels to those found with a glucose stimulus alone (Table 4).

The addition of phorbol 12,13-dibutyrate (1 $\mu$M) did not change the response to $PGE_2$ with glucose (17 mM), in the presence or absence of ionomycin (1 $\mu$M) (data not shown), suggesting that the activation of protein kinase C was not directly mediating the response to ionomycin. The phorbol ester also did not alter the response to mannoheptulose (data not shown).

**Effects of $PGE_2$ and lipoxygenase and cyclooxygenase inhibitors on muscarinic receptor agonist-induced [$^3$H]InsP production.** The stimulation of muscarinic receptors activates phospholipase C and increases InsP production in the islet (1). Although carbamylcholine increased [$^3$H]InsP levels by more than 2-fold (Table 5), the addition of exogenous $PGE_2$ inhibited carbamylcholine-stimulated [$^3$H]InsP accumulation in a concentration-dependent manner (Table 5). One micromolar $PGE_2$ reduced [$^3$H]InsP levels to 77 ± 5% of carbamylcholine-stimulated control values.

The effects of BW755C and NDGA on carbamylcholine-stimulated [$^3$H]InsP levels were similar to the effects on glucose-stimulated islets. BW755C reduced [$^3$H]InsP production

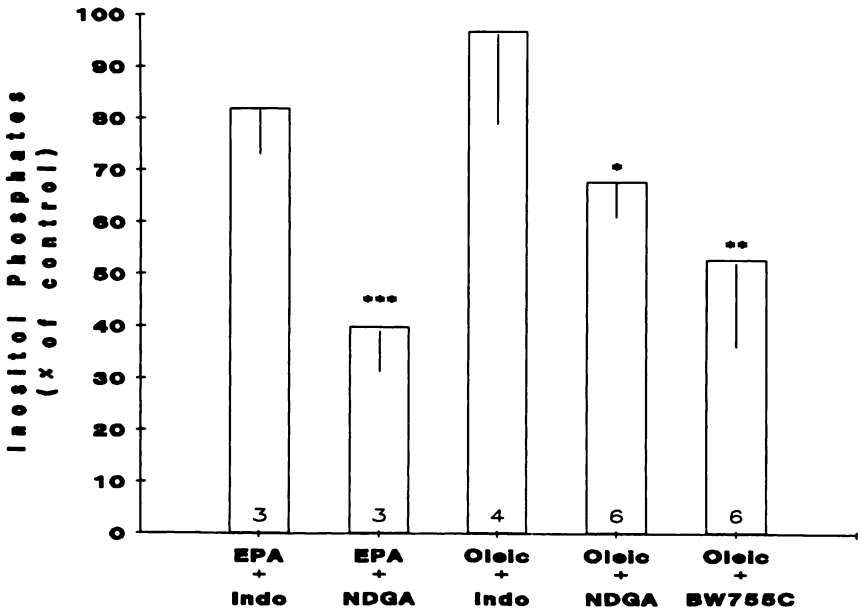


**Fig. 5.** Effects of indomethacin (*Indo*) and NDGA on eicosapentaenoic acid (*EPA*)- and oleic acid-induced [$^3$H]InsP production. Islets prelabeled with *myo*-[$^3$H] inositol were preincubated in KRB buffer in the absence (*basal*) or presence of indomethacin (10 $\mu$M) or NDGA (20 $\mu$M) for 10 min. The incubation was continued for 15 min in the absence or presence of eicosapentaenoic acid (20 $\mu$M) or oleic acid (20 $\mu$M), as indicated. LiCl (10 mM) and *myo*-inositol (1 mM) were then added, and the incubation was continued for 35 min. Values are the mean ± standard error for percentage of the respective controls (eicosapentaenoic acid or oleic acid alone). The number of independent experiments is shown at the base of each *bar*. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, compared with fatty acid-treated control values, as determined by one-way analysis of variance.

TABLE 6

**Effects of BW755C, NDGA, and indomethacin on [$^3$H]InsP levels in carbamylcholine-stimulated islets**

Isolated islets prelabeled with *myo*-[$^3$H]inositol were preincubated in the absence or presence of BW755C (250 $\mu$M), NDGA (20 $\mu$M), or indomethacin (10 $\mu$M) for 15 min. Five minutes after the addition of LiCl (10 mM) and *myo*-inositol (1 mM), carbamylcholine (10 $\mu$M) was added and the incubation was continued for 30 min. Values of [$^3$H]InsP levels are the mean ± standard error for *n* independent determinations.

| Treatment | [$^3$H]InsP levels | *n* |
|---|---|---|
| | *cpm/50 islets* | |
| Carbamylcholine | 1566 ± 97 | 5 |
| Carbamylcholine + BW755C | 774 ± 155[a] | 6 |
| Carbamylcholine + NDGA | 1078 ± 112[b] | 4 |
| Carbamylcholine + indomethacin | 1467 ± 14 | 2 |

[a] $p < 0.01$; [b] $p < 0.05$, compared with carbamylcholine-treated control values, as determined by one-way analysis of variance.

in response to carbamylcholine by more than 50%, whereas the inhibitory effect of NDGA was less marked (Table 6). Indomethacin did not affect [$^3$H]InsP levels in carbamylcholine-stimulated islets (Table 6).

## Discussion

These results demonstrate that glucose, arachidonic acid, and other fatty acids, endogenous fatty acid mobilization, and certain arachidonic acid metabolites affect [$^3$H]InsP production in pancreatic islets. Unsaturated fatty acids and prostaglandins activate phospholipase C and promote InsP generation in other tissues (12, 13, 31), except rat pancreatic acinar cells (14). The present study demonstrates that arachidonic acid enhanced [$^3$H]InsP accumulation in islets in a concentration-dependent manner. In addition, treatment of islets with THC and mobilization of endogenous arachidonic acid (23) was accompanied by increased InsP accumulation. The results also indicate that different classes of cyclooxygenase inhibitors, such as indomethacin (a methylated indole derivative) and ibuprofen (a propionic acid derivative), potentiate [$^3$H]InsP accumulation in the presence of less than maximal secretagogic concentrations of glucose. Thus, the metabolism of arachidonic acid through the cyclooxygenase pathway appears to negatively modulate InsP production.

Prostaglandins are described as negative feedback modulators of insulin secretion, because exogenous prostaglandins inhibit insulin release and cyclooxygenase inhibitors potentiate insulin secretion in response to glucose (16, 17, 19). Prostaglandins may inhibit secretion in part through the suppression of InsP formation, because phospholipase C activation (1–3) and InsP mediate insulin release (5, 32, 33). Because cyclooxygenase inhibitors increased the levels of $Ca^{2+}$-mobilizing [$^3$H]$InsP_3$/$InsP_4$ (7), prostaglandins inhibitory to insulin secretion may suppress InsP levels and $Ca^{2+}$ mobilization. In this and a previous study (18), exogenous $PGE_2$ inhibited islet [$^3$H]InsP accumulation in response to glucose. $PGE_2$ also partially suppresses phosphatidylinositol 4-phosphate hydrolysis in guinea pig neutrophils (34), perhaps through effects on inositol lipid turnover.

$PGE_2$ affects several cellular mechanisms that may have an impact on phosphoinositidase activity. For example, islet phospholipase C activation by glucose is dependent upon $Ca^{2+}$ mobilization (35, 36). Because $PGE_2$ reduces intracellular $Ca^{2+}$ concentrations in cells (37) and reduces cAMP production in islets (38), which reduce $Ca^{2+}$ availability (39), it is possible that $PGE_2$ inhibits phospholipase C activity in part by restricting $Ca^{2+}$ availability. Reduced $Ca^{2+}$ levels in the islet may suppress phospholipase C activity directly or indirectly through the inhibition of phospholipase $A_2$ and reduced fatty acid mobilization. The reversal of the effects of $PGE_2$ by ionomycin suggests that reduced $Ca^{2+}$ availability plays a role in the response. However, muscarinic receptor-mediated InsP generation is also inhibited by $PGE_2$. Unlike with glucose, the stimulatory effects of carbamylcholine on islet phosphoinositide hydrolysis are less dependent upon $Ca^{2+}$ than is glucose stimulation (35). The effects of $PGE_2$ on the carbamylcholine response may be direct, with the $PGE_2$ receptor response mediated by a pertussis toxin-sensitive inhibitory GTP-binding protein (18). Alterations in muscarinic receptor ligand binding in the presence of $PGE_2$ or BW755C have not been excluded. The lack of potentiation of the carbamylcholine response by

indomethacin suggests that endogenous $PGE_2$ does not modulate the muscarinic response on InsP production. Perhaps the levels of arachidonic acid and its metabolites potentially generated from diglyceride hydrolysis are not sufficient to exert a negative feedback effect on phosphoinositidase.

Because $PGE_2$ reduces glucose utilization in islets by a GTP-binding protein-mediated mechanism (24), the $PGE_2$ effect on InsP generation may be transduced through this response. Both $PGE_2$ and mannoheptulose reduced [$^3$H]InsP accumulation, and the responses were antagonized by ionomycin. Thus, $PGE_2$ suppressed glucose metabolism and the correlates of this inhibition probably modulate phosphoinositide hydrolysis. $PGE_2$-induced changes in glucose metabolism may also modulate the phosphoinositidase response to carbamylcholine.

A biphasic cyclooxygenase inhibitor response is suggested by the data showing that, at low glucose concentrations, indomethacin and ibuprofen potentiated glucose-induced InsP production, whereas, at high, maximally secretagogic concentrations of glucose, the cyclooxygenase inhibitors slightly suppressed [$^3$H]InsP accumulation. High glucose concentrations may maximally activate phospholipase C and overcome the inhibitory effects of endogenous prostaglandins. The modest inhibitory effect of the nonsteroidal antiinflammatory agents on phospholipase C may be related to their inhibitory effect on phospholipase $A_2$ (40). The nonsteroidal antiinflammatory agent suppression of glucose-stimulated phospholipase $A_2$ activity and arachidonic acid mobilization may negatively influence InsP generation at high glucose concentrations, in light of the stimulatory effect of fatty acids on InsP generation.

Unlike cyclooxygenase products, lipoxygenase-derived arachidonic acid metabolites are described as positive modulators of insulin secretion, primarily because lipoxygenase inhibitors, including BW755C and NDGA, suppress hormone release (23, 41, 42). BW755C and NDGA also inhibit [$^3$H]InsP accumulation in response to both low and high glucose concentrations, fatty acids and THC, and carbamylcholine. At first glance, these results suggest that lipoxygenase products are positive modulators of [$^3$H]InsP production. However, another inhibitor of lipoxygenase and cyclooxygenase activity, ETYA, evoked a marked stimulation of [$^3$H]InsP formation under basal or glucose-stimulated conditions. This result was unexpected and suggested, first, that ETYA-induced inhibition of prostaglandin synthesis (30) was potentiating the formation of [$^3$H]InsP in the manner of indomethacin and ibuprofen; second, that the 20-carbon unsaturated fatty acid structure of ETYA contributed to enhanced InsP generation; and, third, that BW755C and NDGA exert effects upon InsP accumulation that are perhaps unrelated to their effects on arachidonic acid metabolism.

In order to determine the specificity of the fatty acid and arachidonic acid metabolism inhibitor effects, several unsaturated fatty acids were investigated for their effects on InsP production. Arachidonic acid, linolenic acid, eicosapentaenoic acid, and oleic acid were potent stimuli for [$^3$H]InsP production; docosahexaenoic acid was less potent in this regard, perhaps due to the longer chain length of this fatty acid. Arachidonic acid also increases InsP formation in astrocytes, in a manner independent of eicosanoid synthesis (31). In the latter study, NDGA did not antagonize the effect of arachidonic acid, but the concentration of NDGA was only 25% of that used in this study. In another report, neither NDGA nor indomethacin, at concentrations similar to those used in this study, affected 10-sec $K^+$-stimulated InsP production in islets labeled to steady state for several days with *myo*-[$^3$H]inositol (35). The mechanism accounting for the inhibition by NDGA and BW755C of InsP production in the present study, with different stimuli and time course of InsP accumulation than in the latter report, is under investigation.

The results with fatty acids in the present study are opposite the effects in exocrine acinar pancreatic tissue, where arachidonic acid suppresses InsP production (14) by virtue of the inhibition of inositol incorporation into phosphoinositides. In the present study, the stimulation of [$^3$H]InsP accumulation was obtained in the presence of LiCl, which inhibits the conversion of inositol monophosphate to inositol. This argues that a step(s) before inositol monophosphate generation must be activated by fatty acids in order to promote InsP accumulation. Moreover, because arachidonic acid stimulated [$^3$H]InsP accumulation, even when the fatty acid was added following LiCl addition, it is unlikely that inositol accumulation or InsP or diglyceride production before LiCl addition were responsible for the observation made with arachidonic acid.

Fatty acids probably have unique effects on the InsP pathway in different cell types. In the islet, for example, $Ca^{2+}$-mediated phospholipase C (35) may be affected by the ability of arachidonic acid and other fatty acids to mobilize islet $Ca^{2+}$ (43). In astrocytes, arachidonic acid-stimulated InsP production is inhibited by $Ca^{+2}$ chelation with EGTA (31). The degree of unsaturation of the fatty acid chains did not relate directly to potency in the stimulation of InsP accumulation. Under $Ca^{2+}$-free conditions, arachidonic, linoleic, and docosahexaenoic acids evoke insulin secretion, apparently through their ability to evoke $Ca^{2+}$ mobilization from intracellular stores (43). The effective concentration of the fatty acids in this study (20 $\mu$M) for [$^3$H]InsP production and the concentration of fatty acids evoking $Ca^{2+}$ mobilization and insulin secretion in islets (33 $\mu$M) (43) are similar. And, although docosahexaenoic acid did not significantly increase InsP accumulation, it did modestly increase insulin release under $Ca^{2+}$-free conditions. Thus, several fatty acids appear to increase insulin secretion, $Ca^{2+}$ mobilization, and phosphoinositide hydrolysis in islets; however, these phenomena may be circumstantial with regard to the initiation of hormone release.

The ability of indomethacin to potentiate the [$^3$H]InsP response to arachidonic acid, but not eicosapentaenoic or oleic acids (which do not contribute to classical prostaglandin synthesis), supports the hypothesis that prostaglandin metabolites exert a negative feedback effect on InsP production. Eicosapentaenoic acid may promote the production of $n-3$ cyclooxygenase products, thus reducing classical prostaglandin levels and potentiating the InsP response. The selectivity of the indomethacin response also demonstrates that indomethacin was not directly stimulating phospholipase C activity when potentiating the glucose and arachidonic acid responses.

The lipoxygenase inhibitors BW755C and NDGA, on the other hand, inhibited [$^3$H]InsP production in the presence of eicosapentaenoic acid and oleic acid. The latter results suggest that the antioxidants BW755C and NDGA affect phospholipase C activity directly, in addition to their effects on arachidonic acid metabolism. The antioxidant inhibition of lysosomal phospholipase C (44) and the potential for oxidants to promote phospholipid hydrolysis (45) may also contribute to reduced

InsP accumulation in this study. Reduced [$^3$H]InsP production as well as the inhibition of glucose metabolism (36) and $Ca^{2+}$ uptake (46) and the blockade of the direct effects of $Ca^{2+}$ on insulin exocytosis (47) by NDGA and BW755C indicate the potential for lipoxygenase inhibitors to affect insulin release in several ways.

In summary, glucose-stimulated InsP formation is potentiated by cyclooxygenase inhibitors, which suppress the biosynthesis of prostaglandins. $PGE_2$ inhibits both glucose- and muscarinic receptor-mediated changes in InsP production. $PGE_2$ may suppress islet metabolism, alter $Ca^{2+}$ availability, or transduce changes in GTP-binding proteins that have an impact on phosphoinositidase activity. In contrast to the prostaglandins, arachidonic acid and other unsaturated fatty acids positively modulate islet InsP production. The stimulatory fatty acid response may be the result of changes in $Ca^{2+}$ mobilization, membrane fluidity, or substrate availability to phosphoinositidase. A second messenger role for arachidonic acid in pancreatic islet secretion has been suggested. Glucose activates phospholipase $A_2$ and mobilizes arachidonic acid (48). Arachidonic acid increases insulin release, evokes the release of $Ca^{2+}$ from the endoplasmic reticulum (49) and $^{45}Ca^{2+}$ efflux from islets, and activates protein kinase C (43, 49). At different levels of glucose stimulation and fatty acid mobilization and metabolism in islets, prostaglandins and arachidonic acid may modulate InsP production and insulin secretion in different ways.

**Acknowledgments**

The dedicated and skilled assistance of Suna Bilgin and Bonnie Pace is greatly appreciated.

**References**

1. Best, L., and W. J. Malaisse. Stimulation of phosphoinositide breakdown in rat pancreatic islets by glucose and carbamylcholine. *Biochem. Biophys. Res. Commun.* **116**:9–16 (1983).
2. Laychock, S. G. Identification and metabolism of phosphoinositides in isolated islets of Langerhans. *Biochem. J.* **216**:101–106 (1983).
3. Dunlop, M. E., and W. J. Malaisse. Phosphoinositide phosphorylation and hydrolysis in pancreatic islet cell membrane. *Arch. Biochem. Biophys.* **244**:421–429 (1986).
4. Morgan, N. G., G. M. Rumford, and W. Montague. Studies on the role of inositol trisphosphate in the regulation of insulin secretion from isolated rat islets of Langerhans. *Biochem. J.* **228**:713–718 (1985).
5. Turk, J., B. A. Wolf, and M. L. McDaniel. Glucose-induced accumulation of inositol trisphosphates in isolated pancreatic islets. *Biochem. J.* **237**:259–263 (1986).
6. Tanigawa, K., H. Kuzuya, and H. Imura. Possible role of phosphatidylinositol turnover in insulin release from isolated rat pancreatic islets. *Horm. Metab. Res.* **16**:458–460 (1984).
7. Wollheim, C. B., T. J. Biden, P. D. Lew, and W. J. Schlegel. Calcium mobilization by inositol 1,4,5-trisphosphate during activation of islet, pituitary, and myeloid cells. *Cardiovasc. Pharmacol.* **8**(Suppl. 8:S65–S70 (1986).
8. Prentki, B., B. E. Corkey, and F. M. Matshinsky. Inositol 1,4,5-trisphosphate and the endoplasmic reticulum $Ca^{2+}$ cycle of a rat insulinoma cell line. *J. Biol. Chem.* **260**:9185–9190 (1985).
9. Blachier, F., and W. J. Malaisse. Phospholipase C activation via a GTP-binding protein in tumoral islet cells stimulated by carbamylcholine. *Experientia (Basel)* **43**:601–602 (1987).
10. Dunlop, M. E., and R. G. Larkins. Muscarinic-agonist and guanine nucleotide activation of polyphosphoinositide phosphodiesterase in isolated islet-cell membranes. *Biochem. J.* **240**:731–737 (1986).
11. Dawson, R. M. C., N. L. Hemington, and R. F. Irvine. The inhibition of diacylglycerol-stimulated intracellular phospholipases by phospholipids with a phosphocholine-containing polar group: a possible physiological role for sphingomyelin. *Biochem. J.* **230**:61–68 (1985).
12. Irvine, R. F., A. J. Letcher, and R. M. C. Dawson. Phosphatidylinositol-4,5-bisphosphate phosphodiesterase and phosphomonoesterase activities of rat brain. *Biochem. J.* **178**:497–500 (1979).
13. Irvine, R. F., N. Hemington, and R. M. C. Dawson. The calcium-dependent phosphatidylinositol-phosphodiesterase of rat brain. *Eur. J. Biochem.* **99**:525–530 (1979).
14. Chaudhry, A., S. G. Laychock, and R. P. Rubin. The effects of fatty acids on phosphoinositide synthesis and *myo*-inositol accumulation in exocrine pancreas. *J. Biol. Chem.* **262**:17426–17431 (1987).
15. Robertson, R. P., P. Tsai, S. A. Little, H.-J. Zheng, and T. F. Walseth. Receptor-mediated adenylate cyclase-coupled mechanism for $PGE_2$ inhibition of insulin secretion in HIT cells. *Diabetes* **36**:1047–1053 (1987).
16. Robertson, R. P. *In vivo* insulin secretion: prostaglandin and adrenergic interrelationships. *Prostaglandins* **6**:501–510 (1974).
17. Laychock, S. G., and S. Bilgin. Calcium mobilization, prostaglandin $E_2$, and *alpha*$_2$-adrenoceptor modulation of glucose utilization and insulin secretion in pancreatic islets. *Biochem. Pharmacol.* **38**:2511–2520 (1989).
18. Laychock, S. G. Prostaglandin $E_2$ inhibits phosphoinositide metabolism in isolated pancreatic islets. *Biochem. J.* **260**:291–294 (1989).
19. Robertson, R. P. PGE, carbohydrate homeostasis, and insulin secretion: a suggested resolution of the controversy. *Diabetes* **32**:231–234 (1983).
20. Turk, J., J. N. Hughes, R. A. Easom, B. A. Wolf, D. W. Scharp, P. E. Lacy, and M. L. McDaniel. Arachidonic acid metabolism and insulin secretion by isolated human pancreatic islets. *Diabetes* **37**:992–995 (1988).
21. Turk, J., J. R. Colca, and M. L. McDaniel. Arachidonic acid metabolism in isolated pancreatic islets. III. Effects of exogenous lipoxygenase products and inhibitors on insulin secretion. *Biochem. Biophys. Acta* **834**:23–36 (1985).
22. Metz, S. A., S. Little, W. Fujimoto, and R. P. Robertson. Evidence for lipoxygenase mediation of arachidonic acid-stimulated insulin secretion. *Prostaglandin, Thromboxane, Leukotriene Res.* **12**:271–275 (1983).
23. Laychock, S. G., J. M. Hoffman, E. Meisel, and S. Bilgin. Pancreatic islet arachidonic acid turnover and metabolism and insulin release in response to *delta*-9-tetrahydrocannabinol. *Biochem. Pharmacol.* **35**:2003–2008 (1986).
24. Laychock, S. G. Prostaglandin $E_2$ and *alpha*$_2$-adrenoceptor agonists inhibit the pentose phosphate shunt in pancreatic islets. *Arch. Biochem. Biophys.* **269**:354–358 (1989).
25. Hoffman, J. M., and S. G. Laychock. Choline turnover in phosphatidylcholine of pancreatic islets. *Diabetes* **37**:1489–1498 (1988).
26. Dean, N. M., and J. D. Moyer. Separation of multiple isomers of inositol phosphates formed in $GH_3$ cells. *Biochem. J.* **242**:361–366 (1987).
27. Berridge, M. J., R. M. C. Dawson, C. P. Downes, J. P. Heslop, and R. F. Irvine. *Biochem. J.* **212**:473–482 (1983).
28. Chaudhry, A., R. H. Thompson, R. P. Rubin, and S. G. Laychock. Relationship between Δ-9-tetrahydrocannabinol-induced arachidonic acid release and secretagogue-evoked phosphoinositide breakdown and $Ca^{2+}$ mobilization of exocrine pancreas. *Mol. Pharmacol.* **34**:543–548 (1988).
29. Metz, S., W. Fujimoto, and R. P. Robertson. Modulation of insulin secretion by cyclic AMP and prostaglandin E: the effects of theophylline, sodium salicylate and tolbutamide. *Metabolism* **31**:1014–1022 (1982).
30. Turk, J., J. R. Colca, N. Kotagal, and M. L. McDaniel. Arachidonic acid metabolism in isolated pancreatic islets. I. Identification and quantitation of lipoxygenase and cyclooxygenase products. *Biochim. Biophys. Acta* **794**:125–136 (1984).
31. Murphy, S., and G. Welk. Arachidonic acid evokes inositol phospholipid hydrolysis in astrocytes. *FEBS Lett.* **257**:68–70 (1989).
32. Best, L., and W. J. Malaisse. Nutrient and hormone-neurotransmitter stimuli induce hydrolysis of phosphoinositides in rat pancreatic islets. *Endocrinology* **115**:1814–1820 (1984).
33. Zawalich, W. S. Modulation of insulin secretion from β-cells by phosphoinositide-derived second messenger molecules. *Diabetes* **37**:137–141 (1988).
34. Takenawa, T., and J. Ishitoya. Regulation of inositol phospholipid metabolism and arachidonic acid release: inhibitory mechanisms of arachidonic acid release by prostaglandin $E_2$ and cAMP. *Adv. Prostaglandin Thromboxane Res.* **15**:129–130 (1985).
35. Biden, T. J., B. Peter-Riesch, W. Schlegel, and C. B. Wollheim. $Ca^{2+}$-mediated generation of inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate in pancreatic islets. *J. Biol. Chem.* **262**:3567–3571 (1987).
36. Dunlop, M., A. Christanthou, A. Fletcher, M. Veroni, P. Woodman, and R. Larkins. Effects of inhibitors of eicosanoid synthesis on insulin release by neonatal pancreatic islets. *Biochem. Biophys. Acta* **801**:10–15 (1984).
37. Chouaib, S., R. J. Robb, K. Welte, and B. Dupont. Analysis of prostaglandin $E_2$ effect on T lymphocyte activation. *J. Clin. Invest.* **80**:333–340 (1987).
38. Seaquist, E. R., T. F. Walseth, D. M. Nelson, and R. P. Robertson. Pertussis toxin-sensitive G protein mediation of $PGE_2$ inhibition of cAMP metabolism and phasic glucose-induced insulin secretion in HIT cells. *Diabetes* **38**:1439–1445 (1989).
39. Henquin, J.-C. The interplay between cyclic AMP and ions in the stimulus-secretion coupling in pancreatic β-cells. *Arch. Int. Physiol. Biochim.* **93**:37–48 (1985).
40. Franson, R. C., D. Eisen, R. Jesse, and C. Lonni. Inhibition of highly purified mammalian phospholipase $A_2$ by non-steroidal anti-inflammatory agents. *Biochem. J.* **186**:633–636 (1980).
41. Metz, S. A., M. VanRollins, R. Strife, W. Fujimoto, and R. P. Robertson. Lipoxygenase pathway in islet endocrine cells. *J. Clin. Invest.* **71**:1191–1193 (1983).
42. Metz, S. A., W. Y. Fujimoto, and R. P. Robertson. Lipoxygenation of arachidonic acid: a pivotal step in stimulus-secretion coupling in the pancreatic *beta*-cell. *Endocrinology* **111**:2141–2143 (1982).
43. Metz, S. A. Exogenous arachidonic acid promotes insulin release from intact or permeabilized rat islets by dual mechanisms. *Diabetes* **37**:1453–1469 (1988).
44. Gamache, D. A., A. A. Fawzy,and R. C. Franson. Preferential hydrolysis of

peroxidized phospholipid by lysosomal phospholipase C. *Biochim. Biophys. Acta* **958**:116–124 (1988).
45. Shasby, D. M., M. Winter, and S. S. Shasby. Oxidants and conductance of cultured epithelial cell monolayers: inositol phospholipid hydrolysis. *Am. J. Physiol* **255**:C781–C788 (1988).
46. Lee, T.-C., B. Malone, and F. Snyder. Stimulation of calcium uptake by 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor) in rabbit platelets: possible involvement of the lipoxygenase pathway. *Arch. Biochem. Biophys.* **223**:33–39 (1985).
47. Metz, S. A. Blockade by lipoxygenase inhibitors of $Ca^{2+}$-dependent insulin secretion from permeabilized rat islets. *Biochem. Pharmacol.* **38**:1849–1862 (1989).
48. Laychock, S. G. Fatty acid incorporation into phospholipids of isolated pancreatic islets of the rat: relationship to insulin release. *Diabetes* **32**:6–13 (1983).
49. Wolf, B. A., J. Turk, W. R. Sherman, and M. L. McDaniel. Intracellular $Ca^{2+}$ mobilization by arachidonic acid. *J. Biol. Chem.* **261**:3501–3511 (1986).

**Send reprint requests to:** Dr. Suzanne Laychock, Department of Pharmacology and Therapeutics, 102 Farber Hall, SUNY at Buffalo, School of Medicine, Buffalo, NY 14214.