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PANCREATIC ISLET ARACHIDONIC ACID TURNOVER AND METABOLISM AND INSULIN RELEASE IN RESPONSE TO DELTA-9-TETRAHYDROCANNABINOL

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Abstract—Isolated pancreatic islets from the rat secrete insulin in response to glucose or delta-9-tetrahydrocannabinol (THC). THC stimulated the basal release of insulin and also potentiated the secretory response to glucose. The exposure of control or glucose-stimulated islets to THC inhibited the incorporation of [^{14}C]arachidonic acid (AA) into phospholipids. However, in islets prelabeled with [^{14}C]AA, THC enhanced the glucose-induced loss of AA from phospholipids. The enhanced AA release from islet phospholipids in response to glucose and THC was accompanied by increased synthesis of 12-L-[5,6,8,9,11,12,14,15-^{3}H(N)]-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) and prostaglandin E_2. The lipoxygenase inhibitor 3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline hydrochloride (BW755C) inhibited 12-HETE synthesis and insulin release in glucose and THC-challenged islets; nordihydroguaiaretic acid also inhibited insulin release in THC-treated islets. In contrast, the cyclooxygenase inhibitor, indomethacin, stimulated insulin release. In homogenized islet preparations, THC inhibited acyl-CoA acyltransferase, while it stimulated phospholipase A_2 activity. The stimulatory effects of THC on islet cell AA hydrolysis from phospholipids, lipoxygenase product formation, and secretion suggests that these biochemical sequelae in cell activation are important modulators of insulin release.

The turnover of arachidonic acid (AA) in cell membrane phospholipids of isolated pancreatic islets has been linked with insulin secretion. Not only does the primary insulin secretagogue, glucose, stimulate AA incorporation into phospholipids [1], but glucose also stimulates phospholipase A_2 activity [2] which releases AA from phospholipid pools for metabolism through the cyclooxygenase [3–5] and lipoxygenase [5, 6] pathways. However, the re-esterification of AA into lysophospholipids by acyl-CoA acyltransferases [7] has complicated the elucidation of a model directly relating AA release and metabolism and insulin release.

Delta-9-tetrahydrocannabinol (THC) is an inhibitor of acyl-CoA acyltransferase and stimulates AA release from cells [8–11]. In addition, THC enhances AA metabolism through the cyclooxygenase and lipoxygenase pathways [11–14]. Thus, THC was investigated in isolated pancreatic islet preparations in order to determine if changes in AA turnover and metabolism are related to insulin release.

MATERIALS AND METHODS

Materials. [5,6,8,9,11,12,14,15-(N)-^{3}H]AA (87 Ci/mmole), 12-L-[5,6,8,9,11,12,14,15-^{3}H(N)]-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE), and L-α-1-palmitoyl-2-arachidonyl[*arachidonyl*-1-^{14}C]phosphatidylcholine (40 mCi/mmole) were obtained from New England Nuclear (Boston, MA). [1-^{14}C]AA (58 Ci/mole) was obtained from the Amersham Corp. (Arlington Heights, IL). Research grade collagenase from *Clostridium histolyticum* (0.6 U/mg) was obtained from Serva Feinbiochemica (Heidelberg, West Germany). THC was obtained from the National Institue on Drug Abuse. Emulphor was from the GAS Corp. (Linden, NJ). Indomethacin was obtained from Merck Sharp & Dohme Research Lab (Rahway, NJ), and 3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline hydrochloride (BW755C) was a gift from Dr. P. J. McHale, the Wellcome Research Laboratories (Kent, England). Nordihydroguaiaretic acid (NDGA) was from the Sigma Chemical Co. (St. Louis, MO). All other reagents were laboratory grade or high performance liquid chromatography (HPLC) grade organics.

Preparation and incubation of islets. Isolated pancreatic islets from male rats were prepared by a collagenase technique, as previously described [15]. Equal numbers of islets (100–200 islets for radiolabel incorporation experiments, or 20 islets for insulin release experiments) were incubated as paired samples in Krebs–Ringer bicarbonate (KRB) buffer supplemented with 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) (16 mM), glucose (2.8 mM), and 0.01% bovine serum albumin (BSA) (fraction V; fatty acid free), and the pH was adjusted to 7.4 after equilibration with O_2–CO_2 (19:1) at 37° [1]. In all experiments, islets were preincubated for 20 min, and then the medium was replaced with oxygenated KRB buffer containing radiolabeled AA and/or other agents as indicated.

The time-related release of insulin from islets was determined as previously described [7] by measuring insulin in aliquots of KRB buffer sampled during 0–60 min of incubation in the presence or absence of

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various agents as indicated in the text. Insulin levels were determined by radio-immunoassay [15].

In studies of prelabeled islets for determination of AA recovery in phospholipids, islets were incubated in polypropylene microfuge tubes with KRB buffer (0.25 ml) containing 8.5 mM glucose and 0.25 μCi [^{14}C]AA for 90 min. The islets were then resuspended in 1 ml KRB buffer containing 0.5% BSA and 2.8 mM glucose, and the islets were incubated for 10 min; then the islets were resuspended in fresh KRB buffer, and the wash procedure was repeated two additional times. After washing, the islets were suspended in 1 ml KRB buffer containing 0.1% BSA and 2.8 mM glucose, and incubated for 10 min; this step was repeated one additional time to ensure that each batch of islets contained an equivalent concentration of BSA. Islets were resuspended in a final volume of 0.5 ml KRB buffer containing 0.01% BSA, and aliquots were removed for determination of zero-time insulin levels. Addition of glucose, and THC (20 μM) in Emulphor or an equal concentration of Emulphor alone in paired controls (less than 0.0001% Emulphor in the final volume), was made at time zero. The islets were incubated for 10 min, and aliquots of incubation medium were removed for determination of insulin release by radio-immunoassay [15]. The remaining buffer was removed, and the islets were resuspended in 1 ml of ice-cold KRB buffer containing 0.5% BSA, and centrifuged in an Eppendorf microfuge for 20 sec. The supernatant fraction was removed, the islet tissue was again resuspended in 0.5 ml of ice-cold KRB buffer, and the phospholipids were extracted in chloroform–methanol (1:2, v/v) and isolated by TLC, as previously described [7]. All values for [^{14}C]AA recovery include label quantitated in phosphatidylcholine (PC), phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine.

In studies to determine the effects of THC on the incorporation of labeled AA into phospholipids, islets were incubated in KRB buffer containing 2.8 mM glucose in the presence or absence of THC (20 μM) dissolved in Emulphor or an equal volume of Emulphor in controls (less than 0.0001% of the final volume) for 30 min. At this time, [^{14}C]AA (1 μCi/ml) and glucose (17 mM) were added to each sample, and the incubation was continued for 30 min longer. The incubation medium was then removed, and 1 ml of ice-cold KRB buffer was added to the islets, which were immediately centrifuged in an Eppendorf microfuge for 30 sec; the buffer was removed and replaced with 0.5 ml of ice-cold KRB buffer, and the islet phospholipids were extracted and isolated as described above. Quantitated radiolabel is for total [^{14}C]AA recovered in PC, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine.

Determination of acyltransferase and phospholipase activities. Acyl-CoA acyltransferase activity in islet homogenates was determined as previously described [7]. THC in Emulphor, or Emulphor alone as a control, was added to the assay as indicated in the text, together with 1 μM lysophosphatidylcholine as an acceptor, 0.5 mM ethylene glycol-bis-(β-aminoethyl ether)*N,N'*-tetraacetic acid (EGTA) to inhibit phospholipase A_2, and 0.05 μCi [^{14}C]AA as substrate. Radiolabel recovered as PC was isolated by thin-layer chromatography (TLC) [7] and quantitated by scintillation spectrometry.

Phospholipase A_2 activity was determined in islet homogenates prepared by sonicating islets briefly in sucrose (0.3 M)-HEPES (10 mM) buffer, pH 7.4. Enzyme activity was determined by incubating homogenate (30 ± 4 μg protein/0.1 ml) with $CaCl_2$ (1 mM), [^{14}C]PC labeled in the C-2 position on AA (0.2 μCi/ml), THC solubilized in Emulphor or Emulphor vehicle alone in control samples, and Tris–base buffer, (0.1 M), pH 8.0. Assays were initiated by the addition of homogenate and incubated at 37° for up to 90 min. The reaction was stopped by the addition of chloroform–methanol (1:2, v/v) for extraction of lipids. Phospholipid, diglyceride, and fatty acid fractions were separated by TLC in the solvent system petroleum ether–diethylether–acetic acid (5:5:1, by vol.). Phospholipase A_2 activity was determined as percent hydrolysis of labeled AA from PC. Hydrolysis of AA was linear for up to 90 min in the presence of islet protein, and the rate of hydrolysis was dependent upon the protein concentration. Protein was determined by the method of Lowry *et al.* [16].

Determination of lipoxygenase and cyclooxygenase products. Paired batches of isolated islets were incubated in 0.5 ml KRB buffer containing 8.5 mM glucose and [^{3}H]AA (1 μCi), but lacking BSA, for 60 min. The islets were then incubated in 0.5 ml of fresh buffer containing 2.8 mM glucose for 30 min, and then glucose and [^{3}H]AA (1 μCi) were added in the presence or absence of THC or Emulphor control. A sample with buffer, Emulphor and radiolabel, but lacking islets, was processed in an identical manner as the biological samples as a "blank" to determine autooxidation of labeled AA. Blank values were subtracted from islet values. The incubation was continued for 90 min, and then 4% 1 N HCl was added to the islets which were immediately homogenized at 4°. The homogenate was applied to a SEP-PAK C_{18} column (Millipore), and the column was washed with 15 ml deionized water, followed by 4 ml methanol to elute lipids. The methanolic fraction was dried *in vacuo*, and the recovered lipids were resuspended in solvent appropriate for C_{18} high performance liquid chromatography (HPLC) and lipoxygenase product isolation, or TLC for cyclooxygenase product isolation, as previously described [7, 17]. Fractions eluted from HPLC, which corresponded with [^{3}H]12-HETE standard elution, were collected and counted by scintillation spectrometry. Islet prostaglandins (PG) isolated on TLC which corresponded with the migration of standard PGE_2, as determined by iodine vapor staining, were scraped into scintillation vials and the radioactive content was determined by scintillation spectrometry. Counting efficiency for tritium was 55%, and for carbon 14 it was 95%. Values were corrected for quench. All samples were counted for 10 min at less than 2% error.

Statistical analysis. Statistical analysis was carried out on paired islet samples using Student's *t*-test (two-tailed), or a two-way analysis of variance (ANOVA) employing an unweighted means analysis for unequal cell sizes. Values are the mean ± S.E.M.

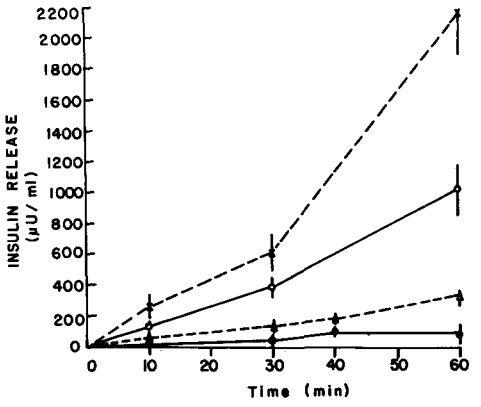

Fig. 1. Insulin release from pancreatic islets exposed to glucose and/or THC. Islets were incubated in KRB buffer containing 2.8 mM glucose (●), 8.5 mM glucose (○), 20 μM THC and 2.8 mM glucose (△), or 20 μM THC together with 8.5 mM glucose (×). Insulin release values are means ± S.E. for eight to fourteen different experimental determinations, and values are normalized per 20 islets. Two-way ANOVA indicated that THC-treated islet insulin release values were different from 2.8 mM glucose values ($P < 0.01$); 8.5 mM glucose values were different from 2.8 mM glucose values ($P < 0.01$; and 8.5 mM glucose values were different from 8.5 mM glucose and THC ($P < 0.05$).

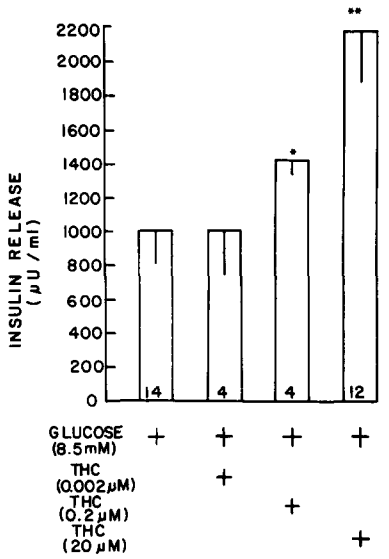

Fig. 2. Concentration-dependent THC potentiation of glucose-induced insulin release. Islets were incubated in KRB buffer containing glucose and THC, as indicated, for 60 min. Values are means ± S.E., and the number of different experiments is shown at the base of the bars. Glucose-stimulated values were compared to glucose- and THC-stimulated values, and probabilities were determined by two-way ANOVA: *$P < 0.05$ and **$P < 0.01$.

for different numbers (N) of independent experimental determinations.

RESULTS

Insulin secretion from intact isolated pancreatic islets increased in a time-dependent manner in response to a submaximal secretory concentration of glucose, or 20 μM THC (Fig. 1). Lower concentrations (0.002 to 2 μM) of THC did not alter basal insulin release. In addition, THC potentiated the insulin secretory response to glucose in a time- and concentration-dependent manner (Figs. 1 and 2).

It was demonstrated previously that glucose increases labeled AA incorporation into islet phospholipids [1]. In the present studies, islets were preincubated in the presence or absence of THC (20 μM) for 30 min, and then [^{14}C]AA and glucose (17 mM), or glucose (17 mM) and THC (20 μM) were added to the islets for an additional 30 min. The incorporation of [^{14}C]AA into phospholipids of islets pretreated with THC was reduced significantly by 48 ± 18% ($P < 0.01$) of glucose-stimulated values (328 ± 29 dpm/100 islets). However, even under basal conditions of 2.8 mM glucose, THC (20 μM) reduced the incorporation of [^{14}C]AA into phospholipids by 58 ± 17% ($P < 0.05$) of control values.

Table 1. Recovery of [^{14}C]AA in phospholipids of prelabeled islets

	Esterified [^{14}C]AA recovery		Insulin release	
Treatment	(dpm ± S.E.)	(N)	(μU/ml ± S.E.)	(N)
Basal	956 ± 336	8		
Glucose (17 mM)	586 ± 112*	5	268 ± 172	4
Glucose (17 mM) plus THC (20 μM)	291 ± 42† (56 ± 12%)	5	620 ± 96† (202 ± 35%)	4

Islets were prelabeled with [^{14}C]AA, and then they were incubated for 10 min with 2.8 mM glucose (basal), or 17 mM glucose in the presence or absence of THC. Phospholipids were extracted, isolated and quantitated. Values for radiolabel recovery in phospholipids and insulin release are normalized per 100 islets. N = number of independent determinations.

* Glucose-stimulated values are compared to basal values. Probability was determined by Student's *t*-test ($P < 0.01$; degrees of freedom 11).

† Glucose plus THC values compared to glucose-stimulated values. Probability was determined by Student's *t*-test (paired) ($P < 0.05$; degrees of freedom N-1).

In other experiments, the loss of esterified AA from phospholipids was determined in islets prelabeled with [^{14}C]AA. Glucose enhanced the hydrolysis of [^{14}C]AA from islet phospholipids, and the presence of THC further reduced recovery of esterified radiolabeled fatty acid in phospholipids (Table 1). In these experiments, THC significantly increased insulin release (Table 1).

Since the reduced recovery of esterified [^{14}C]AA in prelabeled THC-treated islet phospholipids may have been due to either reduced re-esterification of free fatty acid into lysophospholipids by the action of acyl-CoA acyltransferase, or to enhanced phospholipase A_2 activity and enhanced hydrolysis of fatty acids from membrane lipids, the effect of THC on these cellular processes was determined. Islet homogenates contain acyl-CoA acyltransferase activity which esterifies labeled AA to exogenous lysophosphatidylcholine [7]. When THC was added to islet homogenates containing exogenous lysophosphatidylcholine, acyl-CoA acyltransferase activity and [^{14}C]AA incorporation into phospholipid was inhibited in a concentration- and time-dependent manner (Fig. 3). On the other hand, when islet homogenate was incubated with [^{14}C]PC labeled in the C-2 position with AA as a substrate for phospholipase A_2, fatty acid hydrolysis in the presence of THC (20 μM) was increased by 184 ± 42% ($P < 0.05$) of control after 90 min.

Increased release of AA from islet phospholipids in the presence of THC suggested that increased levels of free fatty acid were available for metabolism through the cyclooxygenase or lipoxygenase pathways. To determine if AA metabolites were modulating the increased insulin secretion observed in the presence of THC, the effects of inhibitors of cyclooxygenase and lipoxygenase were investigated. Indomethacin, the cyclooxygenase inhibitor, did not change insulin release in response to glucose (8.5 mM) alone (data not shown), but insulin release in response to glucose and THC was increased by more than 50% (Fig. 4). In contrast, inhibition of islet lipoxygenase activity by BW755C inhibited insulin release in response to glucose and THC by more than 40% (Fig. 4). Another lipoxygenase inhibitor, nordihydroguaiaretic acid, also inhibited glucose and THC-stimulated insulin release by 40% (Fig. 4).

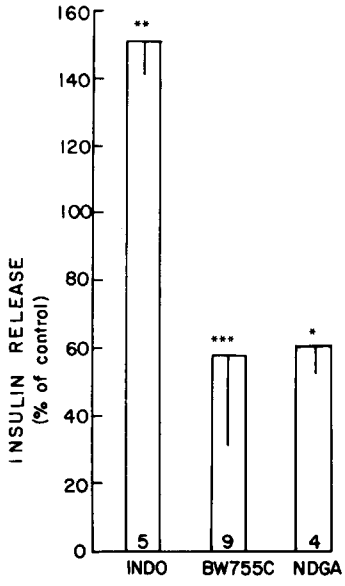

Fig. 4. Changes in insulin release in response to indomethacin (INDO), BW755C or nordihydroguaiaretic acid (NDGA). Islets were incubated in KRB buffer containing glucose (8.5 mM) and THC (20 μM) in the absence (control) or presence of INDO (20 μM), BW 755C (250 μM) or NDGA (20 μM) for 60 min. Values are means ± S.E. for the number of independent experimental determinations (N) shown at the base of each column. Probability was determined by Student's *t*-test (paired): *$P < 0.05$, **$P < 0.02$, ***$P < 0.01$; degrees of freedom N-1.

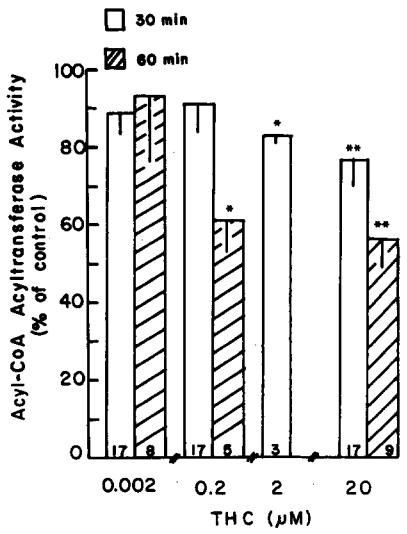

Fig. 3. Inhibition of islet acyl-CoA acyltransferase by THC. Islet homogenate was incubated in the absence (control) or presence of different concentrations of THC, lysophosphatidylcholine (1 μM), and [^{14}C]AA (0.05 μCi) in the acyltransferase assay buffer. Values are means ± S.E. for the number of independent experimental determinations (N) shown at the base of the columns. Probability was determined by Student's *t*-test (paired): *$P < 0.05$, **$P < 0.02$; degrees of freedom N-1.

To further determine if lipoxygenase products were mediating the effects of THC on insulin release, islets were prelabeled with [^{3}H]AA, stimulated, and the AA metabolites were analyzed by HPLC. 12-HETE has been reported to be the major islet lipoxygenase product formed in response to glucose stimulation [5, 6]. Prelabeled islets incubated with glucose plus THC produced 551% more [^{3}H]12-HETE than islets stimulated by glucose alone (Table 2). BW755C inhibited the synthesis of [^{3}H]12-HETE in islets challenged with glucose (8.5 mM) plus THC (20 μM) by 34 ± 10% ($P < 0.02$).

The effects of THC on islet prostaglandin synthesis were also determined. Islets prelabeled with [^{3}H]AA synthesized 78% more [^{3}H]PGE_2 in the presence of THC and glucose than in the presence of glucose alone (Table 2).

Table 2. Synthesis of [^{3}H]12-HETE and [^{3}H]PGE_2 by pancreatic islets

Treatment	[^{3}H]12-HETE (dpm/100 islets ± S.E.)	N	[^{3}H]PGE_2 (dpm/100 islets ± S.E.)	N
Glucose	230 ± 25	3	1176 ± 416	5
Glucose plus THC	1283 ± 237 (551 ± 58%) $P < 0.05$	3	1900 ± 589 (178 ± 26%) $P < 0.05$	5

Islets were preincubated for 60 min in KRB buffer containing 2.8 mM glucose and [^{3}H]AA (1 ±Cm/ml). The islets were then incubated in fresh buffer containing [^{3}H]AA and glucose (8.5 mM) in the presence or absence of THC (20 μM) for 90 min. [^{3}H]12-HETE and [^{3}H]PGE_2 were isolated by HPLC or TLC respectively. The number of different experimental determinations are designated (N). Probabilities were determined by Student's *t*-test (paired) with degrees of freedom N-1.

DISCUSSION

Earlier reports from this laboratory suggested that glucose-stimulated AA turnover in islet cell membrane phospholipids mediates insulin release [1]. Not only does glucose stimulate phospholipase A_2 and acyl-CoA acyltransferase, but the AA released through hydrolysis contributes to prostaglandin and leukotriene (12-HETE) biosynthesis [2, 3, 5, 6]. The stimulatory effects of THC on phospholipase A_2, together with the inhibitory effects of the cannabinoid on acyl-CoA acyltransferase activity [8–10], suggested that THC would be a useful tool to investigate the importance of AA turnover in membranes and AA metabolism in relationship to insulin secretion.

THC not only stimulates basal insulin release, but potentiates the secretory response to a submaximal concentration of glucose. THC stimulates insulin release in the concentration range of 0.2 to 20 μM, and higher concentrations of the drug were avoided due to reported inhibitory effects on enzymes, cell viability, and glucose metabolism [18–20]. THC at the effective secretory concentrations used in this study stimulates phospholipase A_2 and inhibits acyltransferase in islet homogenates. The effects of THC on labeled AA recovery in intact islet phospholipids probably reflect changes in the activity of both phospholipase A_2 and CoA acyltransferase, and a portion of the AA which is released and is not re-esterified into membrane phospholipids is metabolized to cyclooxygenase and lipoxygenase products. In other cell types, THC stimulates phospholipase A_2, cyclooxygenase, lipoxygenase, and adenylate cyclase [11, 14, 19, 20], whereas inhibition of acyltransferase has been reported [21].

The results of the present study suggest that the enhanced insulin release observed with THC in the presence or absence of a glucose stimulus may have been due to the formation of AA metabolites, since AA recovery was reduced under basal or glucose-stimulated conditions. Indeed, when inhibitors of lipoxygenase activity, BW755C and nordihydroguaiaretic acid, were added to islets challenged with glucose and THC, the insulin release was inhibited markedly, in agreement with the findings of other investigators [22–24]. It is probable that BW755C and nordihydroguaiaretic acid inhibit the metabolism of AA released from membranes by the combined actions of glucose and THC on phospholipase A_2. These results support the conclusions of previous studies that lipoxygenase-derived metabolites modulate insulin release in islets [5, 7, 22–25]. In addition, the results suggest that a fraction of the AA hydrolyzed from membrane phospholipids by phospholipase A_2 is reacylated back into phospholipids under ordinary conditions, perhaps to maintain adequate fatty acid levels in membranes or to decrease concentrations of lytic lysophospholipids.

THC also enhances prostaglandin formation in islets, although not to as high a percentage of stimulation as was found for 12-HETE. However, THC can directly inhibit prostaglandin synthetase [26], and this may retard the formation of PGE_2 by islets even in the presence of enhanced AA availability. The inhibition of cyclooxygenase by indomethacin augments insulin release in the presence of glucose and THC. Previous studies have shown that inhibition of islet prostaglandin synthesis enhances insulin release [3, 4, 27]. It is possible that the inhibition of cyclooxygenase caused a redistribution of AA for metabolism by lipoxygenase, and that the enhanced hydroxy- or hydroperoxy-eicosatetraenoic acid formation mediated the increase in insulin release. The present results support the hypothesis that some prostaglandins are inhibitory modulators of insulin release, whereas certain lipoxygenase products contribute to enhanced insulin release. Alternatively, the enhanced levels of free AA in cells stimulated by glucose and THC may be metabolized as a fuel source or affect other cellular events such as calcium mobilization which participate in the amplification of insulin release.

In conclusion, the stimulatory effects of THC on islet cell phospholipase A_2, lipoxygenase product formation and secretion suggest that these biochemical sequelae in cell activation are important modulators of insulin release. THC, by virtue of its ability to mobilize AA in cells, is a useful tool to determine the potential for AA metabolites to mediate various cellular processes.

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