

# Role of Arachidonate Lipoxygenase and Cyclooxygenase Products in Insulin and Glucagon Secretion From Rat Pancreatic Islets

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Rat pancreatic islets incubated in nutrient medium were used to study the role of endogenous arachidonic acid metabolism in pancreatic hormone secretion. Both glucose and fetal calf serum stimulated radioimmunoassayable PGE<sub>2</sub> production and insulin secretion from islets. These effects were abolished by the phospholipase inhibitor p-bromophenacyl bromide or by concurrent inhibition of cyclooxygenase and lipoxygenase by flurbiprofen plus nordihydroguaiaretic acid (NDGA), respectively. Bromophenacyl bromide also inhibited glucagon secretion. When used alone, flurbiprofen caused a significant enhancement of glucose-induced insulin secretion that was attributed to reactive stimulation of lipoxygenase-product formation rather than to selective cyclooxygenase inhibition. NDGA given alone in the presence of stimulatory concentrations of glucose suppressed the normal eight-fold rise in insulin secretion, but caused a marked enhancement in glucagon secretion that could be overcome by simultaneous inclusion of flurbiprofen. We concluded that: (1) Increased metabolism of arachidonic acid in pancreatic islets amplifies the secretion of insulin and glucagon. (2) The lipoxygenase as well as the cyclooxygenase pathways of arachidonate metabolism participate in the amplification of insulin secretion. (3) The observations made in this study are inconclusive with respect to the involvement of the lipoxygenase and cyclooxygenase pathways in glucagon secretion; an inhibitory role for lipoxygenase pathway products is suggested.

**G**LUCOSE, the paramount stimulator of insulin release, enhances the hydrolysis of phospholipids and the metabolism of arachidonic acid in pancreatic islets.<sup>2-4</sup> Phospholipase A<sub>2</sub>, the enzyme that liberates arachidonate from the phospholipid stores stimulates insulin release.<sup>5</sup> Arachidonate metabolism is stimulated also by phorbol esters,<sup>6</sup> furosemide,<sup>7</sup> methylxanthine,<sup>8</sup> and fetal calf serum,<sup>9</sup> which are agents with diverse biological actions. Also, insulin secretion is stimulated by phorbol esters,<sup>6</sup> furosemide,<sup>10</sup> methylxanthines,<sup>11</sup> and fetal calf serum.<sup>12</sup> Conversely, agents associated with inhibition of phospholipid and arachidonate metabolism, including glucocorticoids,<sup>13</sup> diphenylhydantoin,<sup>14</sup> and quinacrine,<sup>10</sup> inhibit insulin secretion. The implication is that changes in membrane phospholipids or increases in arachidonate metabolites may play a stimulatory role in insulin secretion.

Many *in vitro* studies support the view that prostaglandins (PGs) may serve as positive modulators in the secretion of insulin, glucagon, and somatostatin.<sup>15-21</sup> Nevertheless, the physiological significance and even the qualitative role of endogenous cyclooxygenase products as regulators of islet hormone secretion remain to be established.<sup>12,22</sup> At variance with the view of prostaglandins as agents which promote insulin

secretion are the findings that cyclooxygenase inhibitors often increase rather than decrease insulin secretion<sup>23,24</sup> and that elevated prostaglandin levels may sometimes be associated with suppression of insulin secretion.<sup>25,26</sup>

Recently, lipoxygenase enzymes that are involved in arachidonate metabolism have been shown to be glucose sensitive.<sup>27</sup> Lipoxygenase activity in pancreatic islets accounts for substantial production of nonprostanoid metabolites of arachidonic acid.<sup>3</sup> In view of the growing evidence for an important permissive or stimulatory role of lipoxygenase products in secretagogue action in other glands<sup>28</sup> we have undertaken a pharmacological evaluation of a regulatory role for endogenous eicosanoids in islet-hormone secretion.

## MATERIALS AND METHODS

Intact pancreatic islets were isolated from young adult, male, fed Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), by the method of Lacy and Kostianovsky.<sup>29</sup> A standard polystyrene tissue culture plate with 96 0.3-mL round-bottomed wells served as the incubation vessel. Each well contained 0.3 mL Waymouth MD-705/1 tissue culture medium (Gibco, special formulation) supplemented with 1 mg/mL bovine serum albumin. Control "blank" wells received no islets. Five islets were transferred into each of the other wells. Four wells were assigned to each treatment condition.

The experimental protocols were designed to induce various levels of activation of phospholipase A<sub>2</sub> and stimulation of insulin release by employing glucose at concentrations of 4, 6, 9, 13 and 20 mmol/L, and no glucose as a control. Fetal calf serum (FCS 10, 30 or 100  $\mu$ L/mL) was also employed to activate phospholipase A<sub>2</sub>.<sup>9</sup> Whenever FCS was employed, 100 kallikrein inhibitor units of aprotinin was added to the media in order to protect glucagon. In one experiment, exogenous arachidonic acid (Sigma) was added to the incubation media (10, 40 and 100  $\mu$ mol/L) in the presence of 1 mg/mL albumin to ascertain that hormone release occurred when this fatty acid was in abundance. The following drugs were used to inhibit maximally various enzymes involved in arachidonic acid metabolism: (1) 40  $\mu$ mol/L p-bromo-phenacyl bromide (BPB, Sigma) to inhibit phospholipase A<sub>2</sub>,<sup>30</sup> (2) 10  $\mu$ mol/L flurbiprofen (FLR, Upjohn) to inhibit

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cyclooxygenase,<sup>31,32</sup> and (3) 40  $\mu\text{mol/L}$  nordihydroguaiaretic acid (NDGA, Sigma) to inhibit lipoxygenases.<sup>33-36</sup>

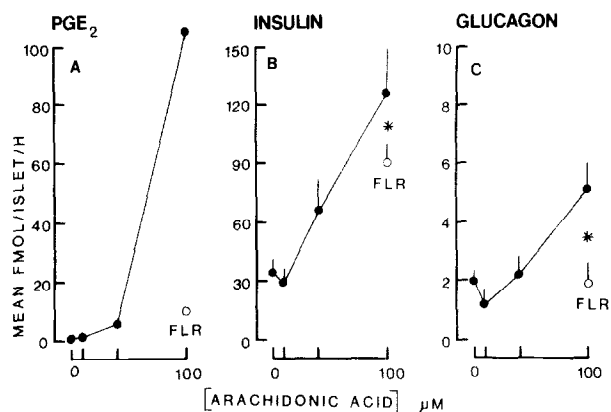
The plates were kept for 20 hours in an incubator at 37 °C and an atmosphere of 5% carbon dioxide and 95% air. The length of the incubation period was calibrated to allow sufficient prostaglandins to accumulate in the media so that their levels could be measured in radioimmunoassay (RIA). Upon completion of the incubations, 10- $\mu\text{L}$  aliquots of the media were submitted to insulin and glucagon RIAs.<sup>37</sup> For the RIA of PGE<sub>2</sub>,<sup>38</sup> 250- $\mu\text{L}$  aliquots from quadruplicate wells were pooled and extracted as follows. An equal volume of acetonitrile was added to each sample, in order to extract lipids and then to dehydrate proteins. The mixture was vortexed and centrifuged to precipitate the protein. The liquid phase was removed and acidified with formic acid (pH 3.5). To remove nonpolar, nonhydroxylated fats, 3 mL of petroleum ether was added; the tube was vortexed and centrifuged. The lower aqueous layer was removed and submitted to petroleum ether extraction a second time. The aqueous layer was mixed with 4 mL ethyl acetate. The mixture was centrifuged, the upper ethyl-acetate layer containing eicosanoids was removed and saved. The aqueous layer was treated a second time with ethyl acetate. The ethyl-acetate layers were combined and dried in air at room temperature. Recovery of prostanoids was estimated by the recovery of <sup>3</sup>H-TXB<sub>2</sub> which had been added to each sample prior to extraction; it was around 80%. When unlabeled PGE<sub>2</sub> was added to incubation media containing either albumin or FCS prior to the extraction process, 75% to 90% of it was recovered in the RIA. The limit of sensitivity of the RIA for PGE<sub>2</sub> was 5.7 femtomoles per tube (fmol/tube). When the media in the islet-free blank wells contained fetal calf serum, the PGE<sub>2</sub> immunoreactivity increased proportionate to the concentration of FCS. Based on these measurements, the concentration of PGE<sub>2</sub> in the FCS was 12 ± 8 nmol/L.

The numbers of observations refer to experiments conducted using separate batches of islets; each observation was made in quadruplicate. The samples were radioimmunoassayed in duplicate; the data represent values after the subtraction of concentrations detected in the islet-free incubation media from the corresponding experimental islet-containing media. They are given as the means ± standard errors of the mean (SEM), and expressed as fmol per islet per hour. This unit of expression is conventional and is intended to adjust for differences in experimental protocols with respect to islet density and duration of incubation; steady secretion rates are not implied. The significance of the differences were determined using analysis of variance among the replicates (four values per observation) and Duncan's multiple-range tests<sup>39</sup> for the hormone results, and Dunnett's *t* test<sup>40</sup> for the PGE<sub>2</sub> data derived from pooled samples (one value per observation representing four incubation wells). A probability of identity of less than 0.05 by two-tailed distribution was accepted as statistically significant.

## RESULTS

### Effect of Arachidonic Acid

To determine whether in this model of islet cells the abundance of arachidonate evokes increased synthesis of PGs and release of insulin and glucagon, the islets were incubated without and with 10, 40 or 100  $\mu\text{mol/L}$  exogenous arachidonic acid in the presence of 9 mmol/L glucose and 1 mg/mL albumin (Fig 1). As compared to incubate levels in the absence of arachidonate, PGE<sub>2</sub>, insulin, and glucagon release increased with 100  $\mu\text{mol/L}$  arachidonate. Only insulin release increased with 40  $\mu\text{mol/L}$  arachidonate; 10  $\mu\text{mol/L}$  of the fatty



**Fig 1.** Release of immunoreactive PGE<sub>2</sub> (A), insulin (B), and glucagon (C) from incubated rat islets in the presence of 9 mmol/L glucose, 1 mg/mL albumin and 0, 10, 40 or 100  $\mu\text{mol/L}$  arachidonic acid (closed circles) in one experiment. In a parallel experiment depicted by the open circles, the media also contained 1  $\mu\text{mol/L}$  flurbiprofen (FLR). Asterisks denote statistically significant differences between the quadruplicate values obtained with 100  $\mu\text{mol/L}$  arachidonate, in the presence or absence of FLR (statistical assessment of the PGE<sub>2</sub> data was not possible because the samples were pooled).

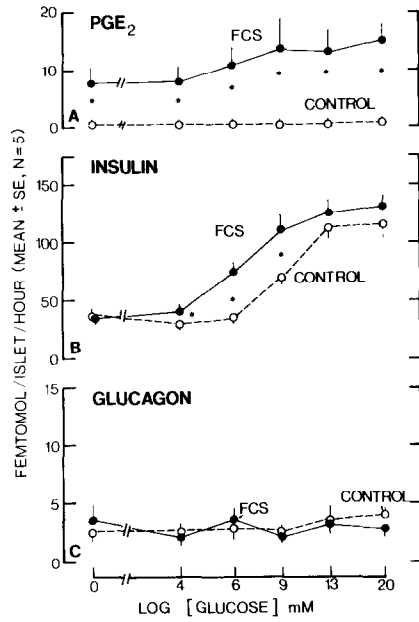
acid had no effect on any of the parameters. In a concurrently conducted experiment, the islets were incubated with 100  $\mu\text{mol/L}$  arachidonic acid plus 1  $\mu\text{mol/L}$  flurbiprofen. With flurbiprofen, the release of PGE<sub>2</sub>, insulin, and glucagon decreased toward or to levels observed in the absence of arachidonate.

### Effect of Fetal Calf Serum

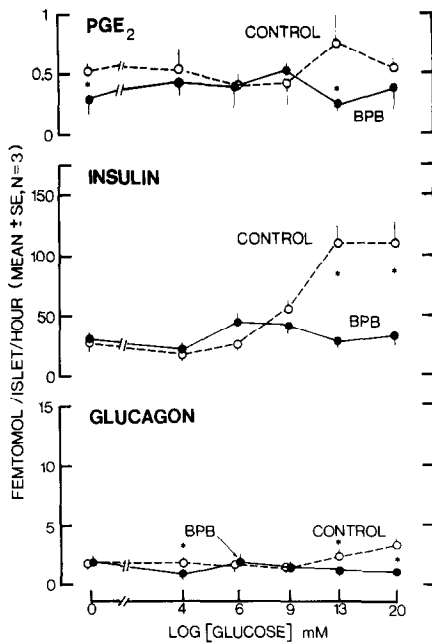
FCS at the concentration of 100  $\mu\text{L/mL}$  was used as a probe to stimulate phospholipase A<sub>2</sub> and thus to induce an abundance of endogenous arachidonic acid (Fig 2).<sup>9</sup> In addition, presumably FCS itself contained some arachidonate that further enriched the arachidonate pool. Albumin (1 mg/mL) rather than serum was the additive in the control wells. With FCS, the release of PGE<sub>2</sub> from islets increased 15- to 17-fold at 0 and 4 mmol/L glucose, and 24- to 34-fold in the presence of 6 to 20 mmol/L glucose. The lowest concentration of glucose that evoked significant increases in insulin release was 9 mmol/L without and 6 mmol/L with FCS. FCS augmented insulin release to 130%, 216% and 161% of controls at 4, 6, and 9 mmol/L glucose, respectively. The release of glucagon did not appear to be influenced by either the prevailing concentration of glucose or by the presence or absence of FCS.

### Effect of p-Bromophenacyl Bromide

In order to elucidate further the role of phospholipase A<sub>2</sub> in islet hormone secretion, BPB was utilized at the concentration of 40  $\mu\text{mol/L}$ , considered to be sufficient to inhibit the enzyme maximally (Fig 3).<sup>30</sup>



**Fig 2.** Release of immunoreactive PGE<sub>2</sub> (A), insulin (B), and glucagon (C) from incubated rat islets at six concentrations of glucose in the presence of 1 mg/mL bovine serum albumin (Control) or 100  $\mu$ L/mL fetal calf serum (FCS). Asterisks denote statistically significant differences between corresponding experimental and control data points. In control experiments, PGE<sub>2</sub> release with 13 and 20 mmol/L glucose was significantly greater than that without glucose.



**Fig 3.** Release of PGE<sub>2</sub>, insulin, and glucagon from incubated islets at six concentrations of glucose without (Control) or with 40  $\mu$ mol/L p-bromo-phenacyl bromide (BPB). Media contained 1 mg/mL albumin. Asterisks denote significant differences between corresponding experimental and control data points. In control experiments, PGE<sub>2</sub> release with 13 mmol/L glucose was significantly greater than that without glucose.

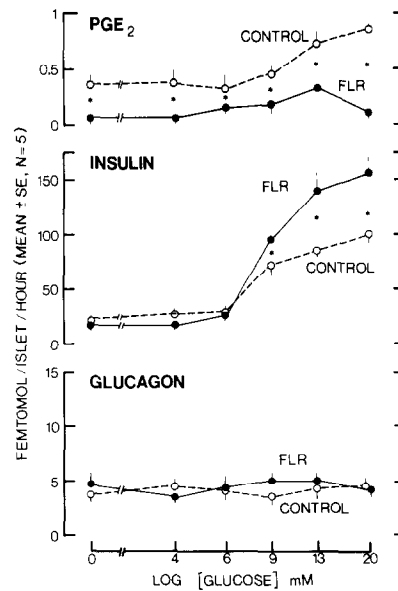
The incubation media contained 1 mg/mL albumin but no FCS. BPB inhibited PGE<sub>2</sub> release in the absence of glucose (56% of control) and with 13 mmol/L glucose (33% of control). Insulin release was inhibited by BPB at the two highest concentrations of glucose (at 13 and 20 mmol/L, 25% and 29% of control, respectively). Glucagon release was also inhibited by BPB: 45%, 49%, and 36% of control, respectively, at 4, 13 and 20 mmol/L glucose.

**Effect of Flurbiprofen**

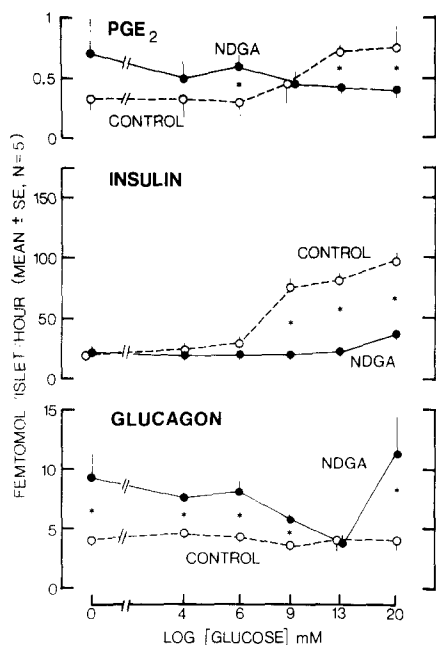
A potent and selective inhibitor of cyclooxygenase, FLR was used at the concentration of 10  $\mu$ mol/L (Fig 4). At all concentrations of glucose, FLR inhibited PGE<sub>2</sub> release in the range of 47% to 13% of control. FLR promoted insulin release when glucose concentration was high (with 9, 13 and 20 mmol/L glucose, 130%, 163%, and 152% of control, respectively). Glucagon release was not significantly affected by FLR.

**Effect of Nordihydroguaiaretic Acid**

NDGA at the concentration of 40  $\mu$ mol/L was employed as a probe to inhibit the lipoxygenases (Fig 5). In the glucose concentration range of 0 to 9 mmol/L, PGE<sub>2</sub> release with NDGA was similar to or moderately greater than that observed during control experiments (104% to 213% of control). At 13 and 20 mmol/L glucose, NDGA inhibited PGE<sub>2</sub> release to 60% and 56% of control, respectively. Insulin release



**Fig 4.** Release of PGE<sub>2</sub>, insulin, and glucagon from incubated islets at six concentrations of glucose without (Control) and with 10  $\mu$ mol/L flurbiprofen (FLR). Media contained 1 mg/mL albumin. Asterisks denote significant differences between corresponding experimental and control data points. In control experiments, PGE<sub>2</sub> release with 13 and 20 mmol/L glucose was significantly greater than that without glucose.



**Fig 5.** Release of PGE<sub>2</sub>, insulin, and glucagon from incubated islets at six concentrations of glucose without (Control) and with 40 μmol/L nordihydroguaiaretic acid (NDGA). Media contained 1 mg/mL albumin. Asterisks denote significant differences between corresponding experimental and control data points. In control experiments, PGE<sub>2</sub> release with 13 and 20 mmol/L glucose was significantly greater than that without glucose.

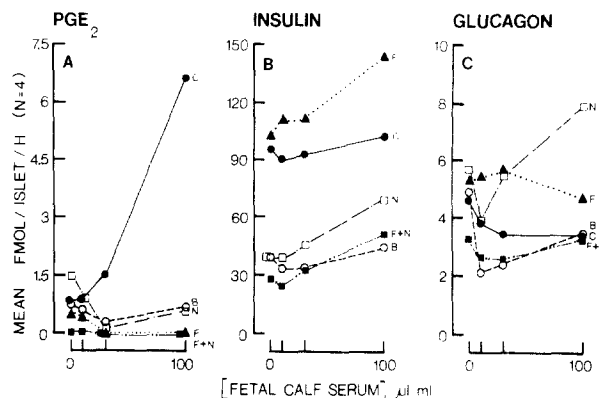
was inhibited by NDGA at high concentrations of glucose (at 9 to 20 mmol/L, 39% to 27% of control). With the exception of 13 mmol/L glucose, glucagon release was greater with NDGA than without it, in the range of 166% to 278% of control.

**Effect of Glucose Alone on PGE<sub>2</sub> Release**

In order to quantify with precision the promotion of PGE<sub>2</sub> release by glucose, the data from the control experiments depicted in Figs 2, 3, 4, and 5 were pooled. In the presence of 1 mg/mL albumin, with 0, 4, 6, 9, 13 and 20 mmol/L glucose, PGE<sub>2</sub> release was 0.33 ± 0.01, 0.43 ± 0.07, 0.44 ± 0.02, 0.46 ± 0.01, 0.68 ± 0.04, and 0.68 ± 0.07 fmol/islet/h (N = 18), respectively. The analysis of variance revealed a significance level of *P* < 0.001. In paired comparisons, the values observed with 6, 9, 13 and 20 mmol/L glucose were different from the value observed in the absence of glucose (*P* < 0.05 to < 0.001).

**Interactions of Fetal Calf Serum and Enzyme Inhibitors**

In another series of experiments, the concentration of glucose was kept constant at the highest level employed in the preceding experiments (20 mmol/L) (Fig 6). The concentration of fetal calf serum was



**Fig 6.** Release of PGE<sub>2</sub> (A), insulin (B), and glucagon (C) from incubated islets in the presence of 20 mmol/L glucose and 0, 10, 30 or 100 μL/mL fetal calf serum. C = control experiments without drug additives. In the other experiments, the following drugs were present in the incubation media: B = p-bromophenacyl bromide 40 μmol/L; F = flurbiprofen 10 μmol/L; N = nordihydroguaiaretic acid 40 μmol/L; and F + N = flurbiprofen 10 μmol/L plus nordihydroguaiaretic acid 40 μmol/L. Standard error bars have been omitted to avoid clutter: statistical information is given in the text.

varied from 0, 10, 30 to 100 μL/mL, in order to induce progressively increasing abundance of arachidonic acid. BPB, FLR, and NDGA were employed individually as in the preceding experiments. In addition, FLR and NDGA were added to the media as a mixture, in order to inhibit respectively the cyclooxygenase as well as the lipoxygenases.

In control experiments, PGE<sub>2</sub> release increased progressively as the concentration of FCS increased (*P* < 0.001 at 30 or 100 μL/mL FCS v no FCS). This increase was inhibited completely by either FLR or FLR plus NDGA (*P* < 0.001). NDGA alone inhibited PGE<sub>2</sub> release at 30 and 100 μL/mL FCS to about 10% of control (*P* < 0.05). Also BPB inhibited PGE<sub>2</sub> release to 70% to 10% of control (*P* < 0.05 to 0.01).

The increases in insulin release associated with the increases in FCS levels were much less than the increases in PGE<sub>2</sub> release; at 100 μL/mL FCS, insulin release was 118% (NS) and PGE<sub>2</sub> release 801% (*P* < 0.001) of corresponding values without FCS. BPB inhibited insulin release to about 40% of controls (*P* < 0.01) at all concentrations of FCS. FLR did not modify insulin release in the absence of FCS. In the presence of FCS, FLR augmented insulin release to 122% to 127% of control, which was statistically significant (*P* < 0.05) only at 100 μL/mL FCS. NDGA inhibited insulin release at all levels of FCS (*P* < 0.01). Also FLR plus NDGA inhibited the release significantly under all conditions. The range of values were 46% to 29% of the corresponding controls without any drugs (*P* < 0.05 to 0.01), and 73% to 63% of the corresponding values with NDGA alone (*P* < 0.05).

Glucagon release was not affected significantly by the changes in the levels of FCS. In the absence of FCS, only FLR plus NDGA inhibited glucagon secretion ( $P < 0.05$ ). In the presence of various concentrations of FCS, the effects of the drugs on glucagon secretion were quite variable and did not display any definite patterns; the only value which was significantly different from the corresponding control was that observed in the presence of NDGA and 100  $\mu\text{L}/\text{mL}$  FCS.

#### DISCUSSION

The results of our studies indicate that arachidonic acid metabolism in pancreatic islets participates in the regulation of secretion of islet hormones, and that perturbations in this metabolism cause complex changes in hormone release.

We monitored the levels of  $\text{PGE}_2$  as an index of biosynthetic activity in the cyclooxygenase pathway of arachidonate metabolism. We regard the information on  $\text{PGE}_2$  levels also as a validation of the occurrence of the anticipated effects of the inhibitor drugs. In order to monitor the levels of immunoreactive  $\text{PGE}_2$  in the media, the incubation period had to be extended considerably beyond the durations necessary to monitor hormone secretion. Thus, the levels of  $\text{PGE}_2$ , insulin, and glucagon may underestimate the secreted amounts as a result of possible degradation. We attempted to minimize the degradation of glucagon by enzymes present in serum, using aprotinin. Based on information provided by Metz et al in a similar incubation system for neonatal rat pancreatic cells,<sup>41</sup> we assume that  $\text{PGE}_2$  was reasonably stable also in our model.

The increases in  $\text{PGE}_2$  release that occurred with exogenous arachidonate indicate that the islet cells can take up and metabolize this fatty acid to PGs. The abolition of the arachidonate-induced increases in  $\text{PGE}_2$  release by flurbiprofen, a potent cyclooxygenase inhibitor, adds credence to this interpretation. The dose-related increases in  $\text{PGE}_2$  production that occurred with glucose or fetal calf serum and particularly with the combination of these agents are consistent with existing evidence that both glucose and FCS activate phospholipase  $\text{A}_2$ .<sup>4,9</sup> The marked reduction of the effects of glucose plus FCS by BPB supports the interpretation that the increased level of activity of phospholipase  $\text{A}_2$  and resultant increase in cytosolic arachidonate accompany the administration of glucose and FCS.

The stimulation of insulin and glucagon release with exogenous arachidonate and the inhibition of this effect with flurbiprofen suggest an amplifying role for the metabolites of arachidonate generated in the

cyclooxygenase pathway. The stimulatory effect of arachidonate on islet-hormone secretion seen in our experiment corroborate the observations of Metz *et al*<sup>42</sup> on insulin secretion from cultured neonatal rat pancreatic cells and our previous demonstration of the stimulation by arachidonate of insulin and glucagon secretion from the isolated perfused rat pancreas.<sup>43</sup> The ability of BPB to inhibit insulin and glucagon release under conditions that promote phospholipase  $\text{A}_2$  activity indicates that the consequent increases in arachidonate levels in the cytosol and ensuing increases in the metabolism of arachidonate play an amplifying role in the secretion of both hormones. The effect of BPB on glucagon release must be interpreted with caution, because glucagon release did not increase in the presence of FCS, a promoter of phospholipase activity. In the FCS experiments, the degrading action of serum on glucagon may have masked any increases, despite the presence of aprotinin in the incubation media. Alternatively, the suppressive action of glucose may have prevented the anticipated increase in glucagon secretion from occurring.

The focus of attention in this study was on the involvement of the cyclooxygenase-activated and lipoxigenase-activated products of arachidonate metabolism in the amplification of islet hormone secretion that occurs when arachidonate is in abundance. As pharmacological probes, FLR was used to inhibit the cyclooxygenase and NDGA the lipoxigenases, so that clues may be gained by the process of subtraction.

The fact that FLR inhibited the cyclooxygenase under all of our experimental conditions is evidenced by the decreases in the biosynthesis of  $\text{PGE}_2$ , the major cyclooxygenase product in islets.<sup>22</sup> At the moment we are not equipped to assess directly whether with NDGA the anticipated inhibition of islet lipoxigenases<sup>3</sup> was achieved. In other systems, at the concentration employed in this study, NDGA effectively inhibited lipoxigenases.<sup>34,35</sup> We have no reason to suspect that the same did not occur in our experiments. An issue of concern is the selectivity of the action of NDGA on islet lipoxigenases, because  $\text{PGE}_2$  release was inhibited by NDGA whenever glucose or fetal calf serum was in abundance. One interpretation of this observation is that NDGA inhibited not only lipoxigenases, but also the cyclooxygenase. Alternatively, the synthesis of prostaglandins decreased secondary to the decreases in lipoxigenase products: Feuerstein et al<sup>44</sup> have shown that leukotriene C, a lipoxigenase product, stimulates prostaglandin release from macrophages. Whatever the mechanisms may have been, in evaluating our findings with NDGA, the decreases in cyclooxygenase activity that sometimes occurred should be kept in mind. Similar considerations are

necessary in evaluating the observed effects of FLR. FLR is one of the most potent and selective inhibitors of cyclooxygenase.<sup>31</sup> Thus, the issue of concern is the probable consequences of FLR-induced inhibition of cyclooxygenase on the activity in the lipoxygenase pathway, rather than a direct effect of FLR on the lipoxygenases. There is evidence that the inhibition of cyclooxygenase promotes the activity in the lipoxygenase pathways by two mechanisms:<sup>36,45</sup> (1) Prostaglandins inhibit lipoxygenase activity, so that decreased synthesis in prostaglandins would allow the activity in that pathway to increase. (2) The inhibition of the cyclooxygenase leads to increased metabolism of arachidonate in the lipoxygenase pathways. Thus, also in interpreting the effects of FLR on islet-hormone release, potential changes in both pathways of arachidonate metabolism must be considered. We believe that by comparing the results with FLR or NDGA alone to those obtained by using the two drugs in combination, the involvement of the alternate metabolic pathway in the observed effects can be deduced.

Ten micromolar FLR enhanced glucose-induced insulin release. This observation is consistent with the findings of several other investigators<sup>46</sup> that cyclooxygenase inhibitors promote glucose-induced insulin release. On the other hand, our present results are in conflict with our own observations in the present experiment with exogenous arachidonate and in other studies that  $1\mu\text{mol/L}$  FLR inhibited insulin release.<sup>22,47,48</sup> Inhibition of insulin release by cyclooxygenase inhibitors was reported also by others.<sup>46</sup> Scrutiny of our data reveals that FLR promoted insulin release when cyclooxygenase was inhibited profoundly and only in the presence of high concentrations of glucose, particularly when fetal calf serum had been added. Availability of glucose or serum are conditions leading to activation of phospholipase  $A_2$  and ensuing increase in available arachidonate. Thus, with strong but not with mild inhibition of cyclooxygenase the circumstances may have been suitable for the shunting of arachidonate into the lipoxygenase pathways. We have shown that administered leukotrienes, which are products of 5-lipoxygenase pathway, stimulate insulin release.<sup>49</sup> Metz et al<sup>42</sup> observed that 12-hydroperoxyicosatetraenoic acid, a 12-lipoxygenase product, evoked insulin secretion. The inhibition of insulin release with NDGA that we documented in this study is consonant with our findings on arginine-induced and glucose-induced insulin release from the perfused rat pancreas.<sup>48,50</sup> Yamamoto et al<sup>5,6</sup> and Metz et al<sup>42,51</sup> made similar observations to support that lipoxygenase pathway products promote insulin secretion. If, in our experiments with  $10\mu\text{mol/L}$  FLR, these compounds and other yet-to-be-identified insulin secretagogues

were produced in abundance, they could have been responsible for the enhancement of insulin release. This paradigm demands that the increases in insulin release that occur upon the inhibition of cyclooxygenase when there is abundant arachidonate be abolished by the inhibition of lipoxygenases. Our results with the administration of FLR and NDGA in combination demonstrate this predicted response. We have shown previously that  $\text{PGE}_2$  as well as other prostaglandins are secretagogues of insulin.<sup>16-18</sup> The fact that the degree of inhibition of insulin release with FLR plus NDGA was greater than that with NDGA alone is an indication that the attenuating effect of decreased prostaglandin synthesis on insulin release was being masked by increased production of insulin secretagogues in the lipoxygenase pathways. Also, BPB inhibited insulin release at a magnitude greater than that induced by NDGA alone and similar to that induced by FLR plus NDGA. The inhibition of prostaglandin synthesis that we documented by the decreases in  $\text{PGE}_2$  levels, as well as the presumed inhibition of synthesis of lipoxygenase products must have contributed to BPB-induced attenuation of insulin release. A similar reasoning is applicable to the interpretation of the inhibitory effects of NDGA on insulin release. In the present set of experiments  $\text{PGE}_2$  levels decreased with NDGA, and the addition of FLR intensified the inhibition of insulin secretion that occurred with NDGA. Thus, if the inhibition of lipoxygenases is the primary mechanism by which insulin secretion was inhibited, the inhibition of cyclooxygenase appears to have assisted in this action. Nevertheless, in other experiments<sup>48</sup> we documented the ability of NDGA to suppress insulin release in the absence of any decreases in prostaglandin biosynthesis, so that the ability of NDGA to inhibit insulin release by mechanisms other than the inhibition of cyclooxygenase cannot be disputed.

We and others have shown previously that a variety of prostaglandins, including  $\text{PGE}_2$ , stimulate the secretion of glucagon.<sup>16-19,52</sup> Furthermore, we and others have observed that cyclooxygenase inhibitors inhibit glucagon release.<sup>19,22,48,53</sup> The reason for failure of FLR to inhibit glucagon release in many of our present experiments, at a time it inhibited  $\text{PGE}_2$  release is not obvious. Analogous to the interpretation of the effects of FLR on insulin release, one may postulate that lipoxygenase pathway products also promote glucagon secretion, and increased production of arachidonate metabolites as a result of the putative substrate shunting could prevent the inhibition of glucagon secretion. This postulate is not tenable, because with NDGA glucagon secretion was increased, and because in another study we did not

observe a clear stimulation of glucagon release by leukotrienes which are secretagogues of insulin.<sup>49</sup> On the other hand, the products of a pathway of arachidonate metabolism other than lipoxygenase may have been involved: Falck et al<sup>54</sup> reported recently that certain epoxyeicosatrienoic acids, which are products of the epoxygenase pathway of arachidonate metabolism, stimulate glucagon release. At the moment no information is available on the activity of this metabolic pathway in the pancreas or pancreatic islets, so that its involvement in the action of FLR we have observed in this study is highly speculative.

NDGA stimulated glucagon secretion and FLR abolished this effect. The information on the involvement of the lipoxygenase pathway products in the regulation of glucagon secretion is very limited and has originated exclusively from our laboratory.<sup>48,49</sup> Based on other observations of the effects of NDGA and exogenous leukotrienes, we concluded that the lipoxygenase pathways play a minor or insignificant role in regulation of secretion of glucagon. Our earlier data do not support or disrepute an interpretation of NDGA effects in the present study that endogenous lipoxygenase products exert an inhibitory influence on glucagon

secretion. The observed abolition of the effect of NDGA by FLR could be an independent action and hence cannot be used as evidence against the putative role of endogenous lipoxygenase products as inhibitors of glucagon secretion. The gaps in knowledge in this area are too large to reach definitive conclusions at this time.

We conclude that increased metabolism of arachidonic acid in pancreatic islets amplifies the secretion of insulin and glucagon. The lipoxygenase as well as the cyclooxygenase pathways of arachidonate metabolism participate in the amplification of insulin secretion. The observations made in this study are inconclusive with respect to the involvement of the two metabolic pathways in glucagon secretion; an inhibitory role for lipoxygenase pathway products is suggested.

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