DISSOCIATION OF VASCULAR RESISTANCE WITH ENDOCRINE PANCREAS SECRETION: THE EFFECTS OF EPOXYMETHANO ANALOGS OF PGH₂.

J.O. Akpan, M.C. Hurley, S. Pek, W.E.M. Lands. Department of Internal Medicine (Division of Endocrinology and Metabolism and The Metabolism Research Unit) and Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48109 USA (Reprint requests to JOA, University of Ilorin, Ilorin, Nigeria).

ABSTRACT

The epoxymethano analogs of PGH₂ caused rapid and persistent increase in perfusion pressure in isolated rat pancreata without significant effect on glucagon and insulin secretory responses to PGH₂ and PGE₂. The changes in perfusion pressure are interpreted as alterations in vascular resistance since the flow rate was kept constant at 2.5 mL per min. PGH₂ alone caused significant elevation in pressure. However, PGH₂ administration superimposed upon an infused epoxymethano analog of PGH₂, decreased perfusion pressure significantly, whereas PGH₂ induced hormone release was not decreased. The analogs neither stimulated nor inhibited the endocrine pancreas secretion. These studies provide evidence for complete dissociation of vascular constriction from pancreatic hormone release and further suggest that the effects of PGH₂ on islet hormone secretion may result from the conversion of PGH₂ to other prostanoids.

INTRODUCTION

Previous reports (1,2,3,4) demonstrate that the synthetic analogs of endoperoxide, 9,11- and 11,9-(epoxymethano)-azoprostacyclen-5,13-dienoic acid are stable analogs of prostaglandin H₂ (15-hydroxy-9,11-peroxidoprostacyclen-5,13-dienoic acid). The epoxymethano analogs of PGH₂ have been shown to inhibit the endoperoxide synthase and thromboxane synthase activity (3,4). The endoperoxide analogs are receptor level antagonists of both platelet aggregation and cyclic AMP lowering actions of PGH₂ (4,5).

Injection of 9-epoxy-11-methano into canine superior mesenteric artery caused dose dependent vasoconstriction and decrease in
blood flow. However, PGH₂ injected similarly, resulted in vasodilation and increase in blood flow (6). Many prostaglandins exert potent pressor effects on the endocrine pancreas (7,8). The mechanism by which some prostaglandins inhibit the endocrine pancreas secretion has been attributed in part to local vasoconstriction (7,8). In these studies, we have observed that 11-epoxy-9-methano analog which exerts potent vasoconstriction on pancreatic vasculature, neither stimulates nor inhibits secretion nor antagonizes insulin and glucagon secretion in response to PGH₂ and PGE₂.

MATERIALS AND METHODS

Pancreata were obtained from young adult male albino rats, weighing from 250-300 g, that were fasted for 18-24 h. The animals were anesthetized with sodium pentobarbital (50 mg/kg) injected intraperitoneally and the pancreas with its intact vasculature was removed and transferred to an extracorporeal perfusion apparatus, as described previously (9) and perfused with Krebs-Ringer-bicarbonate buffer containing 30 uM albumin, 56 uM dextran and 5.6 mM D-glucose, with or without epoxymethano analogs of PGH₂.

PGH₂ was generated enzymatically from arachidonate as described previously (10) and was stored frozen. 11-epoxy-9-methano and 9-epoxy-11-methano analogs of endoperoxide PGH₂ and PGE₂ were donated by Dr. John Pike of the Upjohn Company, Kalamazoo, MI. On the day of use, the epoxymethano analogs were dissolved in 95% ethanol and then diluted with perfusion buffer to the desired concentration. PGH₂ and PGE₂ were dissolved in 95% ethanol and then diluted with perfusion buffer five seconds before use. The final concentration of ethanol in the perfusate was less than 0.95%. We have shown previously that at this concentration ethanol does not affect secretion of glucagon or insulin (9,10,11).

The perfusion flow rate was maintained constant at 2.5 mL per min. Perfusion pressure was monitored continuously using a pressure transducer, an amplifier and a strip-chart recorder set either at 0.2 in (or at 2 in per min during injection of PGH₂). PGH₂ and PGE₂ were administered as a 250 uL bolus over 5-8 seconds through a diaphragm in the influent line without interrupting the flow of the basic perfusion solution. PGH₂ injections were made before, during and after perfusing the pancreata with buffer solution containing 1 uM epoxymethano analogs. The pancreatic portal venous effluent was collected in one-minute fractions; the samples were chilled immediately and aliquoted for radioimmunoassays. Radioimmunoassays for glucagon and insulin were performed using a "double-antibody" method described previously (12). Statistical significance of differences between observed values was determined by two-tailed student t-test. Values are mean ± SEM.
RESULTS

The pancreas was perfused firstly, for 20 minutes with basic buffer solution containing 5.6 mM glucose (described in MATERIALS AND METHODS) without the endoperoxide analogs. The influent perfusate was then switched to a solution containing 1 uM 11-epoxy-9-methano analog of PGH₂ and perfusion was continued further for 60 minutes. The influent perfusate was lastly switched again to the basic buffer solution. PGH₂ or PGE₂ was injected as a 250 uL bolus into the pancreas before, during and after perfusing the pancreata with solution containing the endoperoxide analogs.

![Chart of PGH₂ and endoperoxide analog induced changes in perfusion pressure.](image)

**Fig. 1.** PGH₂ and endoperoxide analog induced changes in perfusion pressure. Flow rate was maintained constant at 2.5 mL/min. Recording speed was set at 0.2 in/min (or increased to 2 in/min during injection of PGH₂ only). PGH₂ was injected at various intervals before, during and after perfusion with the analog. Pressure values are in millimeter of mercury.

In Figure 1 is shown qualitative perfusion pressure recordings obtained during one of the experiments. 11-epoxy-9-methano alone or 9-epoxy-11-methano (data not shown) caused rapid and persistent increase in the perfusion pressure. PGH₂ (10 uM) alone injected before the endoperoxide analog, also exerted significant, although lesser increase in pressure (Figure 1, left panel). PGH₂ (1 uM or 10 uM) injections superimposed upon influent endoperoxide analog, decreased significantly the high perfusion pressure caused by the analog (Figure 1, middle panel). When the influent perfusate was switched off the analog and the pancreas was perfused with basic buffer solution, perfusion pressure gradually returned almost to baseline in five minutes. PGH₂ again
increased the perfusion pressure (Figure 1, right panel). The changes in pressure observed in these studies are interpreted as alterations in vascular resistance since the flow rate was maintained constant at 2.5 mL per minute.

Figure 2 illustrates levels of glucagon and insulin secretion corresponding to PGH$_2$ injections described above and illustrated in Figure 1. The levels of glucagon (solid circle) and insulin (open circle) secretion induced by PGH$_2$ (1 or 10 uM) before, during and after perfusing the pancreata with endoperoxide analog were not significantly different (P<0.10). The release of glucagon occurred more rapidly than that of insulin but the magnitude of the responses in both hormones were similar. Endoperoxide analog alone did not induce glucagon or insulin secretion (Figure 1 and Table 1). Arginine (10 mM) administered to the pancreas at the end of the perfusion caused a significant increase in the levels of both hormones. This indicates that the isolated perfused pancreas was still capable of normal secretory response to a common secretagogue.

In Table 1 are shown values of glucagon and insulin secreted in response to either PGH$_2$, the endoperoxide analog or PGE$_2$; and the corresponding quantitative changes in perfusion pressure. 11-epoxy-9-methano analog of PGH$_2$ (1 uM) alone caused significant
Table 1

Glucagon and insulin secretion during changes in perfusion pressure.

<table>
<thead>
<tr>
<th>CONDITIONS</th>
<th>GLUCAGON (fmol/5min)</th>
<th>INSULIN (fmol/5min)</th>
<th>% mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL&lt;sub&gt;(n)&lt;/sub&gt;</td>
<td>TEST&lt;sub&gt;(n)&lt;/sub&gt;</td>
<td>CONTROL&lt;sub&gt;(n)&lt;/sub&gt;</td>
</tr>
<tr>
<td>PGH&lt;sub&gt;2&lt;/sub&gt; ANALOG ALONE (1uM)</td>
<td>170±30 (14)</td>
<td>163±17 (14)</td>
<td>140±40 (12)</td>
</tr>
<tr>
<td>BEFORE PGH&lt;sub&gt;2&lt;/sub&gt; ANALOG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGH&lt;sub&gt;2&lt;/sub&gt; (1uM)</td>
<td>213±40 (9)</td>
<td>630±70 (9)</td>
<td>140±45 (8)</td>
</tr>
<tr>
<td>PGH&lt;sub&gt;2&lt;/sub&gt; (10uM)</td>
<td>135±20 (9)</td>
<td>1430±200 (9)</td>
<td>190±40 (7)</td>
</tr>
<tr>
<td>PGE&lt;sub&gt;2&lt;/sub&gt; (10uM)</td>
<td>180±50 (4)</td>
<td>1315±300 (4)</td>
<td>175±10 (4)</td>
</tr>
<tr>
<td>DURING PGH&lt;sub&gt;2&lt;/sub&gt; ANALOG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGH&lt;sub&gt;2&lt;/sub&gt; (1uM)</td>
<td>170±40 (13)</td>
<td>580±75 (13)</td>
<td>120±40 (10)</td>
</tr>
<tr>
<td>PGH&lt;sub&gt;2&lt;/sub&gt; (10uM)</td>
<td>140±10 (17)</td>
<td>1500±260 (13)</td>
<td>146±35 (10)</td>
</tr>
<tr>
<td>PGE&lt;sub&gt;2&lt;/sub&gt; (10uM)</td>
<td>220±80 (4)</td>
<td>1430±380 (4)</td>
<td>170±50 (4)</td>
</tr>
<tr>
<td>AFTER PGH&lt;sub&gt;2&lt;/sub&gt; ANALOG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGH&lt;sub&gt;2&lt;/sub&gt; (10uM)</td>
<td>180±30 (9)</td>
<td>1220±190 (13)</td>
<td>170±70 (10)</td>
</tr>
<tr>
<td>PGE&lt;sub&gt;2&lt;/sub&gt; (10uM)</td>
<td>125±60 (4)</td>
<td>1290±400 (4)</td>
<td>150±40 (4)</td>
</tr>
</tbody>
</table>

Hormone values (mean ± SEM) represent five minute-cumulative portal effluent secretion in response to the agents indicated. Pressure values (mean ± SEM) represent quantitative percent change in perfusion pressure above and below (*) reference value.
and persistent elevation in pressure (90% increase above baseline, last column) without significant effect on hormone release (170±30 vs 163±17, glucagon and 140±40 vs 180±50, insulin). Increases in glucagon and insulin levels occurred consistently in response to PGH2 and PGE2 (10 uM) before, during and after perfusing the pancreata with the analog. Although hormone secretion was not significantly altered by the analog during concurrent perfusion of the analog with two concentrations of PGH2 (1 and 10 uM), the perfusion pressure decreased significantly 20±2% and 30±3% respectively below the observed pressure increase caused by the analog alone. Changes in pressure due to PGE2 (10 uM) were inconsistent and variable. The levels of hormone release and changes in pressure (PGH2 only) induced by either PGH2 or PGE2 after 60 minutes exposure of pancreas to the analog, were comparable to those obtained prior to perfusion with the analog (Table 1).

DISCUSSION

This investigation was carried out to determine the relationship between prostaglandin-induced changes in vascular resistance and endocrine pancreas secretion in the perfused pancreas. The results demonstrate that there is no direct association between perfusion pressure in the pancreas and portal venous glucagon and insulin secretion (Figures 1 and 2). Although PGH2 decreased high perfusion pressure caused by 11-epoxy-9-methano analog of PGH2, hormone secretion induced by PGH2 was not significantly affected (Figures 1 and 2). Since the perfusion flow rate was maintained constant at 2.5 mL per minute, the observed changes in perfusion pressure shown in Figure 1 and in Table 1 are interpreted as alterations in vascular resistance.

The data presented in these studies dissociate local pressor and depressor action of prostaglandins from modulation of hormone release, and are in agreement with those of Saunders and Moser (7) who reported that the amount of insulin release by perfused pancreas was unaffected by PGF2α-induced vasoconstriction. Mandelbaum and Morgan (13) manipulated canine extracorporeal circulation both mechanically and by use of vasoactive agents in vivo and reported no change in rate of insulin secretion despite elevated pancreatic blood flow. Growth hormone infusion and mechanical constriction of the inferior pancreatic artery diminished blood flow without proportionate decrease in insulin output in partially depancreatized dogs (14). However, largely because kallikrein, bradykinin and histamine increase capillary circulation, stimulate the basal- and the glucagon-induced insulin secretion in the dog (15), a direct relationship between secretion and alteration in microvasotone of endocrine pancreas has been suggested (15,16). Data shown in Table 1 are in disagreement not only with the concept of direct correlation between changes in vascular tone and insulin release, but provide further evidence (for the first time to the authors' knowledge) to dissociate prostaglandin-induced glucagon secretion from alteration in vascular resistance. We had reported previously that the
perfusion of isolated rat pancreas over 1 to 10 min period with 
PGE\textsubscript{1}, PGE\textsubscript{2} or PGF\textsubscript{2\alpha} augmented the release of glucagon and insulin (11), and also that PGH\textsubscript{2} is a potent secretagogue for both pancreatic hormones (10).

The mechanism of pressor and secretagogue effects of prostaglandins appears to be specific and separate. The attenuation of the endoperoxide analog-induced high perfusion pressure by PGH\textsubscript{2} (Figure 1 and Table 1) could indicate an action on the same receptor by PGH\textsubscript{2} and the analog as previously reported (1,4,5). PGH\textsubscript{2} could have a higher affinity for the receptor but lower pressor activity than the analog. Therefore, concurrent administration of the two results in displacement of the analog by PGH\textsubscript{2}. However, since the perfusion of pancreas with the analog did not enhance hormone release, it is possible that separate prostaglandin receptors exist for regulation of vasotone and modulation of secretion in endocrine pancreas. The secretagogue effect of PGH\textsubscript{2} could also be due to further conversion to other prostanoids. Robertson (17) has reported inhibition by intravenous infusion of PGE\textsubscript{1} of insulin release independent of alterations in vascular resistance or stimulation of the alpha-adrenergic receptors.

The mechanism by which prostaglandins modulate the secretory activity of endocrine glands is often attributed to interaction of their specific receptors with adenylate cyclase (18), since prostaglandins generally elevate levels of the nucleotide in tissues and affect metabolic functions regulated by cyclic AMP. The stimulant effect of prostaglandins endoperoxide on endocrine pancreas secretion could involve alteration in intracellular calcium concentrations although Hertelendy et al. (19) have reported that the prostaglandins are not acting as ionophores. Mishima and Kuriyama (20) reported depolarization of guinea pig stomach longitudinal muscle membrane by PGE\textsubscript{1}, PGE\textsubscript{2} or PGF\textsubscript{2\alpha} suggesting that these prostaglandins may increase calcium influx by stimulating electrical activity of the membrane. Change in membrane depolarization with the associated calcium influx in response to pancreatic endocrine hormone secretagogues is a well-established phenomenon.

We have provided evidence that the epoxymethano analog of PGH\textsubscript{2} exerts rapid and potent effect on vascular tone in perfused pancreas without significant effect on hormone release in response to PGH\textsubscript{2} and PGE\textsubscript{2}. It is concluded that nonpathologic changes in microvasotone in the pancreas do not significantly alter secretory activity of the endocrine pancreas.

ACKNOWLEDGEMENTS

These studies were supported in part by United States Public Health Service grants AM-21192, AM-02244 and AM-20572. The radioimmunoassays for glucagon and insulin were performed in the Ligand Assay Core Laboratory of the Michigan Diabetes Research and Training Center.
REFERENCES


