DISSOCIATION OF VASCULAR RESISTANCE WITH ENDOCRINE PANCREAS
SECRETION: THE EFFECTS OF EPOXYMETHANO ANALOGS OF PGH_2.

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ABSTRACT

The epoxymethano analogs of PGH_2 caused rapid and persistent
increase in perfusion pressure in isolated rat pancreata without
significant effect on glucagon and insulin secretory responses
to PGH_2 and PGE_2. The changes in perfusion pressure are inter-
preted as alterations in vascular resistance since the flow rate
was kept constant at 2.5 mL per min. PGH_2 alone caused signifi-
cant elevation in pressure. However, PGH_2 administration super-
imposed upon an infused epoxymethano analog of PGH_2, decreased
perfusion pressure significantly, whereas PGH_2 induced hormone
release was not decreased. The analogs neither stimulated nor
inhibited the endocrine pancreas secretion. These studies pro-
vide evidence for complete dissociation of vascular constriction
from pancreatic hormone release and further suggest that the ef-
fects of PGH_2 on islet hormone secretion may result from the
conversion of PGH_2 to other prostanoids.

INTRODUCTION

Previous reports (1,2,3,4) demonstrate that the synthetic analogs
of endoperoxide, 9,11- and 11,9-(epoxymethano)-azoprosta-5,13-
dienoic acid are stable analogs of prostaglandin H_2 (15-hydroxy-
9,11-peroxidoprosta-5,13-dienoic acid). The epoxymethano analogs
of PGH_2 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 PGH_2 (4,5).

Injection of 9-epoxy-11-methano into canine superior mesenteric
artery caused dose dependent vasoconstriction and decrease in

blood flow. However, PGH_2 injected similarly, resulted in vaso-
dilation 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 vasocon-
striction (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
PGH_2 and PGE_2.

MATERIALS AND METHODS

Pancreata were obtained from young adult male albino rats, weigh-
ing 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 appa-
ratus, 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
PGH_2.

PGH_2 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 PGH_2 and PGE_2 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. PGH_2 and PGE_2 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 pres-
sure transducer, an amplifier and a strip-chart recorder set
either at 0.2 in (or at 2 in per min during injection of PGH_2).
PGH_2 and PGE_2 were administered as a 250 uL bolus over 5-8 sec-
onds through a diaphram in the influent line without interrupting
the flow of the basic perfusion solution. PGH_2 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 radioimmuno-
assays. Radioimmunoassays for glucagon and insulin were per-
formed 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.

474

The pancreas was perfused firstly, for 20 minutes with basic buf-
fer solution containing 5.6 mM glucose (described in MATERIALS
AND METHODS) without the endoperoxide analogs. The influent per-
fusate was then switched to a solution containing 1 uM 11-epoxy-
9-methano analog of PGH_2 and perfusion was continued further for
60 minutes. The influent perfusate was lastly switched again to
the basic buffer solution. PGH_2 or PGE_2 was injected as a 250 uL
bolus into the pancreas before, during and after perfusing the
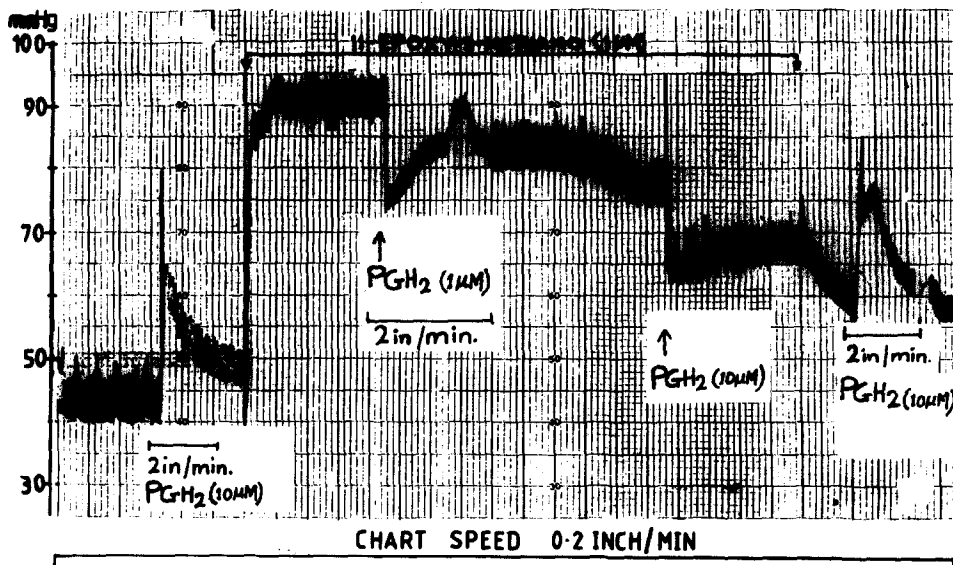pancreata with solution containing the endoperoxide analogs.

CHART SPEED 0·2 INCH/MIN

Fig. 1. PGH_2 and endoperoxide analog induced changes in per-
fusion 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 PGH_2
only). PGH_2 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 persist-
ent increase in the perfusion pressure. PGH_2 (10 uM) alone in-
jected before the endoperoxide analog, also exerted significant,
although lesser increase in pressure (Figure 1, left panel).
PGH_2 (1 uM or 10 uM) injections superimposed upon influent endo-
peroxide 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 grad-
ually returned almost to baseline in five minutes. PGH_2 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 main-
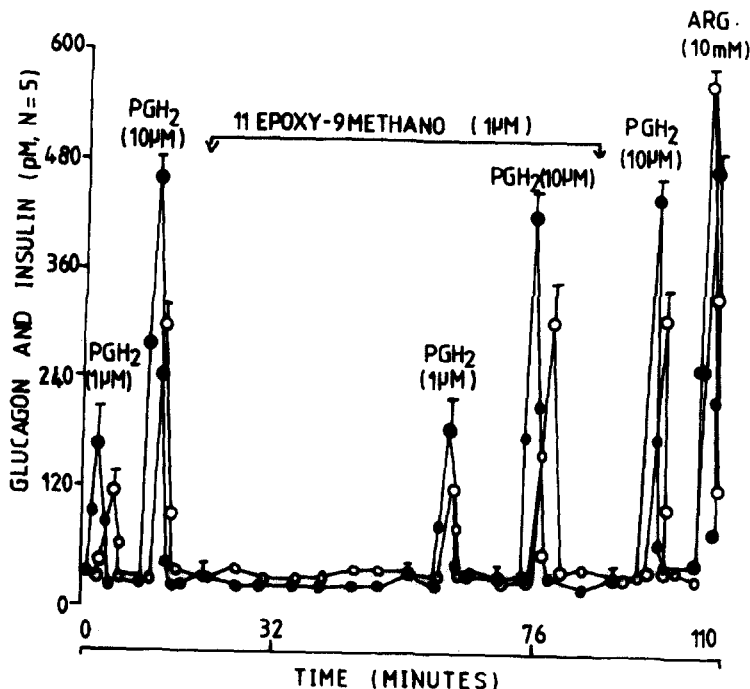tained constant at 2.5 mL per minute.

Fig. 2. Glucagon and insulin secretion during perfusion
of pancreas with PGH_2 and endoperoxide analog.
Hormone values are mean ± SEM for 5 observations.

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, dur-
ing and after perfusing the pancreata with endoperoxide analog
were not significantly different (P<0.10). The release of glu-
cagon occurred more rapidly than that of insulin but the magni-
tude of the responses in both hormones were similar. Endoper-
oxide 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 in-
crease 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.

CONDITIONS	GLUCAGON (fmol/5min)		INSULIN (fmol/5min)		% mmHg
	CONTROL(n)	TEST (n)	CONTROL(n)	TEST (n)	PRESSURE(n)
PGH_2 ANALOG ALONE (1uM)	170±30(14)	163±17 (14)	140±40(12)	180±50 (12)	+90±10(10)
BEFORE PGH_2 ANALOG					
PGH_2 (1uM)	213±40(9)	630±70 (9)	140±45(8)	400±60 (8)	+30±5 (6)
PGH_2 (10uM)	135±20(9)	1430±200(9)	190±40(7)	1100±390(10)	+50±10(6)
PGE_2 (10uM)	180±50(4)	1315±300(4)	175±10(4)	520±120(4)	Variable
DURING PGH_2 ANALOG					
PGH_2 (1uM)	170±40(13)	580±75 (13)	120±40(10)	460±117(10)	-20±2*(7)
PGH_2 (10uM)	140±10(17)	1500±260(13)	146±35(10)	1250±400(10)	-30±3*(8)
PGE_2 (10uM)	220±80(4)	1430±380(4)	170±50(4)	433±190(4)	Variable
AFTER PGH_2 ANALOG					
PGH_2 (10uM)	180±30(9)	1220±190(13)	170±70(10)	1090±330(10)	+30±10(5)
PGE_2 (10uM)	125±60(4)	1290±400(4)	150±40(4)	405±110(4)	Variable

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 base-
line, last column) without significant effect on hormone release
(170±30 vs 163±17, glucagon and 140±40 vs 180±50, insulin). In-
creases in glucagon and insulin levels occurred consistently in
response to PGH_2 and PGE_2 (10 uM) before, during and after per-
fusing 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 PGH_2 (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 PGE_2 (10 uM)
were inconsistent and variable. The levels of hormone release
and changes in pressure (PGH_2 only) induced by either PGH_2 or
PGE_2 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 re-
sults 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 PGH_2 decreased
high perfusion pressure caused by 11-epoxy-9-methano analog of
PGH_2, hormone secretion induced by PGH_2 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 inter-
preted 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 $PGF_{2\alpha}$-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 de-
spite 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 insu-
lin secretion in the dog (15), a direct relationship between se-
cretion and alteration in microvasotone of endocrine pancreas has
been suggested (15,16). Data shown in Table 1 are in disagree-
ment 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 disso-
ciate 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_1, PGE_2 or $PGF_{2\alpha}$ augmented the release of glucagon and insulin (11), and also that PGH_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_2 (Figure 1 and Table 1) could indicate an action on the same receptor by PGH_2 and the analog as previously reported (1,4,5). PGH_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_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_2 could also be due to further conversion to other prostanoids. Robertson (17) has reported inhibition by intravenous infusion of PGE_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_1, PGE_2 or $PGF_{2\alpha}$ 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_2 exerts rapid and potent effect on vascular tone in perfused pancreas without significant effect on hormone release in response to PGH_2 and PGE_2. It is concluded that nonpathologic changes in microvasotone in the pancreas do not significantly alter secretory activity of the endocrine pancreas.

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