PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 233–238 (1982)

Gastric Venous Prostaglandin Concentrations during Basal and Stimulated Acid Secretion in Dog (41337)

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Abstract. A segment of canine gastric fundic *in situ* tissue was perfused at constant flow with femoral arterial blood. Plasma concentrations of 6-oxo-$PGF_{1\alpha}$ (the breakdown product of prostacyclin), PGE_2, and thromboxane B_2 (TXB_2) were measured in the gastric arterial and venous blood by radioimmunoassay. Under nonstimulated conditions, gastric venous plasma contained significantly higher levels of prostanoids than gastric arterial plasma, with 6-oxo-$PGF_{1\alpha}$ being predominant. Arachidonic acid markedly elevated TXB_2 levels in gastric arterial plasma and significantly elevated 6-oxo-$PGF_{1\alpha}$ and PGE_2 and TXB_2 levels in gastric venous plasma following administration into an arterial incubation coil leading to the stomach. However, no increase in gastric venous blood concentrations of 6-oxo-$PGF_{1\alpha}$, PGE_2, or TXB_2 was observed during near maximal pentagastrin- or histamine-stimulated acid secretion. These observations suggest that while the endogenous prostanoids may be involved in the modulation of resting gastric blood flow, their role in mediating the gastric vasodilation associated with stimulated acid secretion is less clear.

Stimulation of gastric acid secretion is accompanied by an increase in gastric mucosal blood flow (1). The gastric mucosa generates several prostaglandins from the precursor arachidonic acid. Moncada *et al.* (2) identified prostacyclin as a major product in the gastric mucosa of a variety of species. Peskar (3) found both PGE_2 and $PGF_{2\alpha}$ were synthesized from arachidonic acid (AA) by the microsomal fraction of human gastric mucosa while Le Duc and Needleman (4) have detected the conversion of AA to PGE_2, 6-oxo-$PGF_{1\alpha}$ (the breakdown product of prostacyclin) and thromboxane B_2 (TXB_2) in the microsomes of canine gastric mucosa. Prostacyclin (5) and PGE_2 (6), infused intravenously, increase the mucosal clearance of a weak base, suggesting that both produce an increase in resting gastric mucosal blood flow and it has been proposed therefore that prostaglandins may be involved with this functional vasodilation (6) associated with stimulated gastric acid secretion.

Inhibition of endogenous gastric prostaglandin synthesis by aspirin or indomethacin produced a reduction in nonstimulated gastric mucosal blood flow. Aspirin reduced gastric mucosal aminopyrine clearance 31% in unanesthetized dogs (7) and left gastric artery blood flow 26% in anesthetized dogs (8). Indomethacin reduced gastric mucosal blood flow in the resting rat gastric mucosa by 49% and dog by 52% (7, 9). Indomethacin likewise reduced mucosal blood flow during stimulated gastric acid secretion in the rat (9) and dog (8). It has also been proposed that the gastric vasodilation induced by histamine may in part be modulated by endogenous prostaglandins, since indomethacin and meclofenamic acid reduced the H_2 receptor component of this vascular response (10). Taken together, these studies suggest that endogenous prostaglandin activity may play a role in the maintenance of nonstimulated gastric mucosal blood flow and may be involved in the mediation of the vasodilation associated with stimulated gastric acid secretion.

Prostaglandin levels in gastric luminal fluid have been studied previously during stimulated gastric acid secretion. Using a

[1] Visiting Scientist from Center for Ulcer Research and Education, VA Wadsworth Medical Center Research Service. Supported in part by NIAMDD Grant AM27465.

0037-9727/82/020233-06$01.00/0

sensitive bioassay system, no prostaglandin-like activity was found in extracts of gastric perfusate collected during basal, pentagastrin- or histamine-stimulated acid secretion in rat (9). Using radioimmunoassay techniques, no change in PGE_2 and PGA_2 concentrations was found in human gastric juice during pentagastrin stimulation, but the total output of prostaglandins tended to increase with the increase in volume of acid secretion (11). In normal and duodenal ulcer patients, the concentration of "PGE" detected in gastric juice by radioimmunoassay during pentagastrin-stimulated acid secretion remained unchanged (12).

The present studies investigate whether there is an arteriovenous difference in prostaglandin concentration across the canine gastric tissue *in situ* and whether there is an increase in gastric venous prostaglandin concentration during pentagastrin- and histamine-stimulated acid secretion.

Methods and Materials. *Animal preparation.* Beagle dogs (8 to 12 kg body weight), fasted for 36 hr but allowed water *ad libitum,* were anesthetized with pentobarbital sodium (30 mg kg^{-1}), intubated, and ventilated with positive pressure (Oxford Ventilator, Penlon Ltd., Abingdon, England). Anesthesia was maintained by intravenous administration of pentobarbital (30 mg) when required. Right and left femoral arteries and the right femoral vein were cannulated. The femoral vein cannula was used for anesthetic and saline administration and the infusion of the gastric acid stimulants. Arterial pressure was measured from the right femoral artery by a Statham pressure transducer (P23G6, Hato Rey, Puerto Rico) connected to a Beckman R411 Dynograph Recorder (Glenrothers Fife, Scotland).

A segment of acid-secretory gastric fundic tissue was encased in a lucite chamber as described by others (13). The branches of the splenic artery and vein which supplied the gastric tissue were isolated, while all other vascular connections supplying the greater curvature of the stomach were ligated. All arterial and venous connections to the spleen were ligated. Each dog was heparinised (500 IU kg^{-1}) and the splenic artery was cannulated with a polyethylene catheter (OD 1.65 mm) close to the gastric tissue, and perfused (10 ml min^{-1}) with blood from a cannulated left femoral artery via a constant-flow roller-pump (Watson–Marlow Flow-inducer). Changes in perfusion pressure, indicative of vascular resistance, were recorded with a transducer connected to the arterial blood line close to the stomach. Resting gastric perfusion pressure was 80–100 mm Hg. An injection port was sited in a delay coil, which allowed 30 sec incubation in the gastric arterial blood before the injected substances reached the stomach. A small port in the arterial line close to the gastric tissue provided access to gastric arterial blood. A short polyethylene cannula (OD 1 mm) was inserted into one of the short splenic connections from the splenic vein which allowed collection of gastric venous blood samples.

The luminal surface of the gastric mucosa in the chamber was perfused with 0.15 *M* NaCl (pH 7.0) at a rate of 4.0 ml min^{-1}. The luminal fluid outflow immediately passed through a Plexiglas block in which was positioned a combined pH and reference electrode (Radiometer, Copenhagen) to measure change in pH during pentagastrin- and histamine-stimulated acid secretion. Acid output was calculated from the change in pH.

Assay of plasma levels of PGE_2, 6-oxo-$PGF_{1\alpha}$, and thromboxane B_2. The gastric preparation was allowed to equilibrate for at least 90 min following the surgical procedures prior to any of the experimental protocol being carried out. Gastric arterial and venous blood samples (5.0 ml) were collected in plastic tubes containing 50 μl of a 36 mg ml^{-1} EDTA solution and 10 μl of a 5 mg ml^{-1} indomethacin solution (final concentration 10 μg ml^{-1}) to inhibit the further biosynthesis of prostaglandins during sample collection and processing. The samples were chilled and immediately centrifuged (10 min at 450*g*) and the platelet poor plasma stored at −20°C. The prostanoids in an aliquot (1 ml) of plasma were extracted initially into acetone, and the neutral lipids were eliminated by *n*-hexane extraction. After adjusting the aqueous phase to pH 4,

the prostanoids were extracted into chloroform. The individual components in the extract were separated by thin-layer chromatography prior to determination of TXB_2, PGE_2, and 6-oxo-$PGF_{1\alpha}$ by specific radioimmunoassay (14, 15). The sensitivity of this assay system was 0.10 ng ml^{-1} for each prostanoid.

Preparation of drugs. Histamine dihydrochloride (BDH, England) was dissolved in 0.15 *M* NaCl and administered by intravenous infusion at a dose of 160 $\mu g\ kg^{-1}\ h^{-1}$ (97 $\mu g\ kg^{-1}\ h^{-1}$ histamine base).

Pentagastrin (Peptavlon, ICI, England) was dissolved in 0.15 *M* NaCl and administered by intravenous infusion at a dose of 8 $\mu g\ kg^{-1}\ h^{-1}$.

Arachidonic acid (Grade I, Sigma Chemical Co.) was dissolved in *n*-hexane (10 mg ml^{-1}) and stored in a light-free container under nitrogen at −40°C. Aliquots (1 vol) were evaporated under nitrogen and dissolved in 0.05 *M* NaOH in methanol (0.5 vol), evaporated, and redissolved in 1 *M* Tris, stored on ice, and remixed prior to each use. Indomethacin (Sigma Chemical Co.) was dissolved (5 mg ml^{-1}) in sodium bicarbonate solution (5%, w/v).

Statistical analysis. Statistical analyses made use of the paired *t* test and $P < 0.05$ was taken as significant.

Results. *Effect of pentagastrin and histamine on gastric acid secretion.* Under resting conditions, only a low rate of acid output into the mucosal perfusate could be detected during the 30-min study period (Table I). Histamine (160 $\mu g\ kg^{-1}\ h^{-1}$) or pentagastrin (8 $\mu g\ kg^{-1}\ h^{-1}$), sufficient to produce near maximal rates of gastric acid secretion, was infused intravenously in randomized order for a 15- to 30-min study period. As shown in Table I, an increase in acid output was detected within 5 min of starting the infusion of either stimulant, which reached stable plateau levels 1000 times that observed under resting conditions within 15 min. Following termination of infusion of either stimulant, acid output returned to resting levels within 30–60 min. After a further resting period of 30 min, the second stimulant was infused intravenously for a period of 15–30 min as before.

TABLE I. ACID SECRETION ($\mu mol\ min^{-1}$) FROM CANINE GASTRIC MUCOSAL FLAP DURING RESTING CONDITIONS AND DURING STIMULATION INDUCED BY INTRAVENOUS INFUSION OF PENTAGASTRIN (8 $\mu g\ kg\ min^{-1}$) or HISTAMINE (160 $\mu g\ kg^{-1}\ min$)[a]

	Pentagastrin	Histamine
Resting	0.003 ± 0.005	0.006 ± 0.002
Stimulation (5 min)	1.28 ± 0.91	0.59 ± 0.47
Stimulation (15 min)	14.40 ± 0.90	25.20 ± 5.30

[a] Results are means ± SE of experiments in four dogs.

Gastric arterial and venous levels of prostanoids. The concentrations of 6-oxo-$PGF_{1\alpha}$, PGE_2, and TXB_2 in samples of plasma from femoral arterial blood taken close to the stomach were at the lower limit of detection (0.1 ng ml^{-1}) in the assay system used. In contrast, the plasma levels of these prostanoids were significantly higher ($P < 0.05$ for each) in samples of gastric venous blood (Fig. 1). The prostacyclin breakdown product, 6-oxo-$PGF_{1\alpha}$, was the predominant prostanoid in the venous blood. The venous concentration of 6-oxo-$PGF_{1\alpha}$ was 3.5 times that of PGE_2.

Effect of arachidonic acid on gastric arterial and venous levels of prostanoids. The prostaglandin precursor, arachidonic acid (AA; 100 μg) was administered as a

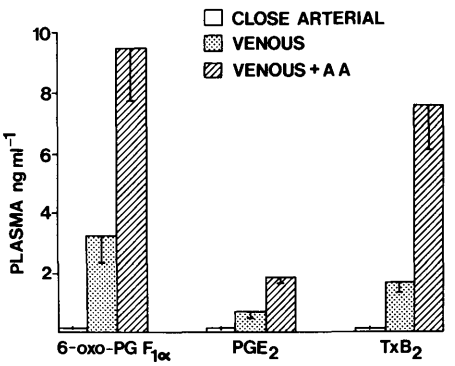

FIG. 1. Levels of prostanoids in close-arterial and gastric venous plasma in the dog, and the change in venous plasma levels following arachidonic acid (AA) administration. AA (100 μg) was injected into a delay coil so as to incubate with arterial blood for 30 sec prior to reaching the gastric circulation. Results, expressed as ng ml^{-1}, are the mean ± SE of four experiments and show a significant arteriovenous difference for each prostanoid ($P < 0.05$ for each).

bolus injection into the gastric arterial blood so as to incubate with the blood in the delay coil prior to reaching the stomach. Samples of venous blood were collected 30–40 sec after AA injection, at a time when the vasoactive action could be detected as an increase in perfusion pressure. Under these conditions, significant increases in the gastric venous plasma levels of 6-oxo-$PGF_{1\alpha}$ ($P < 0.01$), PGE_2 ($P < 0.02$), and TXB_2 ($P < 0.02$) were detected (Fig. 1).

In a further series of six dogs, the effect of AA (100 μg) on arterial plasma levels of prostanoids following 30 sec incubation with arterial blood flowing through the incubation coil was studied. As is shown in Fig. 2, AA significantly increased the arterial plasma levels of TXB_2 ($P < 0.01$) following 30 sec incubation in the delay coil. In contrast, arterial plasma levels of 6-oxo-$PGF_{1\alpha}$ and PGE_2 were not significantly elevated by AA incubation (Fig. 2).

The increase in venous blood 6-oxo-$PGF_{1\alpha}$, PGE_2, and TXB_2 concentrations following the bolus AA injection indicates that the assay system is sensitive to changes in the concentration of these substances and thus validates the technique.

Effects of pentagastrin and histamine on venous levels of prostanoids. Gastric venous blood samples were collected during resting acid secretion and during plateau submaximal rates of acid secretion induced by intravenous infusion of pentagastrin (8 $\mu g\ kg^{-1}\ h^{-1}$) or histamine (160 $\mu g\ kg^{-1}\ h^{-1}$), both 5 and 15 min after starting the infusion. During pentagastrin or histamine infusion, there was no significant change in the venous plasma levels of 6-oxo-$PGF_{1\alpha}$, PGE_2, and TXB_2 (Figs. 3 and 4).

Discussion. These studies indicate that there is a significant arteriovenous (AV) prostaglandin concentration difference across canine gastric tissue *in situ*. The predominant prostanoid measured in gastric venous blood was 6-oxo-$PGF_{1\alpha}$, the hydrolysis breakdown product of prostacyclin. The concentration of 6-oxo-$PGF_{1\alpha}$ in gastric venous blood was found to be three to four times greater than PGE_2. These observations are consistent with those of Moncada *et al.* (2) who observed that prostacyclin is the major product of AA metabolism by cyclooxygenase in the gastric mucosa. These data also support the hypothesis that endogenous prostaglandin activity may play a role in the maintenance of nonstimulated gastric blood flow since there is significant release of vasodilator prostaglandins into the venous blood under nonstimulated conditions. Although this nonstimulated AV difference might be considered artifactual due to the trauma of surgery and gastric tissue mounting, we believe this is not the case since the tissue was allowed 90 min

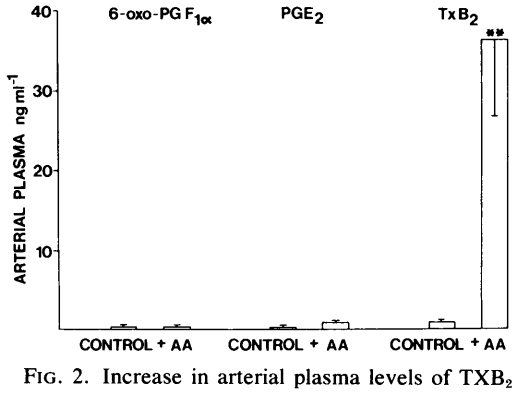

FIG. 2. Increase in arterial plasma levels of TXB_2 following arachidonic (AA; 100 μg) administration to dog blood flowing in a 30 sec incubation coil. Results, shown as ng ml^{-1}, are mean ± SE of experiments in six dogs. (**$P < 0.001$).

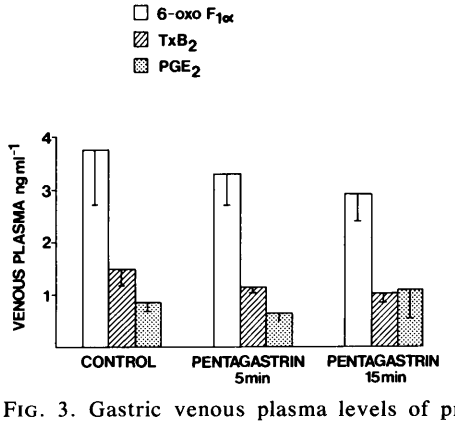

FIG. 3. Gastric venous plasma levels of prostanoids, 5 and 15 min after starting infusion of the gastric acid stimulant, pentagastrin (8 $\mu g\ kg^{-1}\ min^{-1}$, iv). Results, expressed as ng ml^{-1}, are mean ± SE of four experiments.

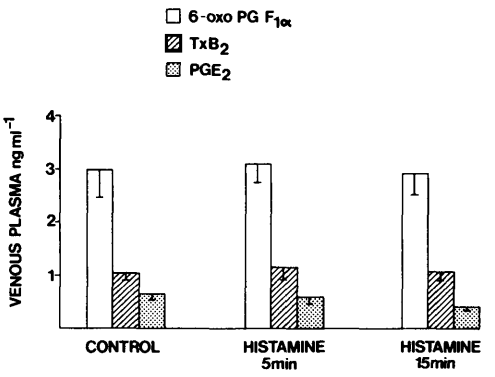

FIG. 4. Gastric venous plasma levels of prostanoids, 5 and 15 min after starting infusion of the gastric acid stimulant, histamine (160 μg kg^{-1} min^{-1}, iv). Results, expressed as ng ml^{-1}, are mean ± SE mean of four experiments.

to recover after preparation before obtaining blood samples. Samples taken under resting conditions some 2–3 hr after surgery showed comparable venous levels of prostaglandins. Further, the relative proportions of these prostaglandins in venous blood are similar to those observed previously in gastric mucosal tissue.

Administration of the prostaglandin precursor, AA, elevated arterial plasma levels of TXB_2 some 40-fold, following incubation in blood flowing through a 30-sec delay coil. The formation of TXB_2, the breakdown product of the biologically active TXA_2 presumably reflects biotransformation of the AA by the blood-borne platelets, known to be a rich source of thromboxane synthetase. In other studies on canine blood flowing through a similar incubation coil system, the formation of TXA_2-like products from AA was detected by the use of isolated vascular bioassay tissues (16). The failure of arterial plasma levels of 6-oxo-$PGF_{1\alpha}$ and PGE_2 to be similarly elevated indicates that under these experimental conditions, those products are not formed in detectable amounts in blood in the absence of gastric or vascular tissue.

When AA was injected into the blood flowing through the gastric circulation, increased levels of 6-oxo-$PGF_{1\alpha}$ and PGE_2 were observed in gastric venous blood, indicating their formation from AA in the gastric tissue. The levels of TXB_2 in the gastric venous plasma were lower than those detected in samples of femoral-arterial blood following AA injection into the incubation coil. Therefore, we have no evidence that thromboxane is formed from AA in gastric tissue. It is not known whether this AV difference in TXB_2 levels reflects uptake, binding, or metabolism of thromboxane during its passage through the gastric circulation. A temporal difference in sampling may also contribute since detection of the vasoactive responses to AA may not be a good index for the transit and distribution of its pharmacologically active products in this complex vascular tissue.

A significant increase in venous blood 6-oxo-$PGF_{1\alpha}$, PGE_2, TXB_2 concentration could not be detected during pentagastrin- or histamine-stimulated gastric acid secretion. These observations could be interpreted to suggest that endogenous prostaglandins may not play a role in the mediation of pentagastrin or histamine-induced mucosal blood flow increase, or functional hyperemia. Another possibility is that the total concentrations of endogenous vasodilator prostaglandins being released from the gastric tissue into the blood, following redistribution of blood between mucosa and muscle, is sufficient to maintain the mucosal vasodilation associated with stimulated gastric acid secretion. However, it is also possible that with stimulated gastric acid secretion, metabolic turnover increases such that generated endogenous prostaglandins are more rapidly metabolized and less likely to be observed in venous blood. The output of prostaglandin metabolites into human gastric juice has recently been detected by Peskar and coworkers (17). Studies on the levels of prostaglandin metabolites in venous blood during stimulation may therefore be of value.

In the present studies, we have shown that the generation and release of prostacyclin and PGE_2 in canine gastric tissue *in situ* is sufficient to create a significant AV concentration difference, with the predominant prostanoid being prostacyclin. This provides some support for the concept that endogenous prostaglandins may contribute to the maintenance of nonstimulated gastric blood flow. However, if endogenous

prostaglandins play a role in the vasodilatation associated with stimulated acid secretion, any local increase in synthesis or release of these parent prostaglandins is not of sufficient magnitude to detect a further increase in gastric AV concentration difference.

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Received July 17, 1981. P.S.E.B.M. 1982, Vol. 169.