Prostaglandin Inhibition of Innervated Antral Motility in Dogs[1] (34371)

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Prostaglandins (PGE) are lipid-soluble, long-chained, unsaturated, oxygenated fatty acids, biologically active and identifiable in human seminal fluid, sheep vesicular glands, and many other organs including the stomach and intestine. Fourteen separate chemically related compounds have been identified and are described as derivatives of a hypothetical molecule—"prostanoic acid." The exact chemical structure of prostaglandins was described by Bergstrom *et al.* (1) and biosynthesis achieved independently by Bergstrom *et al.* and Van Dorp *et al.* (2, 3) Total prostaglandin synthesis has been accomplishd by Beal *et al.* (4) using 3-ethoxy-2-cyclopentenone as the starting material.

The precise physiologic role of naturally occurring prostaglandins in gastrointestinal function has been intensively investigated. Biologic activity may consist of either stimulation or inhibition of motor function in such neuromuscular tissues as are normally spontaneously active. Prostaglandins have been shown to increase spontaneous activity of *in vitro* longitudinal muscle strips and to inhibit spontaneous activity of smooth circular muscle (5, 6). Increased intestinal motor activity after PGE infusion has been inferred from studies in rabbits and mice (7). Abdominal cramping in humans during intravenous PGE_1 infusion has corroborated these observations (8). Robert *et al.* (9) and Ramwell *et al.* (10) have recently demonstrated decreased gastric secretory activity in canine and rat stomachs during PGE_1 infusion. No information is available on the effect of PGE on upper gastrointestinal motor function in the intact unanestheized animal.

The following investigations analyze the effect of PGE_1 on motility patterns of innervated gastric antral pouches in dogs at dose levels previously established as having an inhibitory effect on gastric secretion.

Material and Methods. Eight mongrel dogs weighing between 11 and 22 kg were each prepared with an isolated innervated antral pouch; gastrointestinal continuity was reestablished by side-to-side gastroenterostomy (11). Experiments were commenced after a 3-week recovery period. Prior to study, the animals were fasted overnight (18 hr) except for water, and motility studies were conducted in a quiet room. Antral motility was recorded via a water-filled rubber balloon (4-cc); care was taken to avoid distention of the antrum. After local skin anesthesia was accomplished with 1 cc of 1% Xylocaine, the balloon was secured in position by a purse-string suture applied sufficiently loosely to permit mucus to escape. Pressure changes were measured with a pressure-sensitive transducer and Sanborn polygraph. Respiratory movements were simultaneously recorded on a pneumograph.

Control studies. Control antral motility in the fasting state was recorded for a 10-min period. 2-deoxy-D-glucose (100 mg/kg dissolved in 10 cc of normal saline) was administered by rapid intravenous injection. Subsequent motility changes were recorded for a total of 90 min; the Motility Index (MI = amplitude of contraction $\times$ frequency of contraction) was calculated for seven 5-min intervals (beginning at 15, 20, 25, 30, 40, 80, and 85 minutes) after 2-DG injection. Twelve control experiments were performed on six dogs.

Prostaglandin studies. In 15 experiments, 20 min after 2-DG injection, continuous prostaglandin E_1 infusion (1 μg/kg/min dis-

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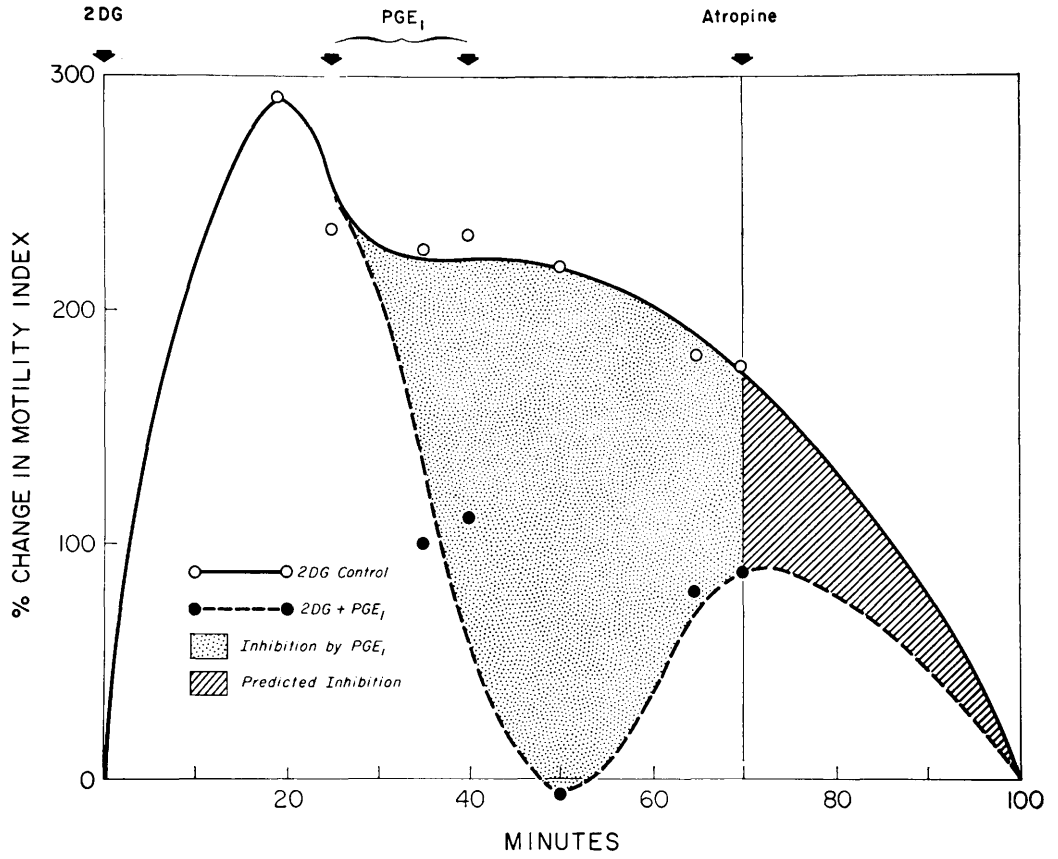

FIG. 1. Substantial inhibition of 2-DG driven antral motor activity is noted within a short period after the onset of PGE_1 infusion. Although each experiment was terminated by intravenous injection of atropine, it is possible to project the degree of inhibition, during an additional 30-min observation period, by extrapolating the curves for 2-DG control and 2-DG with superimposed PGE_1. Baseline motility index (without either 2-DG or PGE_1) in the fasting dog is arbitrarily set at 0%. Twelve control studies (2-DG alone) and 15 experimental studies (2-DG + PGE_1) were performed on six animals.

solved in 60 cc of normal saline) was initiated using a Sigma motor pump (Harvard). The infusion was carried out for from 5 to 20 min and discontinued with complete motor suppression. All experiments were concluded with an intravenous injection of 0.04 mg of atropine sulphate; motility was recorded for an additional 3 to 5 min. Fifteen experiments on six dogs were performed. Continuous EKG recordings were obtained in three animals during PGE_1 infusion; changes in heart rate were monitored.

Blood samples for estimation of blood sugar, serum potassium, and calcium levels were drawn at the start of each experiment and at the initiation of the 20, 40, and 80 min intervals. Blood sugar levels were determined by modification of the Raabo-Terkildsen procedure (12). Serum potassium and calcium were measured by flame photometry and spectrophotometry respectively.

Additional control studies. In four additional control experiments the vehicle used in making stock solution of prostaglandin E_1 (ethanol bicarbonate) was infused at the same rate as the PGE_1 in the prostaglandin studies. In two experiments the vehicle was infused prior to PGE_1 infusion, and in two after PGE_1 infusion.

Results. For purposes of comparison, Mo-

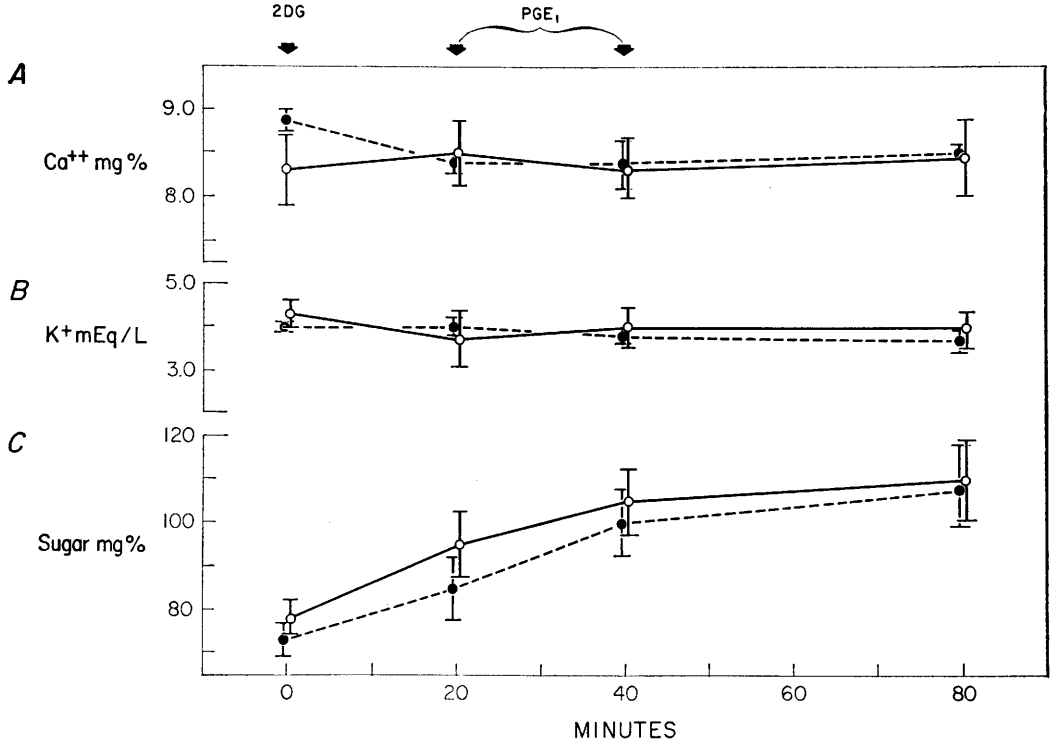

FIG. 2. Changes in serum calcium and potassium after 2-DG alone and with superimposed PGE_1 were insignificant. Blood sugar, predictably elevated by 2-DG, was not significantly altered by superimposed PGE_1.

tility Index was used in the calculation of response differences to 2-DG with and without PGE_1 using the method described by Cooke and Grossman (13). Percentage change in the Motility Index (compared with control) was calculated for each 5-min period as follows:

Percentage change in Motility Index $=$ $[(MI_a\text{-}MI_b)\ MI_b] \times 100$ when MI_b and MI_a represent the Motility Indices before and after the administration of the test drug, respectively.

Control. Control motility patterns from the antral pouches of fasting dogs consisted of three to four low-amplitude contractions per minute. Within 5 min after intravenous injection of 2-DG increase in both amplitude and frequency of contraction was noted in all experiments; in two studies a brief period of amplitude diminution (60–120 sec) was followed by stimulation. Increase in amplitude was the dominant change in motility characteristic. Percentage of change in Motility Index, from control after 2-DG injection, was +288 (±52.5); +232 (±18.7); +228 (±23.7); +235 (±24.3); +217 (±23.5); +183 (±22.2); +175 (±20.7) at 15, 20, 25, 30, 40, 80, and 85 min respectively (Fig. 1).

Prostaglandins. After initiation of PGE_1 infusion (begun 20 min after 2-DG injection) in five experiments, a decrease in both amplitude and frequency of contraction was noted within 5 min. In eight experiments sustained motility inhibition was noted within 15 min. In two experiments only minimum inhibition was noted after 30 min of PGE_1 infusion (Fig. 1).

After cessation of PGE_1 infusion, motor activity returned to or toward control levels. However, the pattern of resumption was not uniform; an average of 40 min was required to acheive a 50% return in MI.

Percentage of change in MI after PGE_1 compared to control 2-DG alone was measured at identical time intervals of 15, 20, 25, 30, 40, 80, and 85 min. Motor activity was

suppressed by PGE_1 to levels of 95 ($\pm$15); 110 ($\pm$22.7); —5 ($\pm$14.9); 76 ($\pm$14.6); and 87 ($\pm$15.5) (Fig. 1). The changes in the Motility Index were statistically significant for all time periods ($p<0.05$).

No change in amplitude or frequency of contraction was noted after ethanol bicarbonate infusion.

Blood Analysis. (A) Calcium. Serum Ca^{++} levels were measured in 20 experiments (10 control and 10 experimental) in the same time intervals as blood sugar and serum K^+. The serum calcium levels were 8.72 ($\pm$0.11); 8.45 ($\pm$0.08); 8.44 ($\pm$0.19); and 8.51 ($\pm$0.11) in the control group; Ca^{++} was 8.33 ($\pm$0.38); 8.53 ($\pm$0.34); 8.36 ($\pm$0.24); and 8.48 ($\pm$0.36) mEq/liter in the experimental group. There was no change of significance in the two groups (Fig. 2A).

(B) Potassium. In 12 control experiments, serum potassium levels were 4.05 ($\pm$0.12); 3.98 ($\pm$0.11); 3.8 ($\pm$0.11); and 3.7 ($\pm$0.12) mEq/liter. The fall in K^+ level after 2-DG was not significant (Fig. 2B).

In 15 experiments with PGE_1 serum potassium was 4.26 ($\pm$0.31); 3.8 ($\pm$0.29); 4.01 ($\pm$0.36); and 3.88 ($\pm$0.33). It is of interest that there was a slight increase in potassium level at the time of PGE_1 infusion, as compared to a slight fall in the control group. This change was again, however, not significant (Fig. 2B).

(C) Sugar. In 12 control experiments, the blood sugar levels were 72 ($\pm$3.8); 85 ($\pm$6.4); 100 ($\pm$7.6); and 108.4 ($\pm$9.0) mg/100 ml at 0, 20, 40, and 80 min respectively. The increase in sugar levels after 2-DG injection was significant ($p<0.05$) (Fig. 2C).

In 15 experiments with PGE_1 infusion, the sugar levels were 77.3 ($\pm$3.1); 95.2 ($\pm$5.4); 105.1 ($\pm$6.6); and 108.8 ($\pm$8.4) respectively. These increases were also significant ($p<0.05$) (Fig. 2C).

There was no significant difference in the two groups after 2-DG with or without PGE_1; although 2-DG significantly elevated blood sugar, the superimposition of PGE_1 did not alter the levels (Fig. 2C).

(D) Respiration. During the 2-DG or PGE_1 infusion, there was no change in respiration.

(E) Heart rate. During control experiments (three experiments in three dogs) mean heart rate was 122/min, increased to 131/min 15 min after 2-DG, and to 168/min 10–15 min after PGE_1. Within 10 min after cessation of the PGE_1 the rate had fallen to 140/min.

Discussion. The precise mode of action by prostaglandins on smooth muscle activity is unknown; a variety of theories have been postulated. Horton *et al.* (14) have suggested an effect in part mediated by way of nervous pathways since the response is partially antagonized by atropine. Coceani *et al.* (15) have suggested that prostaglandins initiate contraction by facilitation of calcium ion influx. Miyazaki *et al.* (16) have emphasized the belief that the primary role is mediated through the muscle membrane and not via intrinsic nervous or contractile systems. Harris *et al.* (17) believed that the inhibitory effect (when observed) is based on suppression of cyclic AMP production. Harris and Alonso (18) have demonstrated a temporary increase in hydrogen ion concentration in gastric secretion, during *in vitro* experiments utilizing frog gastric mucosa, with the addition of 3′ 5′ AMP to the perfusion medium after secretory inhibition by prostaglandins.

Previous studies on the prostaglandin effect on smooth muscle in the gastrointestinal tract have been executed on isolated muscle preparations. Rabbit duodenum and jejunum, hamster colon, guinea pig ileum, chicken rectum and cecum, rat jejunum and stomach fundus are examples. Smooth muscle in these tissues has been shown to contract in the presence of small doses of prostaglandins.

The experiments reported here demonstrated that prostaglandins are capable of profound inhibition of gastric antral motility in unanesthetized dogs after vagal stimulation by 2-deoxy-D-glucose. 2-DG was chosen as the vagal activator because of the potency and clarity of its effect on the vagal centers. It is a proved, powerful initiator of gastric antral motility. In the majority of experiments vagally driven antral motor activity

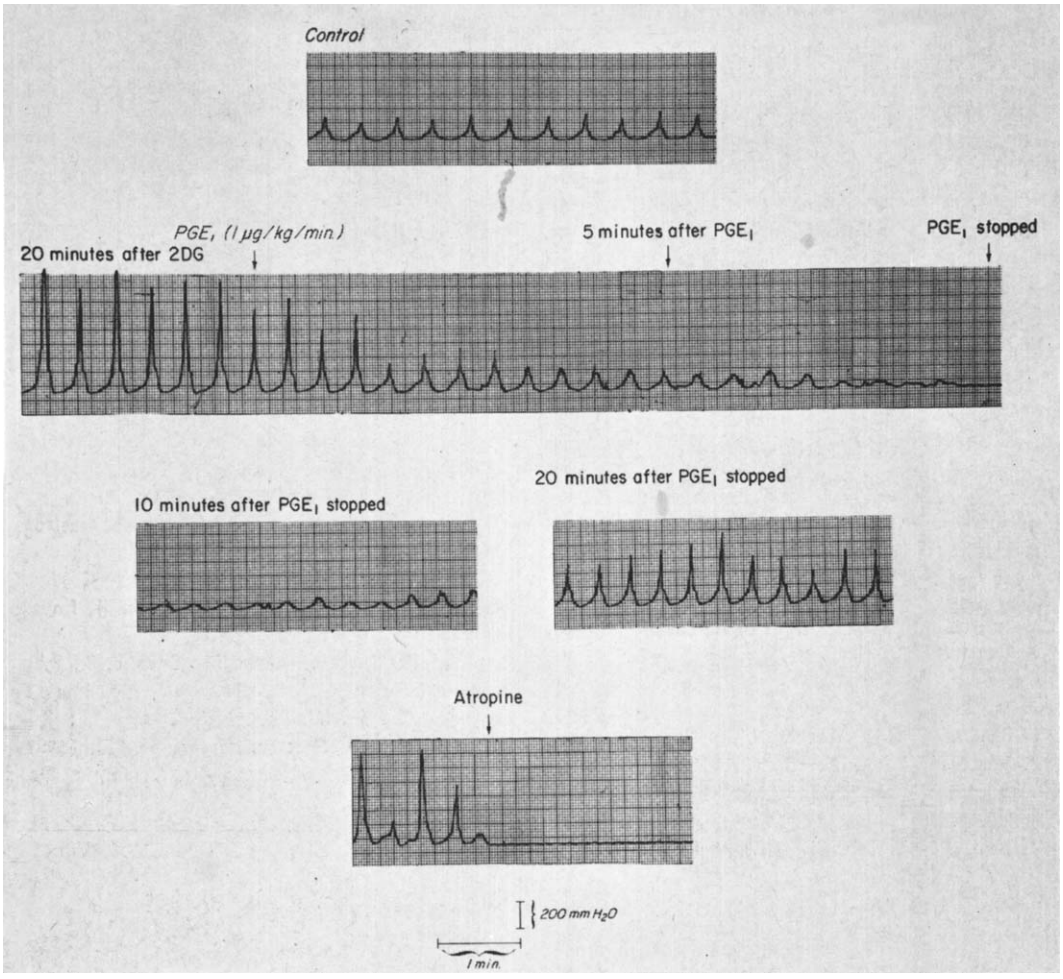

FIG. 3. A typical tracing demonstrating antral motor activity during a baseline control period in the fasting animal, 20 min after intravenous injection of 2-DG and suitable periods after the superimposition of an intravenous infusion of PGE_1. Atropine was given intravenously to terminate all experiments and immediately obliterated all motor activity.

inhibition by PGE_1 occurred within 15 min after initiation of the test agent and persisted at 50% control levels for a variable time subsequent to discontinuation of the infusion. The mechanism of suppressive action appeared, in these studies, to be independent of blood sugar levels, serum potassium, and calcium. The predominant inhibition characteristic affects contraction amplitude rather than frequency; the height of contraction gradually diminishes until none is noted (Fig. 3).

Although no significant change in serum potassium, calcium, or blood sugar was noted, this does not rule out the possibility of intracellular reorganization and distribution of these substances. Since the action of prostaglandins in the intact animal produces a variety of metabolic changes including, under certain circumstances, alterations in blood vessel tone, it remains to be shown whether the inhibitory effects here demonstrated are specific or secondary consequences of otherwise unrelated phenomena. Nevertheless, it is now possible to add the prostaglandin group to the growing list of naturally occurring gastric acid secretory and motor apparatus suppressors.

Summary. PGE_1 has been analyzed in terms of its effect on vagally driven gastric motor activity in dogs; profound inhibition of 2-deoxy-D-glucose stimulated motility was observed.

The effect of PGE_1 on serum calcium, potassium, and blood sugar levels has been monitored; no significant change in calcium or potassium during PGE_1 infusion has been noted. Blood sugar, traditionally elevated by 2-deoxy- D-glucose, was not altered from its usual pattern after PGE_1 infusion.

PGE_1 is capable, in doses previously shown to have an inhibitory effect on gastric secretion, to inhibit gastric motility as well. The precise mode of action is speculated upon but remains undisclosed.

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