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Research Article

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Local Regulation of Blood Flow in the Feline Jejunum

A POSSIBLE ROLE FOR ENDOLUMINALLY RELEASED SUBSTANCE P

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ABSTRACT Serotonin and substance P of gastrointestinal origin have been measured by radioimmunoassay in the bowel lumen under basal and stimulated conditions. To investigate the possibility that local blood flow may be influenced by these endoluminal hormones, 26 cats were studied with exogenous serotonin and substance P infused endoluminally into isolated proximal jejunal segments in vivo. Regional blood flow was measured by using the radioactive microsphere technique before, during, and after the endoluminal instillation of two doses of substance P (3.9 and 30 ng/min) or serotonin (0.9 and 21 μg/min). Neither dose of substance P changed systemic blood pressure. Substance P at the low dose caused an increase in blood flow to the experimental jejunal mucosa (from 53±10 ml/min per 100 g to 102±20 ml/min per 100 g, $P < 0.01$). The higher dose of endoluminal substance P similarly increased blood flow to the experimental jejunal mucosal fraction, and also increased blood flow to the experimental jejunal muscularis fraction (from 17±3 ml/min per 100 g to 23±3 ml/min per 100 g, $P < 0.02$). Serotonin increased blood flow to the experimental jejunal muscularis only at the high dose (17±4 ml/min per 100 g to 25±4 ml/min per 100 g tissue, $P < 0.01$). These results provide evidence for a dose-related local effect of endoluminal substance P on gastrointestinal blood flow.

INTRODUCTION

We have previously demonstrated that the biologic amine serotonin (5-HT)[1] and the undecapeptide substance P (SP) exist in the intestinal lumen in the basal state, and increase in amount during vagal nerve stimulation (1–3). Uvnas-Wallensten (4) has made a similar observation for the antrum. Although SP and 5-HT have been demonstrated in intestinal plexuses, the origin of these endoluminally released hormones is probably the mucosal enterochromaffin cell (EC), which has been shown to contain both 5-HT and SP in its intracytoplasmic granules (5, 6). Previous studies have shown loss of cytofluorimetry (7) and degranulation (8) from EC cells after electrical vagal nerve stimulation. Recently, electron microscopic autoradiography performed by using a tritiated 5-HT precursor, 5-[^{3}H]hydroxytryptophan has indicated that intravenously administered 5-[^{3}H]hydroxytryptophan is taken up by EC cells, and that labeled 5-HT is released into the gut lumen after vagal stimulation (9). Finally, vagal nerve stimulation simultaneously releases motilin, SP, and 5-HT (3), all contained in the EC cell (10).

The role of luminal hormones in the physiology of the intestine remains uncertain. Studies with gastrin have suggested a local trophic effect of exogenously administered gastrin on the intestinal mucosa of the rat (11). Bulbring and Lin (12) reported that luminal serotonin stimulated intestinal peristalsis. No physiologic function for intraluminal SP has yet been demonstrated.

The purpose of this current study was to investigate the possible role of exogenously introduced endoluminal SP and serotonin on local blood flow by using the radioactive microsphere technique.

METHODS

26 cats of both sexes (2.1–4.0 kg) were deprived of food but allowed free access to water for 18 h. Anesthesia was induced with ether and maintained with intravenous chloralose (100 mg/kg) via a femoral venous catheter (PE 90 tubing). Ventilation was controlled via trachecostomy and small animal respirator (Harvard Apparatus Co., Inc., Millis, MA), and temperature was maintained at 38°C. Mean arterial pressure was recorded through a cannula (PE 90) in a femoral artery. The contralateral femoral artery was cannulated (PE 90) and

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[1] *Abbreviations used in this paper*: EC, mucosal enterochromaffin cell; 5-HT, serotonin; SP, undecapeptide substance P.

served as the site of reference blood withdrawal for blood flow determinations. Normal saline was infused via the cannula in the femoral vein and mean arterial pressure remained at a basal 120 mm Hg level throughout the experiment. Through a midline laparotomy, a catheter (PE 90) was placed into the splenic vein and directed into the portal vein toward the liver; its position was confirmed, secured, and it was used for sampling of portal venous blood. The first 15 cm of jejunum distal to the ligament of Treitz was isolated between ligatures, where a soft plastic tube (2 mm i.d.) was introduced and secured proximally. A large hard plastic tube (10 mm i.d.) was introduced and secured distally, preventing increased intraluminal pressure in the isolated segment during perfusion. The proximal tubing was connected to a saline reservoir maintained at 37°C and passed through a peristaltic pump (Buchler Instruments Inc., Fort Lee, NJ) calibrated to deliver 1.0 ml/min into the jejunal segment. The distal tubing was used to collect 5-min samples of luminal perfusate into iced test tubes containing 0.25 ml aprotinin (Trasylol, Delbay Pharmaceuticals Inc., Div. Schering Corp., Bloomfield, NJ). After extraction, the concentrations of 5-HT and SP were measured by radioimmunoassays developed in our laboratory (13, 14). Portal venous blood samples were collected into EDTA tubes containing aprotinin (0.1 ml/ml plasma) at 10-min intervals during the experiment, and were similarly assayed for 5-HT and SP. For the injection of radioactively labeled (15 ± 5 μm) microspheres (3M Co., 3M Center, St. Paul, MN), a catheter (PE 90) was introduced and secured in the left atrium through a thoracotomy.

Saline was infused through the isolated segment before the start of each experiment to assure washout of luminal contents and free flow of perfusate. In half the experiments, a second isolated segment was constructed from the distal adjacent 15-cm jejunum and it was perfused with only saline for the entire protocol by using the identical technique; this second segment provided a control infused segment for blood flow comparisons.

Three consecutive 20-min periods (I, II, III) constituted all experiments. Normal saline alone, warmed to 37°C, was infused through the jejunal segment during periods I and III. During period II, luminal infusate solutions whose concentrations were measured by radioimmunoassay at the entry to the segment, contained either substance P (Peninsula Laboratories, Inc., San Carlos, CA) (low dose: 3.9±0.2 ng/ml [$n = 6$], high dose: 30±6 ng/ml [$n = 7$]) or serotonin (Sigma Chemical Co., St. Louis, MO) (low dose: 919±139 ng/ml [$n = 7$], high dose: 21.6±0.4 μg/ml [$n = 6$]) in saline.

At the end of each 20-min period, regional blood flow measurements were made by using $1.0–2.0 \times 10^6$ microspheres (^{141}Ce, ^{51}Cr, ^{85}Sr, or ^{46}Sc used in random order) injected as a rapid bolus via the left atrial catheter and flushed with 0.5 ml saline; this volume did not affect mean arterial pressure. For 30 s before, during, and after the microsphere injection, the withdrawal pump (Sage Instruments, Cambridge, MA) was used to collect reference blood samples. The radioactivity in the reference blood samples allowed for the calculation of regional blood flow by the formula: regional blood flow = regional cpm × reference blood flow/reference cpm. This technique (including the sampling time) has been verified for small animal work using cats (2) and rabbits (15) in our laboratory. At the termination of the experimental protocol, the animals were sacrificed, and the tissues of interest harvested, separated into mucosal and muscularis fractions, weighed, and counted in a multichannel gamma spectrometer (Packard Instrument Co., Inc., Downers Grove, IL). Harvested tissues included kidney (as a reference organ), duodenum, 15-cm perfused jejunal segment ("experimental jejunum"), distal adjacent 15-cm jejunal segment (nonperfused or perfused "control jejunum"), and 15-cm distal ileum. All flows were expressed as milliliter per minute per 100 g (wet weight) of tissue.

Results were expressed as mean±SEM. Statistical analyses were performed by using the t-test for paired data, with significance accepted at the 5% level.

RESULTS

Endoluminal substance P. During exogenous endoluminal SP infusion at the low dose, there was no change in mean arterial pressure and the only significant increase in blood flow (Fig. 1) occurred in the experimental jejunal mucosa ($P < 0.01$). Neither the experimental jejunal muscularis, nor any other tissue sampled had altered blood flow. Thus, blood flow changes were limited to local mucosal effects in the SP-infused jejunal segment.

During the low dose infusions, the concentration of SP in the jejunal perfusate (Table I) began at 3±2.6 pg/5 min but washed out to zero by the end of period I. Perfusate SP rose to a peak by 10 min into period II, and returned to basal values in period III. The portal venous SP concentration did not change from a mean basal value of 2.1±0.9 pg/ml during or after the SP infusion.

At the high SP dose (Fig. 1), significant increases in blood flow to the experimental jejunal mucosal and the muscularis layers were noted. Control tissues, including kidney, duodenum, control jejunum, and ileum, showed no significant alterations in flow during the experiments. SP-induced blood flow changes were again limited to the tissues in contact with high endoluminal SP concentrations, and these reverted to basal values upon washout of endoluminal SP. During these SP perfusions of the jejunal segment, luminal SP concentrations peaked during period II, and returned to basal during period III (Table I). Portal venous SP concentrations did not change from a mean basal of 3.5±1.8 pg/ml despite the instillation of large amounts of exogenous SP endoluminally.

Endoluminal serotonin. No blood flow changes were noted for the experimental jejunum or any control organs during the low dose studies. The mean concentration of 5-HT at the start of period I was 407±382 ng/ml and washed out to 136±134 ng/ml. During serotonin perfusion, luminal 5-HT levels rose to a peak of 6,231±514 ng/ml during period II, and returned to 418±258 ng/ml at the end of period III. Portal venous 5-HT concentrations did not change from a mean basal concentration of 419±122 ng/ml.

During exogenous endoluminal 5-HT infusion at the high dose the only change in blood flow occurred in the experimental jejunal muscularis fraction, which rose significantly from 17±4 ml/min per 100 g during period I to 25±4 ml/min per 100 g during period II

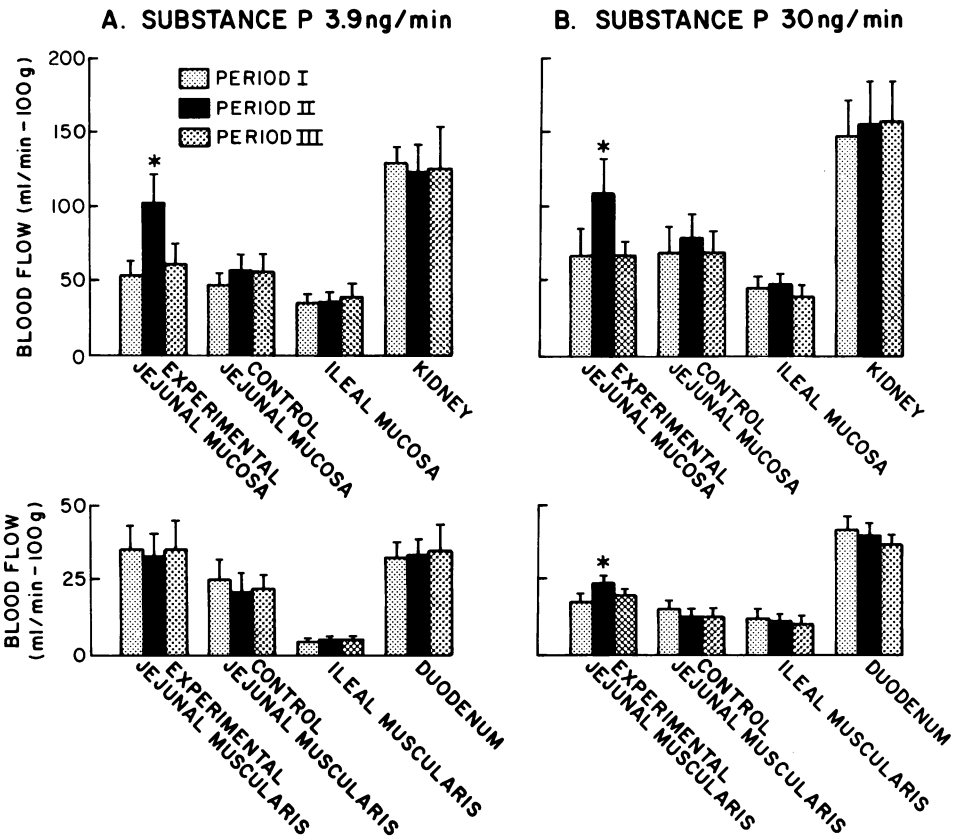

FIGURE 1 The effect of endoluminal infusion of the experimental jejunal segment with substance P (A. $n = 7$; B. $n = 6$) on organ-specific blood flow during period I (saline control), period II (substance P in experimental segment), and period III (saline control). Data are presented as means±SEM. $^{*}P < 0.05$.

($P < 0.01$), and returned to 22±2 ml/min per 100 g during period III. Neither the experimental jejunal mucosa, nor any other control organ had changes in blood flow during this high dose 5-HT endoluminal infusion. In these studies the mean perfusate concentration of 5-HT washed out to 60±60 ng/ml at the end of period I, peaked at 176.2±14.3 μg/ml during period II, and returned to 3.8±1.7 μg/ml at the end of period III. Portal venous 5-HT concentrations did not change from the mean basal value of 520±247 ng/ml.

DISCUSSION

5-HT, SP, motilin, gastrin, somatostatin, vasoactive intestinal polypeptide, and cholecystokinin have all been found within the lumen of the gastrointestinal tract either in the basal state or after vagal nerve stimulation (3, 4, 16, 17). We have previously demonstrated, in a similar feline system, that electrical vagal nerve stimulation causes up to 20-fold elevations in the quantity of 5-HT and SP measured in saline-infused segments of proximal jejunum (1–3). In this study the effects of exogenously introduced 5-HT and SP on local blood flow were studied in the proximal feline jejunum.

Previous work in our laboratory has demonstrated that intravenous infusions of 5-HT and SP, in doses that reproduce either postprandial or carcinoid levels, caused alterations in regional blood flow to the bowel. In these studies, intravenous 5-HT caused increased gastric blood flow, whereas SP increased not only gastric blood flow but also flow to the muscular layers of the stomach and entire small bowel (18, 19). Thus, both 5-HT and SP have been demonstrated to act as circulating mediators of gastrointestinal perfusion.

The current study provided evidence in support of the role of SP as a local mediator of gastrointestinal blood flow, possibly a paracrine effect. At the low dose used, exogenous endoluminal SP caused mucosal, but not muscularis, hyperemia in the experimental jejunal segment, and the increased mucosal blood flow reverted to basal levels after the return of intraluminal

TABLE I

Effect of Endoluminally Infused SP on Luminal and Portal Venous Concentrations of SP Measured by Radioimmunoassay

	Low dose endoluminal SP		High dose endoluminal SP	
	Perfusate SP	Portal venous SP	Perfusate SP	Portal venous SP
	pg/5 min	*pg/ml*	*pg/5 min*	*pg/ml*
Period I				
5′	3±2	—	224±162	—
10′	0	2.1±0.9	29±20	3.5±1.8
15′	0	—	97±89	—
20′	0	2.2±1.4	7±4	3.7±2.6
Period II				
25′	138±76	—	1,641±635	—
30′	1,134±506	2.3±1.0	25,113±11,681	3.6±1.9
35′	963±515	—	37,986±16,148	—
40′	691±527	2.4±1.0	32,922±14,796	2.8±1.8
Period III				
45′	52±39	—	3,988±2,731	—
50′	8±5	2.2±0.8	367±211	2.9±1.6
55′	5±4	—	223±126	—
60′	5±3	1.9±0.9	203±132	2.9±1.9

SP concentration to basal levels. Although the luminal levels of SP were higher than those noted after vagal nerve stimulation, it is difficult to quantitate "physiologic" luminal levels because the mucosal tissue levels and the site of action of SP are unknown. At the high dose, exogenous endoluminal SP caused increased blood flow to both the mucosal and muscularis fractions of the experimental jejunum, suggesting a dose-dependent extension of the local hyperemic effect across the bowel wall. Further support for a local paracrine effect was provided by the lack of blood flow changes in all control organs at both SP doses, and by the stability of portal venous SP levels, ruling out the possibility of absorption of the peptide.

The data from the exogenous endoluminal 5-HT studies do not support a locally controlled hyperemic effect of serotonin on blood flow because of the failure of the amine to influence the mucosal circulation. However, previous reports have noted that intraluminal 5-HT, in doses comparable to our highest dose, stimulated peristalsis when studied in an in vitro system (12). Thus, a possible explanation for the increased muscularis flow is that high concentrations of endoluminally placed 5-HT may have stimulated peristalsis either directly or by enteric neurons, and that this increase in peristalsis may secondarily have increased muscularis blood flow (20).

In conclusion, we have documented a dose-related effect of exogenous endoluminal SP on local gastrointestinal blood flow. Substance P may thus serve as a paracrine mediator of gastrointestinal blood flow.

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