Postnatal changes in gut hemodynamics: a possible role for substance P

PHILIP T. NOWICKI
Department of Pediatrics, The Ohio State University, Columbus 43210; and Wexner Institute for Pediatric Research, Childrens Hospital, Columbus, Ohio 43205

Nowicki, Philip T. Postnatal changes in gut hemodynamics: a possible role for substance P. Am. J. Physiol. 274 (Gastroint. Liver Physiol. 37): G1142–G1150, 1998.—Studies were conducted in young postnatal swine to determine if substance P (SP) participates in the regulation of postnatal intestinal hemodynamics and oxygenation. SP was present in homogenates of whole intestine from postnatal swine in an age-dependent manner as follows: 1 day old and never fed, 126 ± 35; 3 days old and fasted, 148 ± 30; and 14 days old, 51 ± 10 pg/mg protein (P < 0.01, 14- vs. 1- or 3-day-olds). Phenylephrine-precontracted rings of mesenteric artery from 3-day-old subjects mounted for tension recording within buffer-filled myographs demonstrated brisk relaxation in response to SP (EC50, 2 × 10^-10 M). This relaxation was eliminated by mechanical removal of the endothelium or blockade with the L-arginine analog Nω-monomethyl-L-arginine (L-NMMA) and was significantly attenuated by pretreatment with N-acetyl-L-Trp-3,5-bis(trifluoromethyl) benzyl ester (NATB), a highly selective NK-1 receptor antagonist (pA2 5 × 10^-10 M). Infusion of exogenous SP into the mesenteric artery of innervated in vivo gut loops reduced intestinal vascular resistance 35% and increased tissue oxygen uptake 40% in both 3- and 14-day-old subjects. By contrast, blockade of the NK-1 receptor for SP with NATB increased intestinal vascular resistance 19% in 3-day-old subjects but only 5% in 14-day-old subjects (P < 0.01). SP-induced changes in gut vascular resistance were significantly attenuated by prior confinement of NATB or L-NMMA, indicating that the peptide exerted this vascular effect via the NK-1 receptor, which is linked to endothelial cell nitric oxide synthase. Both NATB and L-NMMA attenuated flow-induced dilation within pump-perfused in vitro gut loops from 3-day-old subjects. SP appears to participate in the regulation of the newborn intestinal circulation, especially during the first days after birth.

newborn intestinal circulation; intestinal oxygenation; postnatal transition

At birth the intestine assumes responsibility for nutrient and water absorption and thus rapidly proceeds from a state of relative dormancy to one of intense activity, including a period of brisk postnatal growth (30). The intestinal circulation appears to participate in this transition, inasmuch as the basal blood flow rate is nearly twofold higher after birth than at any other time during postnatal life, consequent to an exceptionally low resting intestinal vascular resistance (5). The mechanisms responsible for this low vascular resistance have not been fully established. Extrinsic constrictor tone delivered by adrenergic efferents, although present at birth, is relatively weak, as evidenced by the limited dilation noted after denervation, and this circumstance most certainly participates in maintaining a low vascular resistance (3). However, resistance across denervated, reservoir-perfused in vitro gut loop from 3-day-old swine is significantly less than that across 35-day-old subjects, indicating that local vascular control mechanisms also contribute to this phenomenon (22). Blockade of nitric oxide (NO) synthesis with L-arginine analogs causes a significantly greater rise in gut vascular resistance in 3- than in 35-day-old swine (21), suggesting that NO participates in creating the age-dependent difference in postnatal gut vascular tone. Although the factors that modulate NO production within newborn gut have not been established, it is possible that vasoactive agents that exert their vascular effects via stimulation of endothelial cell nitric oxide synthase (ecNOS) are present in relatively great abundance in newborn intestine. An ideal candidate for this role is substance P (SP), an undecapeptide member of the tachykinin family. SP is a potent dilator whose effect is primarily mediated via NO production (7), and the peptide is present within the intestine, especially at birth (8). It thus seems reasonable to hypothesize that the low resting vascular resistance within newborn intestine is mediated in part by SP-induced activation of ecNOS.

Four experiments were carried out to test this hypothesis. First, the presence of SP was determined by RIA in intestine homogenates from 3- and 14-day-old swine. Second, the effects of exogenous SP on isometric tension were determined in precontracted, in vitro rings of mesenteric artery from 3-day-old intestine, before and after blockade of the SP NK-1 receptor or treatment with the L-arginine analog Nω-monomethyl-L-arginine (L-NMMA). Third, the effects of exogenous SP infusion on intestinal hemodynamics and oxygenation were determined under in vivo and in vitro conditions in 3- and 14-day-old swine. Fourth, the effects of blockade of the SP NK-1 receptor on gut hemodynamics and oxygenation were determined in 3- and 14-day-old swine and were compared with the effects of NO synthesis blockade.

METHODS

Animal Acquisition and Handling

Studies were conducted on newborn swine, 3 or 14 days old, obtained from a local swine breeding farm on the day before use. All subjects were fasted for 8 h before use. In all instances anesthesia was induced with tiletamine HCl-zolaxepam HCl (6 mg/kg im) and xylazine (4 mg/kg im) and maintained with pentobarbital sodium (5 mg/kg iv given every 60 min or sooner if deemed necessary by vivarium staff). Animals were killed by an overdose of pentobarbital sodium while still anesthetized. All animal care was provided in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 85–23) under the auspices of an experimental protocol approved by the Childrens Hospital...
Research Foundation Institutional Animal Care and Use Committee (protocol 01595AR). All work involving live animals, including surgery and in vivo protocols, was carried out within the Wexner Institute vivarium, an American Association of Accreditation of Laboratory Animal Care approved facility under the supervision of a veterinarian.

Measurement of SP Content in Postnatal Intestine

The small intestine was removed from the ligament of Trietz (i.e., at the end of the duodenum where the intestine exits from the retroperitoneal space) to the ileocecal valve in a single piece. The lumen was cleansed gently but thoroughly by instillation of iced 0.9% saline, followed by gravity drainage and instillation of air. The entire intestine was weighed and a ~10-cm portion of the distal jejunum-proximal ileum was removed; this section corresponded anatomically to that portion of the small intestine used for the creation of the in vivo and in vitro perfused gut loops. The small segment was opened longitudinally, and the mucosal surface was rinsed with iced PBS and gently blotted dry. This step was repeated until the blotting paper no longer demonstrated evidence of chyme. The segment was scraped firmly with a no. 11 scalpel blade so that all layers of the intestine, except the serosal covering, were removed. adequacy of removal was assessed in pilot work by histological examination of the remaining serosa. The tissue was transferred to a preweighed plastic tube, weighed, and mixed with an equal volume (wt/vol) of extraction solution. The extraction solution contained ethanol, deionized water, glacial acetic acid, and trisylol (750:225:15:10). The tissue was homogenized in extraction solution with a pestle at 0°C (3 × 10 s at full speed on ice and then sonicated (2 × 10 s on ice). The resulting homogenate was centrifuged (10,000 × g for 15 min), and the supernatant was removed and dried. The resulting material was resuspended in RIA buffer (0.5 M sodium phosphate with 0.1% BSA, fraction V, pH 7.50), and the RIA was carried out. Protein content was determined on an aliquot of the homogenate by means of the bicinchoninic acid method, using BSA as a standard (28).

Studies of Isometric Tension Within In Vitro Mesenteric Artery Rings

Ring preparation and apparatus. These studies were carried out in mesenteric artery rings harvested from 3-day-old swine. The mesenteric artery trunk was removed en bloc and placed in iced Krebs buffer on a dissecting tray. Rings (3 mm) were cut with care taken to avoid contact with the intimal surface of the vessel, except in rings processed to be endothelial-negative; in these rings the endothelium was removed by gentle swabbing of the intimal surface with a cotton applicator. Rings were mounted between two stainless steel stirrups placed within a water-jacketed 20-ml glass myograph. On stirrup was tethered to a force transducer (Grass Instruments FT-03, Quincy, MA) to facilitate continuous measurement of isometric tension on a multichannel recording device (Grass Instruments model 7 polygraph). The well was filled with Krebs buffer of the following composition (in mM): 118.1 NaCl, 4.8 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25.0 NaHCO3, 11.1 glucose, and 0.026 EDTA. The buffer was maintained at 38°C and continuously aerated with 95% O2-5% CO2. Rings were progressively stretched over 1–2 h to the optimal point on their length-tension curve, as determined by noting the maximal contractile response to 80 mM KCl Krebs buffer. Endothelial integrity was determined in all rings by preconstriction with phenylephrine (10−7 M), followed by >50% dilution in response to acetylcholine (10−7 M)...

Experimental protocol. Two protocols were carried out. In the first protocol, the effective inhibitory concentration of N-acyl-L-Trp-3,5-bis-(trifluoromethyl) benzyl ester (NATB; Peninsula, Belmont, CA) in newborn swine mesenteric artery was determined. NATB is a potent and highly selective antagonist to the NK-1 receptor with an effective inhibitory concentration in the nanomolar range (18). NATB was solubilized in dimethylformamide and subsequently diluted in 0.9% saline on the day of use. The pA2 of the antagonist, i.e., the negative logarithm of the antagonist concentration that reduces the agonist effect noted in an uninhibited system by 50%, was determined by administering different concentrations of NATB (10−11–10−7 M) to distinct pairs of mesenteric artery rings, followed by administration of SP (10−11–10−7 M) to all rings. The effective blocking concentration of NATB was considered to be a full log increment above the pA2 (20) and was used in all subsequent blockade studies. In the second protocol, the effects of NATB (5 × 10−9 M) and l-NMMA (10−4 M) on relaxation of phenylephrine-precontracted rings were compared.

Studies of Effects of SP and NK-1 Receptor Blockade Within Autoperfused In Vivo Gut Loops

Experimental preparation and measurement techniques. These studies were carried out in 3- and 14-day-old subjects fasted for 8 h before use. Subjects were anesthetized and ventilated to maintain normal blood gas tensions. A femoral artery-vein pair was cannulated; the arterial cannulas were directed to a standard pressure transducer to measure systemic arterial pressure, whereas the venous cannula served as a delivery site for crystalloid (5% dextrose in 0.9% saline, 15 ml·kg−1·h−1) and also blood return. A segment of distal jejunum-proximal ileum ~25 cm long was vasaarially isolated from the remainder of the gut so that it was perfused and drained by a single artery-vein pair. The animal was administered heparin, 500 µ/kg, and then the vein was cannulated and the catheter was directed to a beaker primed with 50 ml of heparinized newborn swine blood obtained from a littermate. A flowmeter (Gould, 2.0 mm ID; Cleveland, OH) was placed in the venous circuit to facilitate flow measurement. Blood collected in the beaker was pumped back into the subject at a rate equal to venous outflow, and the venous pressure was kept at 0 mmHg by adjusting the venous catheter with respect to the animal. The artery and peritendial mesenteric nerves directed to the gut loop were not disturbed, except that a narrow (25 gauge) steel needle was inserted 3 mm into the mesenteric artery, perfusing the isolated gut loop, and cemented in place. This portal allowed close intra-arterial infusion of vasoactive agents but did not itself disrupt gut loop hemodynamics. The intestine was covered with plastic film to minimize evaporative heat and water loss, while servocontrolled heating elements maintained the preparation at 38°C. The total oxygen content was determined in paired arterial-mesenteric vein blood samples (Lex-O2-Con, Chestnut Hill, MA), and tissue oxygenation was calculated by means of the Fick equation. To assure that blood oxygen contents had reached steady-state levels before sampling, blood PO2 was sampled twice, at 1-min intervals, before the blood samples for oxygen content determination were obtained. This approach was used in all experimental protocols, including those utilizing the in vitro gut loop preparation that will described.

Experimental protocols. Studies were begun >30 min after surgical preparation was complete, when blood flow and systemic arterial pressure remained ±5% of baseline for >10 min. Care was taken to assure adequate anesthesia before the protocol was begun, and no subject received anesthetic...
during data collection. Each subject was administered either SP (10^-9 M/kg) or NATB (5 x 10^-8 M/kg) into the mesenteric artery. It was determined in pilot work that 5% dimethylformamide in 0.9% saline did not affect the measured variables, and so a formal control group was not studied. Drugs were given as a bolus injection over 2–3 s in a total volume of 1 ml. The corresponding flow rate in the artery at the time of infusion was always >10 ml/min, so that the volume of the injectate had little impact on hemodynamics at the point of infusion. No effort was made to prevent recirculation of administered drugs. Vascular pressure and gut flow rate were monitored continuously for 30 min after infusion, whereas paired arteriovenous blood samples were obtained before and 6 and 30 min after drug infusion. These times were selected to correspond to the peak and steady-state changes in blood flow caused by the infused drugs.

### Hemodynamic Studies Within Denervated, Reservoir-Perfused In Vitro Gut Loops

Experimental preparation. These studies were carried out on 3-day-old swine. Subjects were anesthetized, heparinized, and ventilated as previously described. A segment of proximal ileum-distal jejunum ~25 cm in length was isolated from the remainder of the gut, the single artery-vena pair serving the segment was cannulated, extracorporeal perfusion of the segment was initiated, and the segment was removed from the experimental subject. The gut lumen was cleansed by repetitive gentle infusion of warm saline followed by air. A temperature probe was placed within the gut lumen and connected to servocontrolled heating elements set to keep the tissue at 38°C. The gut loop was weighed before final placement into the heated humidified perfusion chamber so that flow rates could be calculated as a function of tissue weight during the course of the protocols. Arterial perfusion of the intestinal segment was achieved by means of an extracorporeal perfusion apparatus. Blood was obtained from anesthetized, heparinized (500 µ/kg), ventilated swine 90–100 days old on the day of use. The blood was filtered twice (40 µm), re-heparinized (100 µg/ml), and placed into a collection flask. Blood within the collection flask was continuously recirculated (200 ml/min) through a membrane oxygenator (0.6 m²) gassed with 95% air-5% CO₂ (Table 1). A small volume of blood was continuously pumped from the collection flask to a sealed arterial reservoir, where it was warmed (38°C by water bath) and stirred continuously (stir plate) before delivery to the gut loop. Delivery of blood from the arterial reservoir to the gut loop could be achieved in two ways. For controlled pressure perfusion, the reservoir was pressurized with 95% air-5% CO₂ with an air-pressure regulator (Bellofram type 10R, Burlington, MA). This arrangement allowed direct manipulation of arterial pressure as an independent variable (±1 mmHg) and provided pulseless, free-flow perfusion. For controlled flow perfusion, the arterial limb of the apparatus was drawn through a peristaltic pump (Harvard Instruments, Quincy, MA). This arrangement allowed direct manipulation of flow rate as an independent variable (±1 ml/min) and delivered a pulse pressure of 12 ± 1 mmHg. Venous pressure was kept at 0 mmHg by adjusting the height of the venous outflow cannula. Recirculation of blood and drugs administered during the course of the protocols did not occur because the apparatus was run in a single-pass mode.

Measurement techniques. Capillary pressure (Pc) was estimated by the venous occlusion method (10), whereas the capillary filtration coefficient (Kc) was measured gravimetrically by means of the flow-equilibration method of Granger et al. (11); both methods have been previously adapted for use in newborn swine intestine (22). Briefly, the intestinal segment was covered in saline-soaked gauze and plastic film and placed on a wire-mesh pan suspended from a force transducer. When the tissue was isogravimetric (i.e., ± 0.1 g over 5 min) venous pressure was rapidly increased from 0 to 10 mmHg. This perturbation caused a biphasic change in intestinal weight; a rapid phase caused by blood sequestration within the gut loop, followed by a slow phase caused by increased filtration from the vascular to the interstitial spaces. The weight gain noted during the second phase was used to determine Kc. The inflection point between rapid and slow phases of tissue weight gain was determined by noting the development of flow equilibration after venous pressure elevation. Kc was calculated as [(tissue wt₁–tissue wt₂) × 100/Pc]. Blood flow, arterial and venous pressures, and blood oxygen contents were measured as previously described.

Experimental protocols. In vitro gut loops were used in one of three protocols. In the first protocol the effects of a sustained infusion of NATB (5 x 10^-9 M/min) or L-NMMA (10^-4 M/min) before and during sustained infusion of SP (10^-9 M/min) were determined. NATB or L-NMMA was infused for 10 min; thereafter, a confusion of SP was begun and continued for 15 additional minutes. Control loops received an infusion of 0.9% saline for the first 10 min of the protocol, in lieu of blocking agents, whereas still other loops received blocking agents for the entire 25-min period and a sham SP infusion (0.9% saline at 0.2 ml/min) beginning 10 min into the protocol. All drug or sham infusions were delivered at 0.2 ml/min. During this protocol arterial inflow pressure and venous outflow pressure were maintained at 65 and 0 mmHg by continuous adjustment of the arterial reservoir pressure and outflow cannula pressure, respectively. Measurements of Pc, Kc, and arteriovenous O₂ content difference [(a-v)O₂] were made at time 0 and again at 10, 16, and 25 min into the protocol. These times were chosen to correspond to steady-state hemodynamics during blockade infusion and to peak and steady-state hemodynamic changes during subsequent SP infusion. In the second protocol, the effect of continuous SP infusion on the relationship between flow rate and oxygen uptake was determined. Gut loops were perfused under controlled flow conditions, and the pump rate varied from 40 to 120 ml·min⁻¹·100 g⁻¹ in 20 ml/min increments. Each new flow rate was maintained for 3–4 min, during which time arterial pressure, the dependent variable in a controlled flow preparation, reached steady state. Blood samples for oxygen content were drawn just before the next change in flow rate, after repetitive PₐO₂ determinations indicated stability of blood oxygen pressure. Thereafter, an infusion of SP was begun to attain an arterial concentration

### Table 1. Characteristics of blood used for arterial perfusion of in vitro gut loops

<table>
<thead>
<tr>
<th>Hct, %</th>
<th>P₀₂Ar, mmHg</th>
<th>P₀₂O₂, mmHg</th>
<th>O₂ Content, ml O₂/dl</th>
<th>EC₅₀, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days old Reservoir</td>
<td>27 ± 3</td>
<td>96 ± 7</td>
<td>34 ± 2</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>Reservoir</td>
<td>29 ± 4</td>
<td>107 ± 7</td>
<td>35 ± 6</td>
<td>36 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10, 3-day-old and 16 reservoir (i.e., no. of individual donor subjects used). Swine do not express strong red blood cell antigens or a fetal hemoglobin variant (27) but do manifest of individual donor subjects used. Swine do not express strong red blood cell antigens or a fetal hemoglobin variant (27) but do manifest of individual donor subjects used.
of 5 × 10⁻¹⁰ M/l of the peptide. The pump rate was varied, and blood samples were taken as before; in addition, the rate of SP infusion was adjusted after each change in blood flow rate to maintain a steady concentration of peptide within the arterial perfusate. In the third protocol the effects of NATB and L-NMMA on the phenomenon of flow-induced dilation was assessed. Gut loops were perfused under controlled flow conditions at a baseline rate of 100 ml·min⁻¹·100 g⁻¹. The flow rate was then increased 75% by rapidly raising the pump speed. This maneuver was carried out under control conditions and again during continuous infusion of NATB (5 × 10⁻⁹ M/min) or L-NMMA (10⁻⁴ M/min).

Statistical Methods

The protein content of the 10-cm intestinal segment was expressed as a function of the total wet weight of the segment, whereas SP content was expressed as a function of protein content. Ring tension data were expressed as the percent relaxation from phenylephrine-precontraction baseline tension. All ring observations were paired, i.e., the same perturbation was applied to two rings and the mean response was taken. For all data presented herein, n refers to the number of subjects in which an observation was made. All data are expressed as means ± SE. Individual data sets were analyzed by means of an ANOVA technique. In all analyses it was first determined if the F statistic for the combined main effects was significant at the P < 0.05 level, and then post hoc tests (Tukey) were run to determine the sites of significance.

RESULTS

Substantial differences in gut weight and protein and SP content were noted between 3- and 14-day-old subjects (Table 2). The percentage of body mass accounted for by the intestine increased from 7% on day 3 to 19% on day 14 (P < 0.01), clear evidence of the dramatic postnatal growth of the gut.

SP proved to be a potent, NO-dependent relaxing agent in phenylephrine-precontracted mesenteric artery rings. The SP concentration that caused a half-maximal effect (EC₅₀) was 2 × 10⁻¹⁰ M (Fig. 1). The relaxant effect of SP was contingent on the presence of an intact endothelium, whereas the effect of SP was virtually eliminated by pretreatment with L-NMMA (10⁻⁴ M). NATB, a highly selective antagonist to the NK-1 receptor, exhibited a pA₂ of 5 (Fig. 1). The maximal effect (EC₅₀) was 2 × 10⁻⁹ M/kg. Both groups demonstrated a brisk, albeit brief hyperemia in response to the peptide, which was accompanied by a modest drop in systemic arterial pressure in 3-day-old subjects. When expressed as a function of baseline, the extent of the SP-induced hyperemia was similar in both age groups. Intestinal oxygen uptake also increased after SP infusion in both groups. By contrast, blockade of NK-1 receptors with NK-1 receptors with NATB (5 × 10⁻⁹ M/kg) had an age-dependent effect on intestinal hemodynamics (Fig. 3). In vivo gut loops prepared in 3-day-old subjects demonstrated a 19% rise in vascular resistance shortly after NATB infusion, whereas 14-day-old subjects exhibited a modest 5% increase. In both groups the effect was transient. Blockade of NK-1 receptors did not affect gut oxygenation in either group.

Sustained infusions of NATB (5 × 10⁻⁹ M/min) or L-NMMA (10⁻⁴ M/min), followed after 10 min by infusion of SP (10⁻⁹ M/kg), were carried out in gut loops prepared from 3-day-old subjects perfused under controlled pressure, free-flow conditions. Both NATB and L-NMMA significantly increased gut vascular resis-

Table 2. Weight, protein, and SP content of 3- and 14-day-old intestines

<table>
<thead>
<tr>
<th></th>
<th>3 Days</th>
<th>14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, kg</td>
<td>2.63 ± 0.17</td>
<td>3.70 ± 0.37*</td>
</tr>
<tr>
<td>Gut wt, g</td>
<td>60 ± 3</td>
<td>691 ± 36*</td>
</tr>
<tr>
<td>Protein content, mg/g gut</td>
<td>0.17 ± 0.01</td>
<td>0.13 ± 0.02*</td>
</tr>
<tr>
<td>SP content, pg/mg protein</td>
<td>148 ± 30</td>
<td>51 ± 10*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 4 subjects in each age group. Protein content is wet weight after thorough cleansing and blotting. SP, substance P. *P < 0.01, 3- vs. 14-day-old intestines.
tance and reduced perfusion in a sustained manner; tachyphylaxis was not noted up to 25 min after the initiation of blockade (Fig. 4). Infusion of SP dilated the intestine and caused a brisk hyperemia; these effects were blocked by pretreatment with NATB or L-NMMA.

Kf,c and (a-v)O2 increased during L-NMMA infusion but did not change in response to NATB (Fig. 5). Kf,c and oxygen uptake, but not (a-v)O2, increased during SP infusion. These effects were blocked by NATB but not by L-NMMA.

The relationship between blood flow and oxygen uptake in the intestine of 3-day-old subjects is shown in Fig. 6. Under control conditions, oxygen uptake was independent of flow at rates >80 ml min⁻¹ 100 g⁻¹ but declined when flow was reduced below this level. Continuous infusion of SP (10⁻⁹ M/min, adjusted with the...
flow rate to keep the arterial concentration of the peptide steady) shifted the flow-uptake curve upward, especially at higher flow rates. Vascular resistance across 3-day-old in vitro gut loops perfused under controlled flow conditions decreased 14% in response to a mechanical increase in flow rate of 75%, created by increased pump speed (Fig. 7). This reduction was completely eliminated by L-NMMA (10^{-4} M/min). Infusion of NATB (5 \times 10^{-9} M/min) reduced this effect but not as effectively as L-NMMA.

**DISCUSSION**

Several observations support our experimental hypothesis and suggest that SP plays an important role in regulation of intestinal hemodynamics and oxygenation in very young swine. First, the peptide was present in abundance within 3-day-old intestine, but the intestinal peptide content decreased by the 14th postnatal day; this pattern inversely parallels the change in intestinal vascular resistance that occurred at the same time. Second, infusion of exogenous peptide at...
Infusion had to occur because the capillary surface area ery; thus the increase in oxygen uptake during SP vasodilatory effect of SP could not affect oxygen delivery brought about by SP, but also reflected an upward shift in the relationship between blood flow and oxygen delivery. These data were collected under controlled flow conditions, so that the vasodilatory effect of SP could not affect oxygen delivery; thus the increase in oxygen uptake during SP infusion had to occur because the capillary surface area for oxygen diffusion increased or because cellular $P_{O_2}$ fell, increasing the capillary cell $P_{O_2}$ gradient.

Agents that simply vasodilate the intestine, such as isoproterenol (16), do not alter the flow-uptake curve; by contrast, agents that increase oxidative metabolism create the shift noted during SP infusion (16, 17). The dissociation between flow rate and the effect of SP on tissue oxygen uptake is also evidenced by the failure of L-NMMA to compromise gut oxygen uptake or attenuate the SP-induced rise in oxygen uptake under in vitro controlled pressure, free-flow conditions. Thus intestinal oxygen uptake increased in response to SP despite elimination of its vasodilator effect at a site downstream from the NK-1 receptor.

Control of intestinal oxygen transport occurs at two distinct sites: resistance vessels and precapillary sphincters (12). Resistance vessels regulate the rate of blood flow into the microvasculature, a factor that dictates oxygen delivery and hence capillary $P_{O_2}$, whereas precapillary sphincters govern perfusion of individual capillaries and so regulate the surface area available for oxygen diffusion and the diffusion distance (from capillary to cell). Although many factors affect both sites in a qualitatively similar fashion (i.e., increasing both flow and perfused capillary density), independent regulation also occurs (9, 12). Both elements appear responsive to the putative metabolic feedback signal proposed in the metabolic model of local flow regulation; additionally, several agents exert direct control of these elements, especially resistance vessels (9). SP clearly exerted a direct, dilatory effect on newborn physiologically relevant concentrations significantly vasodilated postnatal intestine, under both in vivo and in vitro conditions, suggesting the presence of NK-1 receptors within this vasculature. Third, blockade of NK-1 receptors with a highly selective antagonist increased gut vascular resistance under in vivo conditions in 3- but not in 14-day-old subjects, indicating that endogenous SP participates in setting basal vascular tone in an age-dependent manner. Finally, NK-1 blockade significantly attenuated flow-induced dilation in vitro in intestines from 3-day-old subjects. Flow-induced dilation is an NO-mediated decrease in vascular tone that occurs in response to the mechanostimulus of increased flow or wall shear stress (15). This phenomenon occurs in newborn intestine and may serve to maximize vascular conductance and thus optimize gut oxygen delivery (23). Ralevic and colleagues (27) reported release of SP during flow-induced dilation in a rat hindquarter preparation and speculated that endothelium-derived peptide might participate in this response, inasmuch as it also occurred in rats pretreated with capsaicin to destroy SP-containing sensory afferent nerves. Observations presented herein are consistent with Ralevic's hypothesis.

Infusion of exogenous SP caused a marked increase in intestinal tissue oxygen uptake. This effect was not just the consequence of increased flow, and thus oxygen delivery brought about by SP, but also reflected an increased rate of oxidative metabolism in response to the peptide. The most convincing argument to this effect is presented by Fig. 6, which demonstrates an upward shift in the relationship between blood flow and oxygen uptake during SP infusion. These data were collected under controlled flow conditions, so that the vasodilatory effect of SP could not affect oxygen delivery; thus the increase in oxygen uptake during SP infusion had to occur because the capillary surface area

![Graph](https://example.com/graph.png)
intestinal resistance vessels, an effect that was mediated by NK-1 receptors. Thus SP-induced intestinal dilation was present in the absence of gut parenchyma, occurred at physiologically relevant peptide concentrations, was attenuated by NK-1 receptor blockade, and was virtually eliminated by disruption of NO production. The NK-1 receptor is a G protein-linked seven-transmembrane-spanning receptor that activates the phospholipase C-inositol triphosphate-diacylglycerol signal transduction pathway (14, 25). Activation of this pathway increases endothelial intracellular Ca"³⁺ concentration, which in turn stimulates eNOS activity (19).

In contrast to resistance vessels, the effects of SP on regulation of precapillary sphincters are less clear. Two means used to assess regulation of capillary perfusion are measurement of K"¹", and assessment of (a-v)O₂, although the latter variable must be viewed cautiously. Inasmuch as a rise in (a-v)O₂ can occur passively, in response to a reduction in cellular Po₂ (9, 12). Blockade of NO production with L-NMMA did not inhibit intrinsic regulation of precapillary sphincters. Both K"¹", and (a-v)O₂ increased during L-NMMA infusion, most likely in response to the reduction in blood flow that occurred at that time; such a response would serve to counterbalance the loss of oxygen delivery and to maintain tissue oxygenation (12). These variables increased further during coinfusion of SP, to an extent indistinguishable from controls. These observations clearly indicate that control of capillary sphincter tone in newborn intestine is not an NO-dependent process. K"¹", and (a-v)O₂ did not change during NATB infusion, even though NK-1 receptor blockade compromised gut perfusion in a manner similar to L-NMMA; furthermore, the SP-induced rise in K"¹", (a-v)O₂, and oxygen uptake was blocked by coinfusion of NATB. One interpretation of these observations is that precapillary sphincters are responsive to SP. However, this explanation runs contrary to the established mechanism by which SP exerts its vascular effect, i.e., via NO production, inasmuch as the L-NMMA data do not support a role for NO in exchange vessel regulation. An alternative and more feasible explanation is that the effect of SP on precapillary sphincter tone is indirect. Stated otherwise, the rise in K"¹", and (a-v)O₂ noted during SP infusion are likely mediated by the putative metabolic feedback signal produced by the gut parenchyma in response to the peptide-induced increase in tissue metabolic rate (12).

An important question not addressed by these experiments is the site of origin of vasculature activity in newborn intestine. SP is a neurotransmitter within the intestine, both in the intrinsic gut nervous system (13, 31) and in extrinsic sensory afferent nerves (1, 24). Endothelial production of SP within the gut has also been observed (27) and its release into the vascular space confirmed (26). Localizing the site within 1- to 3-day-old intestine, from which vasoactive SP is derived, would seem a logical next step in this investigation. Additionally, investigation of other vasoactive peptides, such as calcitonin-gene related peptide, might provide fruitful avenues of research. At present it is only possible to conclude that SP is abundantly present within newborn intestine and that it appears to participate in regulation of the gut circulation during early postnatal life.

Charles Miller and David Dunaway provided excellent technical assistance in the completion of this work. This work was supported by the National Institute of Child Health and Human Development Grants HD-25256 and HD-31902. Address for reprint requests: P. Nowicki, Childrens Hospital, 700 Childrens Dr., Columbus, OH 43205.

Received 11 December 1997; accepted in final form 12 March 1998.

REFERENCES


