Shear stress-induced nitric oxide antagonizes adenosine effects on intestinal metabolism

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Han, Chao, Zhi Ming, and W. Wayne Lautt. Shear stress-induced nitric oxide antagonizes adenosine effects on intestinal metabolism. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1227–G1234, 1999.—The influence of nitric oxide (NO) on adenosine-induced metabolic effects was studied in the intestine. Blood flow supplied an in situ-isolated segment of small intestine in anesthetized cats via the superior mesenteric artery (SMA) and was controlled by a vascular circuit. The SMA and portal samples were taken for analysis of oxygen and lactate. Adenosine (0.4 mg·kg⁻¹·min⁻¹, intra-SMA) reduced oxygen consumption by 25.1 ± 2.9 from 73.1 ± 10.8 µmol·min⁻¹·100 g⁻¹ and increased lactate production by 13.3 ± 3.0 from 12.8 ± 4.6 µmol·min⁻¹·100 g tissue⁻¹ during constant-flow (CF, decreased shear stress) but not during constant-pressure (CP, increased shear stress) perfusion. Blockade of NO synthase using N·nitro-L-arginine methyl ester did not affect the metabolic effects of adenosine during CF but eliminated the differences seen between CP and CF perfusion. A NO donor, 3-morpholinosydnonimine, attenuated the metabolic effects of adenosine during CF perfusion. The results suggested that shear-induced NO antagonized metabolic effects of adenosine but that the inhibition of vascular effects by NO was not shear dependent since it occurred in both CP and CF perfusion.

N·nitro-L-arginine methyl ester; 3-morpholinosydnonimine; oxygen consumption; lactate production; superior mesenteric artery

ADENOSINE (Ado) and nitric oxide (NO) are endogenous substances playing important roles in the control of splanchnic blood flow (13, 18). Ado dilates resistance vessels (3) to increase blood flow and oxygen supply to local tissue. NO released from endothelial cells by shear stress results in vascular smooth muscle relaxation through a cGMP-dependent mechanism (14). It has been shown in our laboratory that NO release in the intestine is shear stress dependent (13). An interesting aspect of that study was that shear-dependent NO suppressed the constrictor response to nerve stimulation but did not act on norepinephrine-induced constriction, thus leading to the conclusion that the shear-dependent release of NO caused by these constrictors did not result in a direct effect on vascular smooth muscle but rather acted by decreasing transmitter release.

The metabolic effects of Ado include the suppression of oxidative metabolism leading to a reduction of oxygen consumption (6), as well as the promotion of anaerobic glycolysis (10, 26). These effects of Ado further balance the ratio of oxygen demand and supply. The hemodynamic and metabolic effects of Ado have been interpreted to be protective in different pathological situations (4). NO has been demonstrated in vitro to inhibit aerobic metabolism through inhibition of mitochondrial enzymes (5, 21), but the function of NO on metabolism is still unclear in vivo.

Ado and NO may interact in many physiological and pathological conditions. Ado is released during hypoxic and ischemic conditions. NO synthase activation and increased NO production have been reported under similar conditions (24). NO, as well as Ado, has been suggested to be involved in ischemia-reperfusion (17) and hypoxia-induced vasodilation (16). The potential use of NO and Ado in a therapeutic approach to reduce surgical ischemic-reperfusion injury has been discussed (22).

We found that NO antagonizes the vasodilator effects of Ado (13) and attenuates pressure-flow autoregulation (11) that is known to be mediated by Ado in the superior mesenteric vascular bed. However, the interaction of Ado and NO in control of intestinal metabolism is unknown. On the basis of our early observations that NO antagonizes the dilator effect of Ado (12), we hypothesize that NO also antagonizes Ado effects in the control of intestinal metabolism. In the present study, we tested the hypothesis in an intestinal segment in cats. The effects of Ado on local metabolism and the possible role of NO were investigated. The role of NO was explored by stimulating endogenous NO formation with elevated shear stress, by decreasing endogenous NO production by blocking NO synthase, or by administration of a NO donor. Our results demonstrated that the Ado-induced decrease in oxygen consumption and increase in lactate production in the small intestine were antagonized by either shear stress-released or exogenously administered NO.

METHODS

Surgical Preparation

Cats of either gender, weighing 3.9 ± 0.2 kg, were used in this study. The protocol was approved by the Animal Use Ethics Committee at the University of Manitoba. The animals were fasted for 18 h and anesthetized using pentobarbital sodium (32.5 mg/kg ip). Anesthesia was maintained using a drip-bag infusion (0.78 mg/ml of pentobarbital sodium in saline) via a brachial venous cannulation. Supplemental anesthetics (6.5 mg) and fluid (dextran 40, 10% intravenous solution mixed with equal volume of saline) were also given when required. Body temperature was monitored and maintained at 37.0 ± 0.5°C using a thermal control unit (model 72, Yellow Springs Instruments, Yellow Springs, OH) operating a
heated surgical table. A respirator (Harvard Apparatus, Millis, MA) was used to control ventilation via a tracheal cannulation. The left carotid artery and right femoral vein were cannulated for monitoring systemic arterial pressure and central venous pressure, respectively. Laparotomy was performed through a midline incision. The spleen was removed. The large intestine including ascending, transverse, and part of the descending colon was removed after ligation of the inferior mesenteric artery and vein. The upper end of the intestinal segment was defined by double ligation and transection at the duodenum at a location that allowed pancreatic venous drainage. The superior mesenteric nerve plexus was ligated and cut. The lower abdominal aorta was double cannulated below the renal branches, providing a shunt through a T connector to the superior mesenteric artery (SMA) through a pump-controlled (Masterflex, Cole Parmer Instrument, Barrington, IL) vascular circuit. The SMA was cannulated, and the circuit flow rate was gradually increased to generate a circuit pressure that was slightly higher than systemic pressure to countervail circuit resistance. A venous catheter unit was placed into the portal vein for monitoring portal venous pressure and for blood sampling. The flow rate was measured using a flow-through electromagnetic flow probe (EP608, Carolina Medical Electronics, King, NC). Calibration of the flow probe was carried out at the end of each experiment with the SMA pressure corrected for resistance in the circuit (12). All pressures were monitored by pressure transducers (Gould and Statham, Gould, Oxnard, CA) that had been set to zero at the level of the central vena cava. The pressures and flow rates were recorded on a dynograph recorder (R611, Sensor Medics, Anaheim, CA). The animals were heparinized (200 IU/kg) before the vascular circuit was established. After the surgery, the animals were allowed to stabilize for at least 45 min. At the end of each experiment, the defined intestinal segment was removed from the carcase, and a wet tissue weight was obtained after intestinal contents were cleaned out.

Protocol 1

To study the effect on intestinal metabolism, Ado at a dose providing maximum vascular response (0.4 mg·kg⁻¹·min⁻¹) (12) was infused into the SMA via an infusion line in the circuit during constant-flow perfusion. The SMA flow rate was maintained while circuit pressure dropped because of the vasodilator effect of Ado during the constant-flow perfusion. Blood samples from the arterial circuit sampling line and a portal venous catheter were taken simultaneously, 5 min before the infusion as control and after 5 min of Ado infusion. The infusion of Ado were then repeated after NO synthase blockade by N-nitro-l-arginine methyl ester (L-NAME) given as an intravenous infusion over 10 min (2.5 mg/kg in 10 ml). The first control sample after L-NAME was taken 10 min after completion of administration.

Protocol 2

To assess the role of NO, constant-pressure perfusion was also maintained to elevate the shear stress during Ado infusion. During a constant-pressure perfusion, the blood flow rate was adjusted using the pump to maintain a circuit pressure as close to the control as possible. To compensate for the vasodilator effect of Ado, the blood flow was increased. Blood samples were taken, and Ado infusion, under constant-pressure perfusion, was repeated after NO synthase blockade as described in protocol 1. Protocols 1 and 2 were performed in the same animal. A 10-min interval was allowed between each test. The order of constant-pressure and constant-flow infusion was randomly assigned.

Protocol 3

The effects of exogenous NO were investigated using a NO donor, 3-morpholinosydnonimine (SIN-1), in another group of animals. The effects of Ado infusion under constant-flow perfusion were compared in the absence and presence of SIN-1. Two doses of SIN-1 were given as cumulative bolus injections (0.1 and 0.2 mg/kg iv injected over 1 min). Ado infusion was tested 5 min after each dose of SIN-1.

Protocol 4

To assess the effect of hemodynamic changes on intestinal metabolism, the effects of another vasodilator, isoproterenol (Iso), on intestinal hemodynamics and metabolism were studied in the same experimental design as Ado during constant-flow perfusion. The dose of Iso (0.2 μg·kg⁻¹·min⁻¹) was selected to generate hemodynamic effects similar to those that Ado infusion did. Blood samples from arterial and portal venous sites were taken at 5 min of the infusion.

Sampling and Analysis

The hemodynamics in the SMA during Ado infusion were normally stabilized within 2 min. In a previous study (6), Ado effects on oxygen consumption in cat ileum were found to stabilize in ~2 min. The blood samples were taken at 5 min during Ado infusion. An aliquot of 0.5 ml of blood for each sample was withdrawn into a bubble-free syringe for the assays. Oxygen contents in arterial and venous blood were measured using a Lex-O2-Con-K oxygen content analyzer (Lexington Instruments, Waltham, MA). Blood lactate and glucose concentrations were analyzed (Sports Industrial Analyzer, Yellow Springs International).

Drugs

Ado, L-NAME, and Iso were purchased from Sigma. SIN-1 was purchased from Alexis (San Diego, CA). Ado, L-NAME, and SIN-1 solutions were made fresh in saline before each experiment. Iso was diluted from stock solution (1 mg/ml with 0.2 mg ascorbic acid as preservative) before each experiment. The concentrations of Ado and other drugs were calculated according to the body weight.

Data Calculation and Analysis

Oxygen consumption and lactate production were calculated by arterial-venous concentration difference and blood flow, normalized for the tissue weight and expressed as micromoles per minute per 100 g of tissue. The SMA flow was normalized for the tissue weight and expressed as milliliters per minute per 100 g of tissue. The data were expressed as means ± SE. Student’s t-test or analysis of variance followed by Tukey’s test was used for detection of any difference. Paired t-test was used if applicable. P < 0.05 was used as a criterion for significance to reject the null hypothesis. A computer program, GraphPad Prism, was used to compute the statistics.

RESULTS

Basal Condition of the Animals

Two groups of animals (n = 7 and n = 6) were used in this study. The basal hemodynamics and metabolism of these animals were stabilized within 1 h after the surgery. When stabilized, the systemic and SMA pressures were 116.4 ± 4.4 and 116.6 ± 5.8 mmHg, respectively, whereas the SMA flow was 38.6 ± 4.8
ml·min⁻¹·100 g tissue⁻¹. The basal glucose and lactate levels were 101.1 ± 6.1 mg/dl (5.61 ± 0.34 mmol/l) and 1.95 ± 0.18 mmol/l, whereas the basal oxygen consumption and lactate production across the intestine were 69.2 ± 6.2 and 7.22 ± 3.36 µmol·min⁻¹·100 g tissue⁻¹. The hematocrits at the start of the experiment were 31.4 ± 1.0% and dropped slightly through the experiment (28.1 ± 1.2% at the end, P < 0.05) because of the blood sampling.

Effects of Ado Infusions on Oxygen Consumption and Lactate Production

Infusion of Ado during constant-flow perfusion decreased circuit pressure from 122.0 ± 8.0 to 49.5 ± 5.1 mmHg (n = 7, P < 0.001) (Fig. 1). Oxygen consumption across the perfused tissue was reduced from 73.1 ± 10.8 to 48.0 ± 10.2 µmol·min⁻¹·100 g⁻¹ (P < 0.0001), whereas the production of lactate was doubled (26.1 ± 3.2 vs. 12.8 ± 4.6 µmol·min⁻¹·100 g⁻¹ for control, P < 0.005) as shown in Fig. 2. Infusion of Ado in the constant-pressure perfusion increased the SMA flow from 34.3 ± 7.1 to 138.6 ± 12.4 ml/min (P < 0.001), whereas the perfusion pressure did not change (Fig. 1). However, in response to Ado, the oxygen consumption [76.1 ± 11.8 vs. 72.4 ± 11.6 µmol·min⁻¹·100 g⁻¹, not significant (NS)] and lactate production (20.6 ± 7.9 vs. 16.8 ± 5.2 µmol·min⁻¹·100 g⁻¹, NS) were not altered when shear stress was elevated by constant-pressure perfusion (Fig. 2). Thus infusion of Ado decreased oxygen consumption and increased lactate production in constant-flow (shear stress decreased) but not in constant-pressure perfusion (shear stress increased).

Effect of NO Synthase Blockade on Ado Effects

Administration of L-NAME, a NO synthase blocker, increased circuit perfusion pressure and tended to increase systemic arterial pressure. The SMA pressure reached a plateau in 10 min after L-NAME infusion and lasted for ~3 h. The systemic pressure before and 10 min after L-NAME was 117.1 ± 6.9 and 129.6 ± 6.6 mmHg, respectively (NS). The SMA pressure was more sensitive to the blockade of NO synthase. The perfusion pressure increased from 113.0 ± 5.7 to 162.2 ± 10.3 mmHg (P < 0.01) after L-NAME at constant flow (37.5 ± 7.6 to 34.8 ± 7.7 ml·min⁻¹·100 g⁻¹ after L-NAME). Basal oxygen consumption (83.6 ± 15.0 µmol·min⁻¹·100 g⁻¹ before L-NAME and 81.6 ± 17.4 µmol·min⁻¹·100 g⁻¹ after; NS) and lactate production (12.5 ± 5.4 µmol·min⁻¹·100 g⁻¹ before L-NAME and 14.9 ± 5.5 µmol·min⁻¹·100 g⁻¹ after; NS) across the tissue were not altered by L-NAME.

The vasodilator effects of Ado during both constant-flow and constant-pressure perfusion were potentiated after L-NAME. Ado infusion during constant-flow perfu-
sion decreased SMA pressure by 112.2 ± 11.3 from 162.2 ± 10.3 mmHg (vs. a decrease of 72.6 ± 11.4 from 122.0 ± 8.0 mmHg before L-NAME, calculated as absolute changes, P < 0.01), whereas Ado increased flow rate by 119.6 ± 13.1 from 37.6 ± 9.9 ml·min⁻¹·100 g⁻¹ (vs. an increase of 104.3 ± 14.7 from 34.3 ± 7.1 ml·min⁻¹·100 g⁻¹ before L-NAME, P < 0.05) during constant-pressure perfusion (Fig. 3). The effects of Ado on the intestinal metabolism during constant-flow perfusion were not altered by the blockade of NO synthase. Oxygen consumption was reduced by 25.1 ± 2.9 µmol·min⁻¹·100 g⁻¹ before L-NAME and by 24.0 ± 7.2 µmol·min⁻¹·100 g⁻¹ after L-NAME (NS, Fig. 4). Lactate production was increased by 5.47 ± 0.98 µmol·min⁻¹·100 g⁻¹ (P < 0.005) after L-NAME, which is comparable with that before the blockade of NO synthase. Ado infusion during constant-pressure perfusion after L-NAME, however, reduced oxygen consumption by 17.5 ± 5.8 µmol·min⁻¹·100 g⁻¹, which was significantly different from that before L-NAME (−3.7 ± 6.4 µmol·min⁻¹·100 g⁻¹, P < 0.05, Fig. 4) and not different from the results in constant-flow Ado infusion. The lactate production was increased (by 17.10 ± 5.67 µmol·min⁻¹·100 g⁻¹, P < 0.05) by Ado during constant-pressure perfusion after L-NAME.

Therefore, L-NAME increased basal vascular tone in the SMA and potentiated the vasodilator effects of Ado in both constant-flow and constant-pressure settings. Ado decreased oxygen uptake and increased lactate output only when shear stress was prevented from rising. If shear stress was allowed to increase (constant-pressure perfusion), Ado metabolic action was prevented but could be restored by blocking NO production. Blockade of NO production did not alter basal metabolism, nor did it alter the Ado-induced metabolic action in the constant-flow perfusion where shear stress was not increased.

Effect of SIN-1 on Ado Actions During Constant-Flow Perfusion

The effects of the NO donor were evaluated in the constant-flow perfusion because in that condition shear stress did not increase during Ado infusion and endogenous NO effects were absent. Intravenous bolus injection of SIN-1 caused an immediate drop in systemic pressure and SMA perfusion pressure. The vasodilator response to SIN-1 was less sensitive in the SMA. The systemic arterial pressure dropped from 101.8 ± 4.1 to 71.7 ± 4.0 mmHg (n = 6, P < 0.001) at an SIN-1 dose of 0.1 mg/kg and to 64.0 ± 7.3 mmHg (P < 0.001) at a dose of 0.2 mg/kg, whereas the SMA pressure dropped from 108.9 ± 8.5 to 103.1 ± 10.7 mmHg (NS) and to 82.8 ± 4.3 mmHg (P < 0.05), respectively. This comparison was made at the same SMA flow rates (30.8 ± 3.8 and 29.2 ± 3.9 ml·min⁻¹·100 g⁻¹ at the 2 doses vs. 29.7 ± 3.9 ml·min⁻¹·100 g⁻¹ for control). The vasodilator effect of SIN-1 was sustained for ~1 h in the SMA, whereas it lasted up to 3 h systemically. SIN-1 at either dose had no effect on basal oxygen consumption (64.5 ± 4.2 at 0.1 mg/kg and 58.9 ± 2.8 at 0.2 mg/kg vs. control 60.6 ± 3.8 µmol·min⁻¹·100 g⁻¹, NS) and lactate production (4.1 ± 1.6 at 0.1 mg/kg and 3.5 ± 3.1 at 0.2 mg/kg vs. 6.1 ± 2.1 µmol·min⁻¹·100 g⁻¹, NS).

The infusion of Ado decreased SMA perfusion pressure by 70.0 ± 10.9 (P < 0.005) and 52.5 ± 3.9 (P < 0.0001) mmHg in the presence of 0.1 and 0.2 mg/kg SIN-1, respectively, whereas Ado decreased the pressure by 79.6 ± 8.8 from 116.3 ± 8.1 mmHg (P < 0.0005) in control. Ado-induced maximum vasodi-
lations represented by SMA pressure were greater in the presence of SIN-1 [33.1 ± 1.1 (P < 0.05) and 30.3 ± 0.8 mmHg (P < 0.001)] at a SIN-1 dose of 0.1 and 0.2 mg/kg, respectively, compared with 36.7 ± 1.2 mmHg for control], although the absolute decreases in the pressure induced by Ado were reduced. The effect of Ado on intestinal oxygen consumption was attenuated dose dependently by SIN-1. The reductions of oxygen consumption by Ado infusion were 18.8 ± 4.5 and 9.4 ± 2.2 µmol·min⁻¹·100 g⁻¹ at doses of 0.1 and 0.2 mg/kg, respectively. It was significantly different at a dose of 0.2 mg/kg from the control (21.8 ± 4.5 µmol·min⁻¹·100 g⁻¹, P < 0.05) as shown in Fig. 5. The Ado-induced increase in lactate production in the presence of SIN-1 was attenuated dose dependently (Fig. 5). Thus SIN-1 produced a hypotensive effect in the systemic and superior mesenteric circulation but had no effect on basal metabolism in the intestine. The presence of SIN-1 attenuated the metabolic effects of Ado on oxygen consumption and lactate production in a dose-dependent manner.

Hemodynamic and Metabolic Effects of Iso

Infusion of Iso in constant-flow perfusion caused a decrease in circuit pressure from 110.3 ± 8.2 to 45.7 ± 3.5 mmHg (Fig. 6), which is comparable with the response to Ado. The oxygen consumption across the tissue was reduced by 9.8 ± 2.8 from 64.7 ± 5.3 µmol·min⁻¹·100 g⁻¹ by Iso infusion (Fig. 6). The production of lactate was not altered by Iso infusion.

DISCUSSION

NO, released either by shear stress or by the exogenous donor SIN-1, antagonized Ado effects on intestinal metabolism, but NO was without direct effect on basal metabolism. Ado decreased oxygen consumption and increased lactate production in the small intestine during constant-flow perfusion. The results suggested that Ado suppresses local aerobic metabolism and increases anaerobic metabolism. The effects of Ado on intestinal metabolism were abolished when shear stress increased (in constant-pressure perfusion). The observation that NO synthase blockade by L-NAME restored the effects in this situation strongly suggested an antagonist effect of NO on the metabolic effects of Ado. The data are consistent with a hypothesis that contrasts vascular and metabolic roles and controls by NO: Ado-induced conversion of intestinal metabolism to the anaerobic state is suppressed by shear stress-dependent release of NO (seen only in constant-perfusion state, not constant-flow state), but the suppression of Ado-induced vasodilation by NO is not shear stress dependent, since it occurred in both constant-flow and constant-pressure conditions. The effects of Ado on intestinal metabolism in the absence of increased shear stress (in constant-flow perfusion) were attenuated by the introduction of exogenous NO into the system using SIN-1. These results further confirmed the antagonism
by NO of the metabolic responses to Ado. Furthermore, the observation that neither the NO synthase blockade by L-NAME nor the administration of SIN-1 changed basal oxygen consumption or lactate production suggested that NO was not involved in the control of basal metabolism in the small intestine. In contrast, NO was involved in maintaining basal vascular tone in the SMA.

Methodological Considerations

Surgery. The surgical procedure used in this study was modified from our previous study (12). Removal of the large intestine and transaction at the duodenum were done to ensure that a defined intestinal segment received blood supply exclusively from the SMA. The superior mesenteric nerve plexus was surgically ablated to avoid the effect of nerve reflexes on intestinal hemodynamics and metabolism. We used a double aortic cannulation for the source of circuit blood instead of femoral arterial cannulations, thereby helping to maintain hindlimb perfusion and control of basal lactate level.

Control of shear stress states. To assess the involvement and effect of NO, the responses to Ado were evaluated under different shear stress states, which would result in a different endogenous NO production, because shear stress is a powerful stimulus of endothelial NO synthase (14). When Ado was infused during constant-flow perfusion, the circuit flow rate was held steady so that a drop in perfusion pressure would be seen in response to vasodilation. Shear rate would decrease with the decrease in perfusion pressure in this condition. On the other hand, to maintain the perfusion pressure during Ado infusion in the constant-pressure perfusion, the flow rate has to be increased to compensate for the vasodilation response. Shear rate in the constant-pressure condition, therefore, would increase when the flow rate increased. The theoretical derivation of this conclusion has been presented (8). The effect of constant flow or pressure on shear stress during vasodilation is different from the situation when a vasoconstrictor is infused. We have previously discussed (13) that the vasoconstriction during constant-flow perfusion would increase perfusion pressure and elevate shear rate, whereas, when vasoconstriction was induced under constant pressure, the flow was reduced and shear rate would not be altered. Furthermore, when shear stress is increased by a vasoconstrictor during constant-flow perfusion, the site where shear rate increases is the site of constriction. When a vasodilator is infused to elevate shear rate in constant-pressure perfusion, the site where shear rate increases the most is the site with least dilation either downstream or upstream of the most dilated site. We would expect that the site where shear stress increased the most in our experiment is more likely the capillary site downstream, considering the shear stress dependency of the metabolic effects.

Influence of NO on Basal Hemodynamics and Metabolism

We used a NO synthase blocker, L-NAME, to block the production of NO. In our previous studies (12, 13) a dose of 2.5 mg/kg was used to block NO synthase. In the present study, the increase in basal SMA perfusion pressure after L-NAME indicated a successful blockade of NO synthase. To verify our interpretation of the L-NAME data, we used a NO donor, SIN-1, to introduce exogenous NO into the system. SIN-1 is a synthetic compound that degrades chemically to release NO spontaneously (15).

We confirmed that NO contributes to regulation of vascular tone in the SMA (12, 13). The blockade of NO synthase increased basal SMA perfusion pressure to a greater extent than systemic pressure. The administration of a NO donor, on the other hand, decreased perfusion pressure in the SMA to a lesser extent than it did in the whole system. These results might indicate a higher basal level of NO involved in the regulation of basal vascular tone in the superior mesenteric vascular bed. However, systemic blood pressure effects may simply have been compensated for by efficient baroreflexes (7) while the intestine had been denervated. The basal intestinal metabolism was not altered by either the blockade of NO synthase or the administration of SIN-1, although they both changed basal vascular tone in the SMA. These results suggested that NO was not primarily involved in the regulation of basal metabolism in the superior mesenteric vascular bed.

NO has been demonstrated in vitro to interact with mitochondrial enzymes (5, 21) and inhibit energy metabolism. It has been reported that NO reduced oxygen consumption in isolated myocardial (23) and skeletal muscle slices (19). However, the results of in vivo studies are very inconsistent. Blockade of NO synthase was reported to increase oxygen consumption in the hindlimb of dogs (9, 19) and in conscious dogs (20). In contrast, a study in rat ileum (2) demonstrated that L-NAME did not change oxygen consumption. The basal oxygen consumption of the whole body was not influenced by sodium nitroprusside, a NO donor (25), and was not changed by L-NAME (9, 20) in anesthetized dogs. The inconsistency of published studies could simply result from the limitations of different preparations such as an in vitro versus in vivo study, the influence of anesthesia (20), and the dose of L-NAME used to block NO synthase. A study in dog jejunum (1) showed that L-NAME increased oxygen consumption only at doses >10 mg/kg and the effect was time dependent. These doses are much higher than that used in our study (2.5 mg/kg). We infused the drug over a 10-min period and started tests in another 10 min to avoid any transient effects as reported (1).

Consideration of Ado Hemodynamic Effects

The metabolic effects of Ado could possibly be secondary to its vasodilator effects. In this regard, we compared Ado effects with another vasodilator, Iso. The infusion of Iso at the dose to produce similar hemody-
namic effect as Ado did decreased oxygen consumption by only 9.8 µmol·min⁻¹·100 g⁻¹ (compared with a reduction of 26.1 µmol·min⁻¹·100 g⁻¹ by Iso) and did not increase lactate production. Thus, the similar hemodynamic response produced by Iso could not mimic the metabolic action of Ado. In the presence of SIN-1, Ado reduced perfusion pressure to an even lower level, but the effects on metabolism were attenuated. Furthermore, when NO production was blocked, Ado suppressed gut metabolism whether blood flow was elevated or held steady, thus also indicating a lack of dependence of the Ado effect on oxygen supply. The hemodynamic changes were not likely to be the cause of Ado metabolic effects.

Influence of NO on Ado Effects

The potentiation by L-NAME and the inhibition by SIN-1 of vasodilator effects of Ado in the SMA are consistent with our previous findings (12), suggesting antagonism by NO of Ado vasodilator effect. Ado infusion during constant-flow perfusion decreased oxygen consumption and increased lactate production in the intestine. However, Ado did not alter the metabolic profiles during constant-pressure perfusion. Therefore, when blood flow is held constant, thus avoiding an increase in shear stress, Ado suppressed gut metabolism, but if the dilation induced by Ado was allowed to result in elevated blood flow (shear stress increased), Ado did not suppress gut metabolism. The NO synthase antagonist L-NAME did not alter basal gut metabolism, nor did it alter the ability of Ado to suppress gut metabolism in the constant-shear stress condition. However, when shear stress was allowed to increase in response to the Ado-induced dilation, NO appeared to be released and to suppress the metabolic effect of Ado. Ado metabolic action was fully restored by blocking NO release in this condition. We were able to demonstrate NO inhibition of Ado metabolic action by provision of NO through the NO donor SIN-1 in the absence of shear stress changes. Thus the observation that Ado suppression of gut metabolism was eliminated by increased shear stress and restored by blocking of NO production or mimicked by exogenous NO in the absence of shear stress supports the following conclusions. Basal NO production does not directly alter gut oxygen uptake or lactate output. NO released by shear stress or supplied exogenously does suppress the metabolic effects of Ado. The NO effect on gut metabolism in this situation is thus indirect through suppression of Ado action.

The picture that is emerging on the basis of our studies of the in vivo, integrated intestinal role of NO is much more subtle than originally anticipated. To briefly recapitulate our working hypotheses, Ado and Iso cause shear stress-independent release of NO that acts on vascular smooth muscle to inhibit the Ado- or Iso-induced dilation; this NO does not result in metabolic action. This NO is thus likely not released at the capillary level. Ado suppresses aerobic metabolism, but, if the Ado leads to a sufficient increase in blood flow to elevate shear stress, NO is released and results in suppression of the metabolic action of Ado; this NO most likely comes from capillary endothelium. Basal levels of NO do not have a metabolic effect but do maintain a vasodilated tone. Vasconstriction through sympathetic nerve activation or norepinephrine infusion leads to NO release from the constricted site, but the NO does not appear to act directly on vascular smooth muscle. Rather, NO appears to inhibit the vascular responses to nerve stimulation by inhibiting transmitter release because effects of exogenously administered norepinephrine were not inhibited by shear stress-induced release of NO (13). Thus the vascular role of NO is both direct, through regulation of basal tone, and indirect, subsequent to suppression of neurotransmitter release and subsequent to suppression of other vasodilators. The metabolic role is indirect in that NO inhibits the metabolic effects of Ado, but this NO is released subsequent to increased blood flow, probably at the capillary level, and NO does not play a role in basal metabolic regulation.

In conclusion, the present study suggests that Ado-induced suppression of intestinal metabolism was antagonized by shear-induced NO, whereas the antagonism by NO of Ado vasodilator effects was not shear stress dependent. Basal release of NO (which is not likely to be shear stress dependent) is involved in the control of basal tone but not primarily in the control of basal metabolism in the intestine.

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