Disparity in Osmolarity-Induced Vascular Reactivity

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Conventional peritoneal dialysis solutions (PDS) are vasoactive. This study was conducted to identify vasoactive components of PDS and to describe quantitatively such vasoactivity. Anesthetized nonheparinized rats were monitored continuously for hemodynamics while the microvasculature of the jejunum was studied with in vivo intravital microscopy. In separate experiments, vascular reactivity of rat endothelium-intact and -denuded aortic rings (2 mm) was studied ex vivo in a standard tissue bath. In both studies, suffusion of the vessels was performed with filter-sterilized isotonic and hypertonic solutions that contained glucose or mannitol as osmotic agents. PDS served as a control (Delflex 2.25%). Hypertonic glucose and mannitol solutions produced a significant vascular reactivity in aortic rings and instantaneous and sustained vascular relaxation at all levels of the intestinal microvasculature. Similarly, lactate that was dissolved in a low-pH isotonic physiologic salt solution produced significant force generation in aortic rings. Whereas isotonic glucose and mannitol solutions had no vasoactivity in aortic rings, isotonic glucose produced a selective, insidious, and time-dependent vasodilation in the intestinal preemuscular arterioles (18 ± 0.2% of baseline), which was not observed in the larger inflow arterioles (100 μm). This isotonic glucose–mediated vascular relaxation can be attenuated by approximately 50% with combined adenosine A2a and A2b receptor antagonists and completely abolished by adenosine A1 receptor inhibition. By using two different experimental techniques, this study demonstrates that hyperosmolality and lactate are the major vasoactive components of clinical peritoneal dialysis solutions. The pattern and the magnitude of such reactivity are dependent on vessel size and on the solutes’ metabolic activity. Low pH of conventional PDS is not a vasoactive component by itself but renders lactate vasoactive. Energy-dependent transport of glucose into cells mediates vasodilation of small visceral arterioles by an adenosine receptor-mediated mechanism and constitutes a significant fraction of PDS-mediated vascular reactivity in the visceral microvasculature.

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In a recent study, we attempted to investigate the molecular mechanism of PDS-induced vascular relaxation. In this study, vascular rings from the aorta and superior mesenteric arteries were exposed to a conventional PDS under controlled conditions. The PDS contracted these arteries by an endothelium-independent mechanism that involved a vascular smooth muscle–derived prostanoid pathway (10). We therefore hypothesized that the pattern and the magnitude of vascular reactivity produced with conventional peritoneal dialysis and other hypertonic solutions are determined primarily by the hyperosmolality and the solutes’ metabolic activity. We further hypothesized that the role of other components of the dialysis solution is subordinate. The aim of this study was to identify the major vasoactive components of conventional PDS under controlled experimental conditions. This is required before investigation of the molecular mechanisms and signal transduction pathways involved in such vasoactivity.

Materials and Methods

General Animal Care and Surgery
Male Sprague-Dawley rats (Harlan, Inc., Indianapolis, IN) were housed in Association for Assessment and Accreditation of Laboratory Animal Care–approved facilities and were maintained on standard rat diet and water ad libitum for at least 1 wk before use. All animal care and experimental procedures conformed to “Principles of Laboratory Animal Care” of the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” of the US National Academy of Science as published by the National Institutes of Health (NIH publication 80-23, revised 1987) and were previously approved by the Institutional Animal Care and Use Committee of the University of Louisville and the Louisville Veterans Administration. Experiments were performed on rats (200 to 210 g) that had fasted overnight. Anesthesia was induced with intraperitoneal pentobarbital (60 mg/kg) and maintained with supplemental subcutaneous injection as needed. Body temperature was maintained at 37 ± 0.5°C with a rectal probe and a servocontrolled heating pad. Surgery was carried out after loss of the blink and withdrawal reflexes. Tracheostomy was performed to reduce airway resistance, and the animal was allowed to breathe room air. The right femoral artery was cannulated with a PE-50 catheter to provide continuous monitoring and online recording of arterial BP.

Bathing Solutions

Microvascular Studies. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). The intestinal segment was suffused continuously during tissue preparation and equilibration with a non-vasoactive modified Krebs’ solution that contained 6.92 g/L sodium chloride, 0.44 g/L potassium chloride, 0.37 g/L calcium chloride, and 2.1 g/L sodium bicarbonate at a pH of 7.4 and osmolality of 285 mOsm/L. Isotonic glucose and mannitol solutions were prepared as Tris-buffered physiologic salt solution (PSS). The isotonic glucose solution contained 36.29 mM Tris-HCl, 13.71 mM Tris-base, 11.1 mM glucose, 88.98 mM NaCl, 5.87 mM KCl, and 2.55 CaCl2/2H2O. The isotonic mannitol solution had the same components except that mannitol was substituted for glucose. Hypertonic mannitol solutions were prepared either as a 5% mannitol in Krebs’ solution or as a 10% mannitol in deionized water. All solutions were filter-sterilized and prewarmed to 37°C before use. A conventional 2.25% dextrose-based dialysis solution (Delflex; Fresenius USA, Inc., Ogden, UT) that contained 5.67 g/L sodium chloride, 3.92 g/L sodium lactate, 0.257 g/L calcium chloride, and 0.152 g/L magnesium chloride at a pH of 5.5 and an initial osmolality of 398 mOsm/L served as control for the hypertonic solutions.

Macrovascular Studies. Vascular ring studies are typically conducted in a nonvasoactive PSS. PSS composition in millimolars was 118 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, and 11.1 glucose. All aortic rings were maintained at 37°C and bubbled with a 95% O2 and 5% CO2 gas mixture to yield a pH of 7.4 (gas bubble dispersion surface; Radnoti Glass Technology, Monrovia, CA). Properties of the test solutions are illustrated in Table 1. A conventional 2.25% dextrose-based dialysis solution (Delflex) served as control.

Drugs
Phenylenediamine hydrochloride [PHE; 1-(5-oxohexyl)-3,7-dimethylxanthine], acetylcholine hydrobromide (ACH), isoproterenol, and sodium nitroprusside were purchased from Sigma Chemical. PHE and ACh were used to test the integrity of the vascular endothelium for each aortic ring at the start and at the end of each experiment according to protocol. Isoproterenol and sodium nitroprusside, respectively, were used in the tissue bath to retard peristalsis and to assess the maximum dilation capacity of the intestinal microvasculature. All endothelium-intact rings demonstrated >60% of ACh-induced relaxation (81% ± 3.19), and all endothelium-denuded rings demonstrated <5% of the ACh-induced relaxation (2.7% ± 1.66). Three selective adenosine receptor antagonists were used: 8-Cyclopentyl-1,3-dipropylxanthine, an adenosine A1 receptor antagonist; 8-(3-chlorostyryl) caffeine, an adenosine A2a receptor antagonist; and alloxazine, an adenosine A2b receptor antagonist. The final concentration in the tissue bath for 8-cyclopentyl-1,3-dipropylxanthine, 8-(3-chlorostyryl) caffeine, and alloxazine were 200 nM, 200 nM, and 600 µM, respectively. These concentrations were 200 nM, 200 nM, and 600 µM, respectively. These concentrations were 200 nM, 200 nM, and 600 µM, respectively. These concentrations were 200 nM, 200 nM, and 600 µM, respectively.
represent at least three times the 50% effective inhibitory concentration for each adenosine receptor antagonist as determined by information provided by the manufacturer.

**Study I: Microvascular Reactivity**

**Experimental Procedure.** The peritoneal cavity was exposed through a midline abdominal incision of 1.5 cm, and a 2- to 3-cm segment of jejunum was withdrawn gently from the peritoneal cavity with its neurovascular supply intact. The segment was opened along the antimesenteric border by electrocautery. The enteric contents and mucus were removed gently from the mucosal surface. The animals were positioned on a specially designed polyurethane board. The opened jejunum was suspended, serosal side up, over a viewing port in a tissue bath with 4-0 silk sutures. The nonvasoactive bathing solution was maintained at 37°C and bubbled with nitrogen and carbon dioxide to maintain the pH at 7.4. Isoproterenol was added to the bathing solution in a very dilute concentration (0.01 μg/ml) to retard peristalsis. This dose of isoproterenol is below the threshold that alters vascular smooth muscle tone (11).

The animal board was positioned on the stage of a trinocular microscope for direct in vivo intravital microscopy. Microvascular images were transmitted through the microscope to a photodiode array in an optical Doppler velocimeter (Microcirculation Research Institute, Texas A & M University, College Station, TX) to measure center-line red blood cell velocity for the calculation of blood flow in the intestinal A1 inflow arteriole. The microvascular image then was transmitted to a digital camera (Hitachi Denshi, Models K-P D51/D50), which provided 30 images per second to a computer. The digitized microvascular images were stored as streamline video in the computer hard drive for later measurement of microvascular diameters with calipers.

Criteria for an acceptable microvascular preparation during intravital microscopy included a baseline mean arterial pressure >90 mmHg, a center-line red blood cell velocity in a first-order arteriole >20 mm/s, and an active vasomotion in the intestinal premucosal A3 arterioles. We used a standard nomenclature for intestinal microvessels, as originally described by Bohlen and Gore (11). Briefly, first-order arterioles (A1) arise from a mesenteric arcade artery to traverse the mesenteric border of the bowel wall and then penetrate through the muscle layers to the submucosal layer. In the submucosal layer, second-order arterioles (A2) arise from the A1 to run along the longitudinal axis of the bowel. First- and second-order venules parallel the A1 and A2. A2 give rise to branching second-order arcade vessels as well as to smaller third-order arterioles (A3). The A3 vessels branch at a right angle from A2 to form distal A3 (dA3), which terminates in the mucosa as a central villus arteriole. Along their course, A3 also give rise to smaller proximal A3 that supply the seromucosal layers of the bowel wall.

**Experimental Protocol and Measurements.** The intestinal segment was allowed to equilibrate for 40 min in the tissue bath. During this time, the segment was sufaxized continuously with the nonvasoactive Krebs solution. BP, heart rate, rectal and bath temperatures, and bath pH were monitored continuously (Digi-Med Signal Analyzers, Louisville, KY) and recorded every 5 min. Microvascular data consisted of A1, proximal A3 (pA3), and dA3 arteriolar diameters and center-line red cell velocity in the inflow A1. Baseline measurements were considered valid when the variability in the measurement was <0.5%. After baseline measurements, the nonvasoactive Krebs’ solution was aspirated from the tissue bath and a test solution (see bathing solutions) was randomly added into the tissue bath. Microvascular data points were measured initially at 2 min after the addition of the test solution and then at 10-min intervals during the subsequent 90 min. At the conclusion of the experiment, one dose of ACh (10^-4 M) was administered topically in the tissue bath and microvascular data time points were taken at 1-min intervals over 10 min to assess endothelial cell function and endothelial-dependent vasodilation. Finally, a single dose of sodium nitroprusside (10^-4 M) was administered in the tissue bath to assess the endothelium-independent maximal dilation capacity.

**Study II: Macrovascular Reactivity**

The experimental and timeline protocols of these studies are depicted in Figure 1. Briefly, after induction of anesthesia, the thoracic aorta was excised and submerged in a Petri dish filled with a PSS. One half of the thoracic aorta was denuded of endothelium by passing a fine-glass rod, approximately the size of the inner diameter of the aorta, to and fro once through the lumen. The other half of the thoracic aorta was regarded as endothelium-intact aorta. The presence/absence of viable endothelium in these rings was verified with an endothelium-dependent ACh relaxation according to protocol. The aortic segments closer to the aortic arch and ones closer to the diaphragm behave differently to several agonists than the middle segments (unpublished data). Therefore, only the middle 8 mm of the thoracic aorta was used in these experiments.

The endothelium-intact and endothelium-denuded segments of the aorta were divided to produce two 2-mm rings each. Two wires (stainless steel of 0.012 inner diameter) were passed through the lumen of each ring and closed on them to form two wire triangles. One triangle was attached to a fixed hook, and the other triangle was attached via a stainless wire to a force transducer, which was connected to a tissue force analyzer. Each vascular ring preparation was suspended in an individual 20-ml tissue bath, which was filled with PSS. Each vascular ring was stretched to produce an initial passive tension called “preload” of 1.0 g in a bath filled with PSS. Each ring was treated with 1.0 μM ACh and 1.0 μM phenylephrine to saturate “nonreceptor binding...
sites" for the agonists and then washed with PSS for 20 min. After
another 40 min of vascular ring equilibration, the preload of each
vascular ring was readjusted to the initial 1.0-g preload level. Individual
rings were contracted in a sequential manner with six cumulative
phenylephrine doses to give phenylephrine bath concentrations of 0.01
through 3.0 μM (in three steps). Each ring then was relaxed with 3.0 μM
ACH for 10 min to demonstrate ACh-induced relaxation and viability of
the endothelial cells. Hyperosmolar solutions (see bathing solution)
were added randomly in the tissue bath to replace the PSS, and the
resultant vascular tension was recorded. Both endothelium-intact and
-denuded vascular rings were studied in a paired manner design.

Statistical Analyses
All data are presented as mean ± SEM unless stated otherwise.
Percentage change of the vessel diameter from baseline was assessed
with one-way ANOVA and Dunnett multiple-range test to evaluate
changes from the baseline within the same animal. Two-way ANOVA
was used to assess the relationship between vascular reactivity and
arteriolar type. The maximal force of contraction to the peritoneal
dialysis and other hyperosmolar test solutions, the maximal force
of contraction to PHE, and the maximal relaxation to ACh was deter-
minded for each ring from computer-stored digitized raw data. Differ-
ences between groups were assessed with two-way ANOVA and Bonferroni posttest. ACh-induced endothelium-dependent relaxation of
>60% or <5% was used to determine presence/absence, respectively,
of a viable vascular endothelium. A result was considered to be signif-
icient when the probability of a type 1 error was \( P < 0.05 \).

Results
Microvascular Reactivity
Effect of Solute’s Metabolic Activity. D-Glucose, unlike
mannitol, is a metabolically active solute that can be trans-
ported readily and actively into cells. As shown in Figure 2A,
isotonic d-glucose causes a differential reactivity in the intesti-
nal microcirculation (\( n = 12 \)). This reactivity is characterized by
dilation of the smaller premucosal A3 (8 to 15 μm, pA3, dA3,
respectively). This is in contrast to the largely transient initial
constriction observed in the larger inflow A1 (100 μm). The
maximum vascular response during a 90-min exposure of the
intestine to the isotonic glucose solution, expressed as percent-
age from baseline, was A1 (−10.58 ± 1.06% recorded at 10 min),
pA3 (17.17 ± 1.66% recorded at 80 min), and dA3 (19.09 ±
2.41% at 80 min). There was no significant vascular reactivity
when the intestine was exposed to the isotonic mannitol solution
(\( P > 0.05 \); \( n = 12 \)). The averaged 90-min vascular reactivity
in mannitol was −0.13 ± 1.26%, −2.90 ± 1.18%, and −4.45 ±
1.07% from baseline diameter in the A1, pA3, and dA3, respec-
tively. Isotonic glucose-mediated premucosal intestinal arterio-
lar dilation is an insidious and time-dependent response (Figure
2B, top). This vascular reactivity was partially attenuated by
−50% in pA3 and −45% in dA3, with combined adenosine
A2a and A2b receptor antagonists (\( P < 0.05 \); \( n = 12 \); Figure
2B, middle), and completely abolished when the adenosine A1
receptor was inhibited (\( P < 0.01 \); \( n = 12 \); Figure 2B, bottom).
There was no effect of the adenosine receptor subtype inhibition
on the isotonic glucose-elicted selective constriction of the
A1 inflow arterioles.

Effects of Osmolarity Perturbation. The effect of osmolar-
ity perturbation on the intestinal microvasculature is depicted

Figure 2. (A) Effect of solute’s metabolic activity on intestinal micro-
avascular reactivity. Isotonic glucose but not mannitol produced a
differential vascular reactivity in intestinal microcirculation charac-
terized by a significant selective vasodilation of the smaller premucosal
arterioles (8 to 15 μm) and a vasoconstriction of the larger inflow A1
arterioles (100 μm). *\( P < 0.01 \) by ANOVA and Bonferroni post test
versus isotonic mannitol; §\( P < 0.01 \) by ANOVA and Bonferroni post
test versus A3 premucosal arterioles. (B) Mechanism of isotonic glu-
cose–induced microvascular reactivity. BL, baseline arteriolar diam-
eter; A1, intestinal inflow arteriole; pA3 and dA3, intestinal proximal
(p) and distal (d) A3 premucosal arterioles. *\( P < 0.01 \) by ANOVA and
Bonferroni post test versus BL. §\( P < 0.05 \) by ANOVA and Bonferroni
post test versus BL.
in Figure 3. Osmolarity was enhanced with either the addition of a 5% mannitol to a nonvasoactive Krebs’ solution to obtain a final osmolality of 560 mOsmol/L (n = 6) or a 10% mannitol to deionized water to obtain a final osmolality of 575 mOsmol/L (n = 6). A conventional 2.5% dextrose-based PDS (Delflex) with osmolality of 398 mOsmol/L served as control (n = 6). Enhancement of osmolarity caused a generalized vasodilation at all levels of the intestinal microvasculature. Although the osmolarity of the hypertonic mannitol solutions was significantly higher than that of the dialysis solution, the dialysis solution induced a significantly greater vasodilation in the premucosal arterioles (36.85 ± 3.84 and 44.38 ± 6.63%), compared with the 5% mannitol (30.6 ± 4.7 and 22.4 ± 3.5%) and the 10% mannitol (33.7 ± 3.1 and 27.5 ± 2.7%) in the premucosal pA3 and dA3, respectively. In addition, all hyperosmolar solutions tested in this series equally doubled the blood flow of intestinal inflow A1.

**Macrovascular Reactivity**

**Effects of Osmolarity Perturbation.** Seven solutions were tested in this series (n = 12 each; Table 1). Of these solutions, only the solutions made hypertonic with either d-glucose or d-mannitol produced a significant vascular reactivity in the aorta as measured by force generation (Figure 4). The magnitude of this aortic vascular reactivity was largest with the conventional PDS and quantitatively higher in hyperosmolar solutions that contained ions, compared with identical hyperosmolar solutions that lacked ionic contents (Figure 4), suggesting a significant role of the ionic contents of the solution in determining the magnitude of the prevailing vascular response. There was no vascular effect of isotonic glucose or mannitol solutions on the aorta (data not shown).

**Effects of Vascular Endothelium.** In all of the hypertonic solutions tested in our study, endothelium removal significantly attenuated the magnitude of the solution-mediated aortic contraction by ~32% (range ~20 to ~54%; P < 0.05). Such attenuation was maximally seen in endothelium-denuded aortic rings that were exposed to the 5% glucose (~37%) and the 5% mannitol (~54%) in PSS (Figure 5).

**Effects of Solution pH.** The role of pH in hyperosmolality-induced aortic reactivity seems to be influenced by the aortic endothelium and the ionic composition of the solution (Figure 6). Of all of the hyperosmolar solutions tested in this series, the dialysis solution produced the highest aortic ring contraction, regardless of the endothelium (Figure 6). In the aortic denuded
rings, the conventional PDS produced a -23% less contraction force than rings with intact endothelium. This pattern was also preserved in denuded aortic rings suffused with 5% mannitol (-19%), 5% glucose (-33%), 10% mannitol (-27%), and 10% glucose (-15%), when the pH of these hyperosmolar solutions was adjusted to <6 pH units (n = 7 for each solution). Low pH accounted for 4.5% (P < 0.05) of the total variation in the vascular reactivity produced by the 5% glucose or mannitol, which contained other ions (Figure 6, top). In comparison, in the 10% osmotic solute (glucose or mannitol) in deionized water (Figure 6, bottom), low pH accounted for only 0.3% (P > 0.5) of the total variation, which is accounted for exclusively by the vascular reactivity (15.3%; P < 0.001). In the presence of ions, low pH seems to attenuate vascular reactivity by 30% in denuded rings and by 10% in endothelium-intact rings regardless of the main osmotic solute (Figure 6, top). In contrast, in low pH deionized solutions, hyperosmolality as a result of glucose enhanced aortic reactivity in endothelium intact rings by 16% and attenuated that of endothelium-denuded rings by 14%, whereas in hyperosmolality as a result of mannitol, aortic reactivity in endothelium-intact rings was enhanced by 8% and attenuated by 37% in endothelium-denuded rings (Figure 6, bottom).

Effects of the Buffer Anion. In this series, sodium lactate was dissolved in an isotonic PSS to match the lactate concentration of PDS. Addition of sodium lactate (0.392 g/L) to the nonvasoactive PSS yielded a pH > 8 pH units. At this high pH, which was maintained in the tissue bath during the experiment, the maximum contraction force in aortic rings with intact endothelium was 0.01 ± 0.02 g (n = 12; P > 0.1) versus -0.02 ± 0.02 g (n = 12; P > 0.1) in endothelium-denuded rings. When tissue bath pH was adjusted to 5.03, there was a significant contraction force in both endothelium-intact aortic rings (0.33 ± 0.11 g; n = 12; P < 0.05) and endothelium-denuded aortic rings (0.45 ± 0.16 g; n = 12; P < 0.01). This pH-dependent lactate-induced aortic contraction was significantly greater in the endothelium-denuded rings (P < 0.05; Figure 7).

Discussion

The salient findings of these studies are that (1) conventional PDS produce an instantaneous and sustained vasodilation at all levels of the intestinal (visceral) microvasculature; (2) the pattern and magnitude of such dilation is dependent on vessel size and on the osmotic solute’s metabolic activity; (3) energy-dependent transport of glucose into cells mediates an insidious vasodilation preferentially on small visceral arterioles by an adenosine receptor–mediated mechanism, which constitutes a significant fraction of a glucose-based PDS-mediated reactivity in the visceral microvasculature; (4) hyperosmolality is the major vasoactive component of the conventional PDS, whereas the lactate buffer anion system of this solution is vasoactive only at low pH; and (5) low pH and other ionic contents of conventional PDS modifies the magnitude of vascular reactivity instigated by these solutions.

Technique

Our studies were performed with a standard tissue bath procedure under well-controlled experimental conditions. The tissue under investigation, a small segment of the intestine (2 to 4 cm) or aortic rings (2 mm), was positioned in a relatively large tissue bath in which temperature, pH, P_{O_2}, P_{CO_2}, and osmolality were monitored and controlled while we simultaneously made direct observations of the intestinal microcirculation or continuously record the change in force of individual aortic rings. During the equilibration period, the small intestinal segment and the aortic rings were suffused continuously with a

![Figure 6. Effect of pH perturbation on hyperosmolality-induced macrovascular reactivity. *P < 0.01 versus endothelium-intact aortic rings; §P < 0.05 versus controlled pH by ANOVA and Bonferroni post test.](Image 46x511 to 294x735)

![Figure 7. Lactate-induced macrovascular reactivity. En (+), endothelium intact; En (-), endothelium denuded. *P < 0.01 versus high pH, §P < 0.01 versus endothelium-intact aortic rings by ANOVA and Bonferroni post test.](Image 308x106 to 560x286)
nonvasoactive PSS, which results in variability of <0.5% in vessel diameter and force generation measurements. This small variability in the measurements indicates that changes in vessel reactivity in our studies can be attributed only to a specific experimental intervention rather than to a baseline change in vascular reactivity. The experimental design of our studies does not allow for the simultaneous determination of solute and water transport across the blood-peritoneal barrier. Therefore, other issues such as the effect of bioincompatibility and dialysis solution composition on transperitoneal exchange are not addressed in this study.

**Vascular Reactivity**

In our studies, two standard techniques were used to identify the vasoactive components of conventional PDS and quantitatively describe their vasoactivity. All hyperosmolar solutions, including our control PDS, produced significant vascular reactivity in aortic rings and intestinal microvasculature. In addition, sodium lactate dissolved in isotonic PSS produced a significant vascular reactivity in aortic rings only when H⁺ concentration in the solution was increased. Results of our studies were consistent with literature data, which supported the concept that hyperosmolality and lactate are the major vasoactive components of PDS and other hyperosmolar solutions (1,2,8,12–15).

Mortier et al. (9) found that in the rat mesentery, conventional and new bicarbonate-buffered re-sterilized PDS that contain high-glucose degradation products (GDP) dilate mesenteric arteries (250 to 350 μm), whereas new bicarbonate-buffered solutions that contain low GDP were nearly nonvasoactive. Although none of the measured GDP was singled out as a possible potent vasoactive agent, the authors postulated that a possible combination of the measured GDP is likely the cause of the dilation response. In their study, multiple solutions with or without nitroglycerin (10⁻⁴ M) were randomly tested in the same animal. In addition, the vascular reactivity to the test solution was observed in mesenteric arteries, whereas a change in blood flow in a smaller network arteriole was interpreted to mirror a change in the diameter of the mesenteric artery. Our experience with intravital microscopy of the intestinal microcirculation suggests that testing multiple solutions or pharmacologic manipulations of the tissue bath drastically affects baseline microvascular hemodynamics. In particular, endothelium-independent NO donors such as sodium nitroprusside and nitroglycerin cause a transient maximum dilation followed by a rapid drop in the arterial BP and the local blood flow as a result of systemic absorption of the drug. The vascular reactivity (approximately 20% of baseline) of mesenteric arteries to conventional PDS seems to be of the same magnitude seen in the intestinal A1 in this (Figure 3) and previous studies (8). However, the interpretation of the results as to what component of the dialysis solution is vasoactive differs between our study and that by Mortier et al. (9). There are several potential explanations that could account for the difference in interpretation of the results between the two studies. It is well established that vessels of similar size use different mechanisms for endothelium-dependent regulation of vascular tone depending on vascular bed (16). Similarly, the relative contribution of agonist-stimulated NO and endothelium-dependent hyperpolarizing factor to endothelium-dependent relaxation seems to differ between genders (17), arteriolar size within the same vascular bed (18), and arterioles from different vascular beds (16,19,20). Consistent with these experimentally validated observations is the differential microvascular response within the same vascular bed to specific events such as hemorrhage and sepsis (21,22) or exposure to agonists such as serotonin, angiotensin, and activated complement (23–25). In addition, our experiments were performed in a well-defined vascular bed that has a unique microvascular architect, which is identical in all rats (11). In contrast, the mesentery is relatively void of cells and possesses a poorly defined microvascular network, which accounts for much of the variations in local blood flow in different segments and for the variety of effects of vasoactive agents on the dynamics of the mesenteric microcirculation (26,27).

Data of this study indicate that an isotonic glucose solution causes an insidious and preferential vasodilation in the smaller pre mucosal intestinal arterioles and a slight but significant constriction of the larger inflow arterioles. This unique vasoactive property of the isotonic glucose is mediated by an adenosine receptor mechanism. In contrast, the intestinal microvessels remained at baseline during exposure to an isotonic mannitol solution. However, hypertonic mannitol solutions either as 5% in Kreb’s or as 10% in deionized water caused an instantaneous and sustained dilation similar in magnitude to the Delflex-induced dilation at all levels of the intestinal microvasculature. Although mannitol is an efficient osmotic agent, it is a metabolically inert solute that is totally excluded from cells. These data clearly demonstrate that hyperosmolality is a major vasoactive component by itself but that the mechanisms of this dilation effect may differ depending on the specific metabolic activity of the osmotic solute. Isolated cannulated and pressurized skeletal muscle arterioles elicit a dilation response proportional to increasing concentrations of glucose added to a superfusion solution (5). Similar changes in arteriolar diameter were obtained in response to superfusion with sucrose or mannitol (5). These data suggest that in in vitro models, arteriolar vasodilation occurs in proportion to the degree of hyperosmolality. This contrasts with the aortic vascular reactivity in this study in which the least aortic vascular reactivity was obtained with solution’s osmolality >550 mOsmol/L. Therefore, translation of the in vitro data to the in vivo situation should be approached tentatively. This does not refute that such in vitro vascular models are the gold standard for assessing vascular control mechanisms and the signal transduction pathways of these mechanisms. These and previous data do not support a linear relationship between hyperosmolality and vascular reactivity. However, it seems that the magnitude of such reactivity is modified by the H⁺ concentration, ionic contents, and other vasoactive components of the solution such as lactate, as well as by the specific solute metabolic activity. Indeed, earlier intravital videomicroscopy studies of the rat’s cremaster muscle have shown that the magnitude of arteriolar dilation evoked by hyperosmolar solutions of dextrose, sucrose, or sodium chloride was similar but that the dilation rate constant differs...
among the three hyperosmolar solutions (2). Other perfusion studies of the dog’s forelimb (28) and cat’s ileum (29) have found that both the magnitude and the time course of the dilatory effects of hyperosmolar dextrose and sodium chloride solutions differed significantly.

These data on the intestinal vasoactivity suggest that hypertonic glucose–based solutions dilate the intestinal microvasculature by at least two mechanisms: One is an instantaneous microvascular vasodilation related to the osmotic stress, and the second is a more insidious, time-dependent vasodilation, stimulated by an energy-dependent transport of glucose into cells. This pathway is accounted for exclusively by an adenosine receptor–mediated mechanism as demonstrated in our study. For the vasodilation related to the osmotic stress, we suggest that during crystalloid-induced osmosis, the osmotic water flux through the transendothelial water-exclusive channels (aquaporin-1) is the primary mechanism whereby the endothelium is being stimulated to instigate vasodilation effects. Initial cell shrinkage caused by osmotic-driven water flow results in a relative increase in cellular ionic contents, especially, Ca$^{2+}$ and K$^+$, which are known to stimulate endothelium-dependent dilation pathways. Simultaneously, such initial cell volume decrease triggers a regulatory volume increase characterized by net water and ionic uptakes as well as stimulation of organic osmolyte transporters to restore the original cell volume (30,31). It has been shown that during conditions of osmotic stress, there is activation of Ca$^{2+}$-activated K$^+$ channels and ATP-sensitive K$^+$ channels. Such activation results in a dilation response that can be nearly completely abolished by specific inhibition of these channels (5). Large arteries and other inflow arterioles minimally express aquaporin-1 (32) and possess insufficient adenosine receptor subtypes (33). This explains the subordinate magnitude of dilation seen in these macrovessels compared with the marked dilation observed in the smaller premucosal intestinal arterioles in these studies.

It is generally conceived that peritoneal dialysis–induced vasodilation occurs only initially during a hypertonic dwell. Carlsson and Rippe (34) attributed an inflation of the permeability surface area product (PS) of small solutes to an initial vasodilation during a hypertonic dwell. However, our data suggest that a significant fraction of the dilation response is sustained preferentially in the small precapillary arterioles for as long as these vessels are exposed to an isotonic solution that contains glucose. The influence of this precapillary vasodilation on the mass transfer area coefficient (MTAC; or PS) for small solutes during the late phase of the dwell, where the osmotic gradient has dissipated, remains to be determined. It is likely that relaxation of these small vessels in response to exposure to conventional PDS is detrimental in the number of perfused capillaries and in the modulation of the effective capillary surface area available for exchange during peritoneal dialysis. Thus, the instantaneous submaximal vasodilation at all levels of the intestinal microvasculature including the inflow feed A1, which doubles its blood flow in the initial phase of the dwell when osmolality is high, could provide a plausible explanation for the high PS for small solutes during the early phase of the dwell. With dissipation of the osmotic gradient with time as a result of glucose absorption, a subordinate vasodilation is preferentially maintained in the smaller intestinal premucosal A3, whereas the feed A1 at most restore their baseline diameter and blood flow, which explains the relatively lower PS for small solutes during the late phase of the dwell. In contrast, in peritoneal transport rate studies in humans, the addition of a clinical dose of sodium nitroprusside to a dialysis solution produced no effect on peritoneal fluid kinetics, a slight increase in MTAC for small solutes, but a great increase in macromolecular clearances (35). Similarly, in rabbits, a clinical dose of sodium nitroprusside exclusively enhanced peritoneal macromolecular clearance (36). These clearance data might reflect a change in peritoneal microvascular permeability rather than a vasodilation-mediated modulation of the functional peritoneal surface area in terms of capillary recruitment. For recruitment of functional capillary surface area by dialysis solution–induced dilation, a contact between the dialysate and peritoneal tissue must be established. We have shown that less than half of the mouse anatomic peritoneum is in contact with a large volume of solution in the peritoneal cavity (37) and that agitation or use of surfactant-supplemented dialysis solution increases the fraction of contact area, resulting in enhanced transperitoneal exchange (37,38). Although the visceral peritoneum accounts for approximately 60% of the anatomic peritoneum, its fractional contribution to the overall PS for small solutes is only of the order of 30%, whereas the major fraction of PS for small solutes is accounted for by the much smaller parietal peritoneum (38). This is attributed to the complex geometry of the visceral peritoneum that encompasses “macro-unstirred” pockets of fluid, which equilibrates faster than the rest of the dialysate, limiting small-solute diffusion (38). Furthermore, in single-membrane models, permeability and surface area are multiplicatively linked to form the lumped parameter MTAC, which is difficult to separate. Therefore, pharmacologic targeting of this lumped parameter in an attempt to improve adequacy is more likely to change permeability and surface area simultaneously. Thus, efforts to improve dialysis adequacy should be directed toward improving the wetted peritoneal surface area, which is the anatomic peritoneum in contact with the dialysate, and to create favorable mixed conditions at the visceral peritoneum.

In conclusion, using two different experimental techniques, we demonstrated that hyperosmolality and lactate are the major vasoactive components of clinical PDS. The pattern and the magnitude of such reactivity are dependent on vessel size and on the solutes’ metabolic activity. Low pH of conventional PDS is not a vasoactive component by itself but renders lactate vasoactive. Energy-dependent transport of glucose into cells mediates vasodilation of small visceral arterioles by an adenosine receptor–mediated mechanism and constitutes a significant fraction of PDS-mediated vascular reactivity in the visceral microvasculature. Further investigation is required to define the signal transduction pathway and the molecular mechanisms of hyperosmolality-induced vascular reactivity.


33. Li N, Harris PD, Zakaria ER, Matheson PJ, Garrison RN:


