Long-term Microvascular Response to Hydralazine in Spontaneously Hypertensive Rats

PHILLIP M. HUTCHINS, THOMAS H. MARSHBURN, SARAH J. MAULTSBY, COLLEEN D. LYNCH, THOMAS L. SMITH, AND JERRY W. DUSSEAU

SUMMARY Chronic microcirculatory alterations produced by prolonged use of a vasoactive drug were repeatedly observed in the same skeletal muscle vessels of the dorsal microcirculatory chamber. Arterioles and venules with diameters averaging from 70 to 90 µm, the size range contributing most to peripheral vascular resistance, were measured daily for 6 days to determine differences in diameter, tortuosity, and number of branches. Hydralazine was given as a subcutaneous pellet (2.5 mg), with a release life of 21 days. Hydralazine caused a 39% dilation in arterioles of Wistar-Kyoto rats (WKY) at 3 hours but only an 8% dilation in those of spontaneously hypertensive rats (SHR). At 6 hours, arterioles in both groups were similarly dilated (30-33%). Beyond 6 hours, both SHR and WKY arterioles returned to their prehydralazine control diameter, even though arterial pressure was still reduced. By Day 6, in WKY, but not SHR, there was an increase in the tortuosity of arterioles and a tendency for an increase in their number. Venous diameter was also increased on Day 6, consistent with the fluid retention effect of hydralazine. These data indicate that some so-called vasodilators may cause long-term alterations in growth of vessels rather than an increase in vessel caliber. (Hypertension 12: 74-79, 1988)

KEY WORDS • microcirculation • hypertension • hydralazine • vasodilator

VASODILATORS have been used extensively in the treatment of essential hypertension.1,2 The beneficial effects of vasodilator therapy is generally obtained during long-term use. However, previous microvascular studies of vasodilator action have used acute, anesthetized preparations.3 This study was designed to investigate the skeletal muscle microvascular effects of long-term treatment with a nonspecific vasodilator in normotensive and hypertensive animals. The longitudinal observations to be reported were obtained, using the same microvascular bed, in conscious animals studied over a 6-day period.

Reports from our laboratory and others have shown an altered hemodynamic pattern and microvascular reactivity in hypertension that is consistent with a disturbed long-term regulation of the microvasculature.4-7 These findings suggest that short-term and long-term microvascular reactions to vasodilator application would be expected to differ. Therefore, the hypotheses tested in this study were threefold: 1) Short-term vasoactivity changes in the microvasculature are predominantly caused by short-term changes in vessel caliber; 2) long-term cardiovascular regulation in the microcirculation is effected mainly by long-term changes in the number of arterioles in parallel and in individual vessel length; 3) both the acute and chronic response of the microvasculature to vasodilator application are altered in the spontaneously hypertensive rat (SHR) because of a disturbed local control system.

Materials and Methods
Rats used in this study (6 Wistar-Kyoto rats [WKY] and 6 SHR) were purchased from Harlan Sprague-Dawley (Indianapolis, IN, USA). They were allowed free access to food (Purina Rat Chow,Ralston Purina, St. Louis, MO, USA) and water, with a 12-hour light/dark cycle.

Surgical Procedure for Implantation of Dorsal Microcirculatory Chamber
Dorsal microcirculatory chambers (Carolina Medical Electronics, King, NC, USA) were implanted in the rats when they weighed 150 to 160 g, approximately 2 weeks before caudal artery cannulation. The rats were given an injection of trimethoprim (40 mg)/sulfadiazine (200 mg) (Di-Trim, Syntex), at a dose of 0.1 ml/100 g body weight, 2 hours before

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operation. Animals were anesthetized with a 1:1 mixture of ketamine hydrochloride (100 mg/ml) and xylazine (20 mg/ml) at a dose of 0.1 ml/100 g body weight, injected intramuscularly. Supplemental anesthesia with sodium pentobarbital (2.5 mg/100 g i.p.) was given as needed.

The technique for the preparation of the skeletal muscle chamber was described previously; however, an updated description, detailing the current procedures, will be included here. The removal of hair from the surgical field was accomplished by means of animal clippers and a chemical depilatory agent (Nair, Carter-Wallace, New York, NY, USA). Any residue from the depilatory agent was removed with a wet gauze pad. The area was then cleansed thoroughly with an antimicrobial agent (povidone-iodine, Betadine scrub and Betadine surgical disinfectant, Parke Davis, Sandy, UT, USA).

Four rats were placed dorsal side up on the surgery board. Four 3-0 silk sutures were inserted through the skin and underlying skeletal muscle along the length of the vertebral column. An outline for the right-window incision was patterned by a circular dermal punch. Skin and cutaneous muscle were dissected away from the underlying skeletal muscle and then cut, leaving a hole approximately 1.5 cm in diameter. The rat was then covered with a sterile surgical drape to prevent contamination of the surgical field. Placing the rat left side down, the sutures were tied to a supporting D-shaped frame extended over the surgery board to expose at least 3 cm of skin on the right implant site. Any connective tissue remaining was dissected and removed, leaving exposed the left-side cutaneous maximus muscle. Trauma due to dehydration was minimized by moistening the tissue with a 0.9% sodium chloride solution. The right side of the microvascular chamber was then positioned, and holes for securing screws made. This side was then fitted with 1/22 nylon screws and placed in final position, with the screws gently eased into the respective holes for securing screws made in the tissue. Sterile gauze was placed over the chamber half and, while supporting the body and chamber, the animal was flipped over on the axis of the supporting D-frame to expose its left side. Openings for the screws on the left side were cut in the tissue, and the screws pushed through. The window opening on the left side was marked as it was on the right side. A small incision was made to expose the underlying cutaneous maximus muscle. This muscle was carefully dissected from the skin. Exposed tissue was kept moist with 0.9% sodium chloride solution. After freeing the muscle layer from its connective tissue on the underside of the skin, a cut was made along the edge of the outline and the skin discarded. The stainless steel pins of the right window were punched through the cutaneous maximus with an 18-gauge Luer-stub adapter, and the left window was fitted to the right window. Air was eliminated by injecting 0.9% sodium chloride solution into the incision site. Stainless steel nuts were fitted to the nylon screws and tightened to seal the chamber. The holding sutures were removed from the D-frame, and the animal was placed ventral side down on the board. A chamber clamp was used to keep the chamber stationary until the two halves could be sutured securely together with 2-0 monofilament stainless steel surgical wire. A small amount of cyanoacrylate glue was applied to each screw to keep it secure. A petroleum-based antibacterial ointment (neomycin sulfate/polymyxin B sulfate, Neo-109) was applied topically to the skin where the holding sutures had been placed. A second injection of Di-Trim was given 24 hours after the first.

This surgical procedure differs somewhat from our previous description in that the muscle enclosed in the chamber is only one layer thick; thus, the deep vessels are more clearly visible. In addition, the chamber is tied together with stainless steel rather than polyamide polyfilament (Vetafil) sutures (S. Jackson, Washington, DC, USA). One week postimplantation, the preformed microvasculature has stabilized and no appreciable change in microvessel patterns or dimensions occurs in the untreated animal for several months.

Surgical Procedure for Cannulation of Caudal Artery

Rats were chronically instrumented with caudal artery catheters approximately 2 weeks after the dorsal chamber implantations. The animals were anesthetized as just described. All surgical procedures were performed under sterile conditions. The catheter was inserted into the caudal artery according to the following procedure.

The hair of the proximal part of the tail was clipped, and a small part of the skin covering the ventral tail artery and tail vein was shaved. This area was cleaned with hexachlorophene (pHisohex) and Betadine scrub and swabbed with Betadine solution. A midline incision was made on the ventral side of the tail, and the ventral tail artery was exposed. The artery was then ligated and cut, and a polyethylene PE-10/PE-60 catheter (Clay-Adams, Parsippany, NJ, USA) was inserted and advanced 8 to 9 cm. The distal, PE-10 (10 cm) section of the catheter was wedged to the PE-60 section to render maximal frequency response. The catheter was checked for patency. The wound was then closed using Vetafil sutures. A protective stainless steel cuff was placed around the tail to cover the wound and protect the catheter. Care was taken not to compress the tail. In over 95% of the implantations, no apparent changes were seen in the appearance of the tail. Those rats exhibiting excessive reddening or sloughing of the tip of the tail were excluded from the present study population.

The catheter, surrounded by stainless steel spring stock, was connected to a flow-through swivel (Instech Labs, Horsham, PA, USA), blood pressure transducer, and perfusion pump (infusion rate,
Microvascular Photography and Vessel Analysis

The dorsal microvascular chamber was photographed with a Nikon F3 camera, 55-mm Micro Nikkor lens, and two each PK-11 and PK-13 extension rings (Garden City, NY, USA). The rats were photographed while still connected to the hemodynamic monitoring setup and only lightly restrained by hand. Illumination for focusing was from a fiberoptic light source (Cole-Parmer, Chicago, IL, USA), with the principal photographic light from an automatic Sunpak flash (Model 444D, Berkey, Woodside, NY, USA), transilluminating the back flap chamber. The 35-mm photographs were enlarged to an 11-in. square for computer-based digitization and analysis. Only vessels that were clearly visible in the photographs for each period were included in this study. A typical vessel would traverse over 50% of the dorsal microcirculatory chamber width. The individual vessels in the photographs were digitized on a GTCo DigiPad 11 x 11-in. digitizing tablet (resolution of 0.001 in., or 104 μm, on the enlargements; Model 5, Rockville, MD, USA). The same individual vessels were repeatedly analyzed over the 6-day period with a custom software package that computed the number of branches, diameter, and tortuosity (an index of straightness or growth within the chamber). Tortuosity was defined as the actual length of the vessel between the end nodes or branch points divided by the straight line length between these two end nodes. The end nodes were usually at the circumference of the dorsal microcirculatory chamber and thus more fixed in position than other areas within the chamber.

Vasodilator Administration

The animals were approximately 12 weeks of age at the time of the hydralazine pellet implantation. Hydralazine tablets for implantation were obtained from Innovative Research of America (Rockville, MD, USA), in a total dosage of 2.5 mg/tablet. The manufacturer presents data showing that this proprietary formulation gives a reasonably constant release over a 21-day period, well beyond the 6-day period of this study. The manufacturer recommends the implantation of these pellets in the nape of the neck by subcutaneous puncture with a trocar. We found that a small cut with a sharp scalpel and placement of the tablet in the subcutaneous area of the neck with a forceps produced a neater and easier implantation. Blood pressure and heart rate return to control values within minutes after implantation of placebo tablets.

Protocol

The rats were allowed to recover from the arterial cannulation surgery for 4 or 5 days. Control hemodynamic data and microvascular photographs were obtained at 0900, and the hydralazine pellet was implanted at 1000 on the same day. Microvascular photographs were obtained 3 and 6 hours and 1, 2, 3, and 6 days after the pellet implantation. The daily photomicrographs were always obtained at 0900 for comparison to control and to eliminate any diurnal variance. Hemodynamic data were recorded continuously during the analysis period.

Statistics

The data were analyzed for differences with time by analysis of variance. Individual time point differences from the control value were detected with a paired t test for repeated observations.

Results

The 24-hour average of the WKY mean arterial pressure was 106.82 ± 0.72 mm Hg, while arterial pressure in SHR averaged 144.12 ± 0.97 mm Hg (p < 0.001). Heart rate averaged 332.40 ± 5.37 beats/min for the WKY and 322.90 ± 4.12 beats/min for the SHR (p > 0.05). These values are similar to those reported previously in 12-week-old, unanesthetized, continuously monitored WKY and SHR.

After the implantation of hydralazine, the mean arterial pressure fell to a minimum of 70.6 mm Hg in the WKY and to 90.8 mm Hg in the SHR. This represented an approximately 35% decrease in each animal strain. The minimum occurred during the first hour for the WKY and between the first and second hour after the hydralazine implantation for the SHR. Mean arterial pressure gradually returned toward preimplantation levels (Tables 1 and 2). By Day 6, it was not different from the control value in either WKY or SHR. Heart rate initially decreased in the WKY, whereas an increase was observed in the SHR during the first 6 hours after hydralazine implantation.

The reaction of WKY arterioles to the hydralazine is shown in Table 1. The WKY control (prehydralazine) arteriolar diameter averaged 69.8 μm and would perhaps be classified as "distributing arterioles" in a more traditional nomenclature. As can be seen in Table 1, the arterioles dilated only at the 3- and 6-hour periods, in spite of a decreased mean arterial pressure at later time points. There was
a tendency for the number of parallel arterioles to be increased both early (opening of previously closed vessels) and at 6 days (possibly growth of new vessels). There was a decrease in tortuosity at the 3- and 6-hour periods as the arterioles dilated and became straightened. The increase in tortuosity at Day 6 would be consistent with an enhancement in vessel growth.

The SHR arteriolar response to hydralazine is illustrated in Table 2. The average control diameter of the SHR arterioles was 81.2 μm in diameter. The early response, unlike those of the arterioles, was a retraction in caliber, as illustrated in Table 2. The average control diameter was 91.0 μm, also classifying them as distributing arterioles. The acute dilation and opening of arterioles was similar to that seen in the WKY, although not as pronounced and somewhat delayed. As in the WKY, the arteriolar dilation did not continue for the full blood pressure-lowering period of hydralazine. There was no tendency toward increased arteriolar growth by Day 6 after the administration of hydralazine.

The venular effect of hydralazine in the WKY is exhibited in Table 3. The WKY venules averaged 81.2 μm in diameter. The early response, unlike those of the arterioles, was a retraction in caliber, most likely due to the shift of blood volume to the arterial side of the circulation. There was a tendency for venular dilation at 6 days, which is consistent with the fluid retention properties of hydralazine.

Table 4 demonstrates the results of hydralazine administration on the venules of the SHR, which averaged 91.0 μm in diameter. The pattern of early diameter retraction was similar to that in the WKY, although not as considerable. In the SHR there was also a decrease in the number of open venules at Days 3 and 6. Unlike the WKY, there was no increase in diameter at Day 6.

Discussion

The WKY and SHR used in this study weighed approximately 225 g and had mean arterial pressures of 105 and 145 mm Hg, respectively. These values are consistent with our previous findings in unanesthetized rats and the published literature from the animal supplier. As the SHR blood pressure had increased nearly two thirds of its projected rise, these rats were in the late developmental phase of spontaneous hypertension. The heart rates obtained are also in agreement with those measured in unanesthetized, unrestrained WKY and SHR.

Hydralazine caused comparable reductions (35%) in mean arterial pressure in the WKY and SHR. There was a gradual return to control, prehydralazine pressure values by the sixth day, which was likely brought about by the well-known fluid retention properties of hydralazine. In other studies this effect has been prevented by the simultaneous administration of a diuretic, but it would have complicated the microvascular analysis in this study. The short-term vasoactive effect of hydralazine on diameter and vessel number was to increase both (although not quite significantly in number), whereas its effect on blood pressure was in the opposite direction. The direct, hydraulic effect of a blood pressure reduction is to reduce vessel diameter and number of open vessels. Therefore, the blood pressure changes seen here

**TABLE 1. Effects of Hydralazine on WKY Arterioles**

<table>
<thead>
<tr>
<th>Time of measurement</th>
<th>MAP</th>
<th>Heart rate</th>
<th>Diameter</th>
<th>No. of branches</th>
<th>Tortuosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Hour 3</td>
<td>67.6±2.4*</td>
<td>73.2±4.3*</td>
<td>139.2±17.4*</td>
<td>111.4±12.5*</td>
<td>70.5±9.0*</td>
</tr>
<tr>
<td>Hour 6</td>
<td>73.5±1.1*</td>
<td>94.3±4.6</td>
<td>130.1±10.0*</td>
<td>118.8±14.6*</td>
<td>79.1±7.6*</td>
</tr>
<tr>
<td>Day 1</td>
<td>88.8±1.0*</td>
<td>101.2±0.9</td>
<td>96.6±5.6</td>
<td>117.5±15.2</td>
<td>96.4±5.7</td>
</tr>
<tr>
<td>Day 2</td>
<td>93.9±1.6*</td>
<td>99.8±0.4</td>
<td>100.5±3.9</td>
<td>95.0±5.0</td>
<td>95.0±6.2</td>
</tr>
<tr>
<td>Day 3</td>
<td>94.9±1.9*</td>
<td>96.5±1.0*</td>
<td>104.2±4.7</td>
<td>109.2±13.2</td>
<td>96±6.1</td>
</tr>
<tr>
<td>Day 6</td>
<td>98.4±3.1</td>
<td>95.1±0.8*</td>
<td>104.0±8.3</td>
<td>111.9±17.4</td>
<td>124.9±11.1*</td>
</tr>
</tbody>
</table>

All values are percentage of control ± SEM. MAP = mean arterial pressure.
*p < 0.05, compared with control values.

**TABLE 2. Effects of Hydralazine on SHR Arterioles**

<table>
<thead>
<tr>
<th>Time of measurement</th>
<th>MAP</th>
<th>Heart rate</th>
<th>Diameter</th>
<th>No. of branches</th>
<th>Tortuosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Hour 3</td>
<td>74.2±1.5*</td>
<td>125.3±5.7*</td>
<td>108.9±7.0</td>
<td>108.3±10.2</td>
<td>63.9±12.4*</td>
</tr>
<tr>
<td>Hour 6</td>
<td>81.4±1.0*</td>
<td>122.7±4.2*</td>
<td>133.5±6.7*</td>
<td>113.9±11.1</td>
<td>78.5±13.7</td>
</tr>
<tr>
<td>Day 1</td>
<td>87.0±0.6*</td>
<td>101.2±2.4*</td>
<td>110.3±11.0</td>
<td>97.2±10.6</td>
<td>85.0±35.8</td>
</tr>
<tr>
<td>Day 2</td>
<td>93.3±1.3*</td>
<td>94.9±2.0</td>
<td>100.6±13.5</td>
<td>91.7±8.3</td>
<td>89.5±31.5</td>
</tr>
<tr>
<td>Day 3</td>
<td>99.0±1.2</td>
<td>95.8±1.5</td>
<td>95.5±10.5</td>
<td>97.2±10.6</td>
<td>86.6±33.3</td>
</tr>
<tr>
<td>Day 6</td>
<td>102.6±4.0</td>
<td>92.3±2.2</td>
<td>94.2±9.4</td>
<td>86.1±7.4</td>
<td>97.4±28.9</td>
</tr>
</tbody>
</table>

All values are percentage of control ± SEM. MAP = mean arterial pressure.
*p < 0.05, compared with control values.
TABLE 3. Effects of Hydralazine on WKY Venules

<table>
<thead>
<tr>
<th>Time of measurement</th>
<th>Diameter</th>
<th>No. of branches</th>
<th>Tortuosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Hour 3</td>
<td>83.4±13.6</td>
<td>89.6±8.8</td>
<td>85.9±8.1*</td>
</tr>
<tr>
<td>Hour 6</td>
<td>86.1±11.7</td>
<td>89.3±7.5</td>
<td>80.1±9.0*</td>
</tr>
<tr>
<td>Day 1</td>
<td>102.8±4.9</td>
<td>103.7±5.2</td>
<td>%4±4.9</td>
</tr>
<tr>
<td>Day 2</td>
<td>103.9±3.8</td>
<td>104.2±4.9</td>
<td>98.2±4.9</td>
</tr>
<tr>
<td>Day 3</td>
<td>99.7±4.9</td>
<td>101.1±5.8</td>
<td>95.2±12.9</td>
</tr>
<tr>
<td>Day 6</td>
<td>124.4±14.9</td>
<td>95.8±3.7</td>
<td>99.1±6.5</td>
</tr>
</tbody>
</table>

All values are percentage of control ± SEM.
*p < 0.05, compared with control values. For mean arterial pressure and heart rate values, see Table 1.

would tend to reduce the observed responses rather than be responsible for them.

The control diameters of the arterioles and venules of the SHR were slightly, but not significantly, larger than those seen in the WKY. These values are also in accord with observations by ourselves and others in both acute and chronic, unanesthetized preparations. This finding, by itself, is an indication that the short-term regulation of the observed microvasculature operates at approximately the same internal diameter set point. In this manner, the blood vessel is at the appropriate diameter for its position along the vascular tree and can exert bidirectional control. The wall stress, however, would likely be elevated, in that the higher arterial pressure in the SHR is not attenuated proximal to these vessels. According to the hypothesis of Gore, the position along the vascular tree of "optimal wall stress" for vasoactive agent reactivity would be shifted upstream. This shift could potentially explain the difference in the extent and time course of the vasodilatation after hydralazine treatment in WKY and SHR arterioles.

The venous diameter changes appeared to follow purely passive, stress-relaxation-induced responses to the predictable venous pressure fluctuations. After the administration of hydralazine, venous diameter was decreased early in the SHR and, possibly, in the WKY, as would be expected with the shift of blood to the arterial side and the concomitant reduction in venous pressure. The increase in WKY diameter at Day 6 is consistent with fluid retention and an increase in venous pressure. Why the SHR venules did not exhibit this response is an interesting question that is not answered by this study.

Our laboratory and others have reported a reduction in the vasodilating capacity of SHR arterioles. Some investigators have ascribed this to a structurally based defect, while others have suggested that it is a functional defect. In all likelihood, the defect is both structural and functional. Our current data do not allow for the separation of the respective contributions of structural and functional alterations. In any event, the arteriolar diameter returned to control values by the first day after hydralazine implantation, in spite of continued blood pressure reduction. This finding is further evidence that the long-term regulation of the microcirculation is not effected by changes in arteriolar diameter. Long-term control of the microcirculation and the long-term response to the "vasodilator" hydralazine appear to be brought about by adjustments in the number of parallel vessels and tortuosity. The Day 6 increase in tortuosity in the WKY arterioles was not seen in those of SHR. Again, this finding is consistent with an altered long-term microvascular regulation in the SHR. In fact, there was a tendency for SHR arterioles to exhibit a reduction in number of arterioles on Day 6.

Since we were not able to obtain measurements of plasma levels of hydralazine, the possibility exists that plasma levels were declining due to a gradually decreasing absorption from the pellet. Pellet implantation generally results in slow connective tissue encapsulation, which could reduce absorption. The differences in the time course and magnitude of the microvascular responses to hydralazine may be due to the way hydralazine is absorbed and distributed in the SHR versus the WKY. As stated previously, however, the manufacturer of the pellets has measured relatively constant plasma levels for 21 days after implantation.

In summary, hydralazine causes a true vasodilation early after administration that is not maintained into the first day. The long-term effects of hydralazine appear to be changes in vessel number or tortuosity (or both), reflecting alterations in vessel growth. Compared with the WKY, the SHR has a reduced short-term and long-term response to hydralazine administration.

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