PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 163, 326–330 (1980)

Response of Small Arteries in the Rat Cremaster Muscle to Decreases in Perfusion Pressure (40771)

R. J. MORFF AND H. J. GRANGER

Department of Medical Physiology, College of Medicine, Texas A&M University, College Station, Texas 77843

The diameters of the small arteries feeding a microcirculatory bed are determined by the interaction of a number of controlling factors. Neural, humoral, and physical factors all interact to modulate the arterial diameter based on the prevailing local and systemic conditions. The neural and humoral systems are undoubtedly active mechanisms involved in feedback control systems to regulate tissue flow. However, changes in diameter as a result of physical forces, namely, changes in intralumenal pressure, may simply be a local response resulting from changes in vessel wall stresses. There are a number of experimental and pathological conditions in which studies of microvascular diameters are accompanied by significant decreases in systemic pressure. For example, previous studies (1, 2) have shown that severe systemic hypoxia in the rat produced a substantial decrease in arterial pressure, and direct measurement of small artery diameters in the rat cremaster muscle showed significant decreases in diameter during the hypoxic forcing (3). Since the decreases in artery diameter are accompanied by decreased systemic pressure, it is logical to assume that at least a portion of the diameter change could be due to simple mechanical relaxation of the vessel wall caused by decreased intralumenal pressure.

To determine the responses of the small arteries in the rat cremaster muscle to decreased intralumenal pressure, we performed graded occlusions of the abdominal aorta to reduce the pressure perfusing the cremaster muscle, while measuring the diameter of the cremasteric microvessels. The results obtained should help to determine the contribution of changes in intralumenal pressure to the changes in diameter of these small vessels during conditions which involve substantial decreases in perfusion pressure such as hemorrhage, systemic hypoxia, anaphylactic shock, or hypotension.

Materials and methods. The results reported in this study were obtained using male, Sprague–Dawley rats anesthetized with sodium pentobarbital (50 mg/kg, ip). Alterations in the pressure perfusing the cremaster muscle were induced by partial, graded occlusion of the abdominal aorta. A midline abdominal incision was used, and the intestines pushed aside to expose the aorta. A short loop of umbilical tape was then placed under the aorta, just above the bifurcation, and this loop was brought out through the abdominal incision. By clamping the ends of the umbilical tape with a hemostat and gently rotating the loop, graded occlusions of the aorta could be induced, decreasing the perfusion pressure downstream. Blood pressure was obtained by cannulating the left femoral artery with polyethylene tubing (PE 100) pulled to a fine taper, and filled with a heparinized saline solution (0.9% NaCl, 10 units/ml heparin). The cannula was connected to a straingauge transducer (Statham P23Db) and the mean arterial pressure was recorded on a chart recorder (Grass Instruments Model 7D). The blood pressure at the femoral cannula responded rapidly to changes in the diameter of the abdominal aorta and could be controlled by altering the degree of aortic occlusion.

To determine the changes in vascular diameter associated with changes in perfusion pressure the small artery in the right cremaster muscle was directly observed using an intravital television microscopy system. The surgical preparation of the cremaster muscle employed is a modification of the techniques developed by Majno

0037-9727/80/030326-05$01.00/0

(4) and Baez (5), and is described in detail elsewhere (6). Briefly, the scrotal sac was cut longitudinally and the testicle was gently teased away from the sac. The testicle was then cleared of connective tissue to expose the underlying cremaster muscle, which was kept moist with a modified Krebs solution (described below) throughout the surgical procedure. Next, the cremaster muscle was cut longitudinally from the distal tip to the external inguinal ring, taking care to position the cut as far as possible from the major artery and vein which entered the muscle. After this incision, the testicle was gently pulled away from the cremaster muscle and was pushed through the inguinal canal into the abdominal cavity. The rat was then placed on his back on a heating pad and positioned on a Plexiglas board fitted with a cremaster bath chamber. The cremaster muscle was secured in a relatively flat position over a cover glass within the chamber by five ties of 5-0 suture which were placed at equal intervals around the margin of the cut muscle. The bath chamber was then filled with a modified Krebs solution (25.5 m*M* $NaHCO_3$, 112.9 m*M* NaCl, 4.7 m*M* KCl, 2.5 m*M* $CaCl_2 \cdot 2H_2O$, 119 m*M* $MgSO_4 \cdot 7H_2O$, and 11.6 m*M* dextrose) which was heated intermittently by electrical current which passed through a coil of nichrome wire submerged in, but electrically isolated from, the bath chamber. Using this heater system, bath temperature was controlled at 34.5°C. Bath pH was monitored and controlled at 7.40 ± 0.05 units by bubbling CO_2-N_2 gas mixtures through the bath solution. Bath PO_2 and PCO_2 were approximately 40 and 60 mm Hg, respectively, in all of the experiments in this study.

To observe the cremaster circulation the image of the transilluminated muscle was projected through the third port of a trinocular microscope into a television camera, and the video image (with approximately 800× total magnification) was observed on a TV monitor. The internal diameter of the vessels studied were determined by measuring the diameter directly in the TV screen with a clear plastic rule. The exact magnification was determined using a stage micrometer after each experiment and a calibration factor was calculated to convert the measured diameter (mm) to the actual vessel diameter (microns). Diameter measurements were recorded at 30-sec intervals throughout each experiment.

The experimental protocol consisted of a 10-min control period followed by a 10-min occlusion period. Throughout both periods, mean arterial pressure at the femoral cannula was recorded continuously along with the diameter measurements. After the control period the abdominal aorta was partially occluded to reduce the femoral pressure to a new value, and the pressure and diameter were recorded for 10 min. If more than one occlusion period was observed in any one animal, a 10-min recovery period was observed after release of the previous occlusion to allow the pressure and vessel diameter to return to control values.

To compensate for differences in control pressures and diameters, the mean pressure and mean diameter during the control period were determined, and the changes which occurred during occlusion were calculated as percentage changes from these control values. Statistical differences between the control and occlusion period values were determined using the paired Student's *t* test.

Results. The responses of the small arteries in the rat cremaster muscle to reduction in perfusion pressure were observed during 31 periods of partial aortic occlusion in 10 experimental animals. Table I presents the body weights and the average values during the initial control period for the mean arterial pressure (MAP) and small artery diameter (AD) for each of the experimental animals. The average MAP for all 10 animals was 109 ± 16.4 mm Hg (all data in this manuscript will be expressed as mean ± 1 standard deviation), while the mean diameter for the small arteries for all animals was 121 ± 14.8 μm. In all cases, the small artery in which responses were determined was the main artery supplying the cremaster, which was visualized as close as possible to the exit from the inguinal canal.

In general, decreases in perfusion pressure were accompanied by rapid and sustained decreases in small artery diameter, and the artery diameters returned rapidly to

TABLE I. CONTROL PRESSURES, DIAMETERS, AND WEIGHTS [a]

Animal No.	Weight (g)	MAP (mm Hg)	AD (μm)
1	186	85	126
2	184	110	144
3	225	112	141
4	121	108	124
5	95	88	94
6	158	125	121
7	137	125	126
8	154	135	106
9	191	95	117
10	176	110	115
$\bar{x}$ ± SD	163 ± 37.9	109 ± 16.4	121 ± 14.8

[a] This table presents the body weight and the average blood pressures and small artery diameter studied. The first column gives the animal number; the second column the animal's body weight in grams; the third column the average mean arterial pressure (MAP) during the control period (mm Hg); and the last column the average diameter (μm) of the small artery during the control period. The average values for all 10 animals ($\bar{x}$ ± SD) are shown at the bottom of each column.

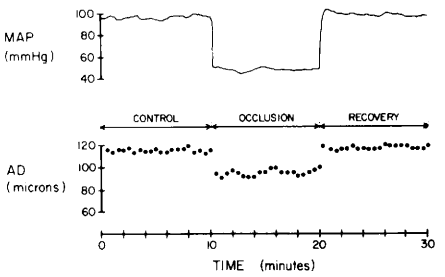

FIG. 1. Typical experimental result. This figure shows the response of the small artery in the cremaster muscle to decreased perfusion pressure. The upper record is mean arterial pressure (MAP) in millimeters Hg, recorded from a femoral artery cannula. The lower record is the diameter (AD) of the small artery in micrometers. The first 10 min represent a control period. During the second 10-min period the abdominal aorta was partially occluded, reducing the femoral pressure to about 50 mm Hg. During the final 10-min period the occlusion was released and the femoral pressure returned to the control level. Artery diameter decreased during the occlusion period and returned to control levels upon release of the occlusion.

control values upon reinitiation of normal perfusion pressure. A typical response during one occlusion period is shown in Fig. 1. This figure shows the pressure recorded in the femoral artery and the diameter (recorded at 30-sec intervals) of the small cremaster artery during control, occlusion, and recovery periods each of 10 min duration. Figure 1 shows that a rapid decrease in perfusion pressure from about 97 to about 50 mm Hg resulted in a rapid reduction in small artery diameter from approximately 117 to 95 μm in this animal. Femoral artery pressure and small artery diameter remained relatively constant during the occlusion period, and returned rapidly to control, without discernible overshoot, when the partial occlusion was released.

A summary of the data for all 31 occlusion periods is presented in Fig. 2, where the percentage decrease in artery diameter from control values (D_{AD}) is plotted as a function of the percentage decrease in perfusion pressure (Dp). Each point within this figure represents the results of one occlusion period. The percentage decrease in blood pressure for each occlusion was determined by sampling the blood pressure record at 30-sec intervals and calculating the average values for each occlusion period and for the immediately preceding control period. Percentage decreases in artery diameter were calculated in a similar

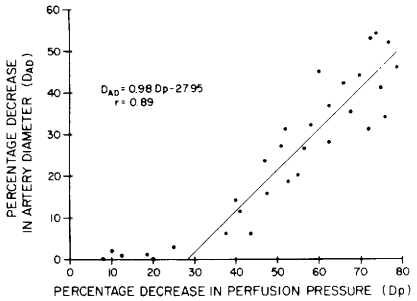

FIG. 2. Regression of percentage decrease in artery diameter (D_{AD}) vs percentage decrease in perfusion pressure (Dp). Each point on this figure is the result for one occlusion period. The six data points to the left of the figure ($Dp < 30\%$) were not significantly different from zero ($P > 0.01$). The response (D_{AD}) for all other occlusion periods ($Dp > 30\%$) were significant ($P > 0.01$). The regression line for these significant points is shown in the figure, along with the regression equation and the correlation coefficient ($r = 0.89$). This correlation was significant.

fashion. Subsequent occlusions in the same animal were not initiated if the blood pressure was unstable, or if the mean pressure during any control period differed by more than 5% from the mean of any previous control period. Examination of the data presented in Fig. 2 showed that substantial decreases in small artery diameter accompanied decreases in perfusion pressure. Statistical analysis revealed that the percentage decreases in artery diameter for the six data points (from three different animals) below a 30% decrease in blood pressure were not significantly different from zero response ($P > 0.05$), whereas all data points for pressure decreases greater than 30% did represent a significant decrease in small artery diameter. Therefore, a linear regression equation was determined, using the method of least squares, for the data points with greater than 30% blood pressure reduction. This analysis yielded the equation:

$$D_{AD} = 0.98Dp - 27.95$$

where D_{AD} = percentage decrease in small artery diameter, and Dp = percentage decrease in perfusion pressure. This line has been plotted in Fig. 2. The correlation coefficient for this equation, $r = 0.89$, indicates a significant correlation ($P < 0.01$) between the amount of blood pressure decrease and the amount of artery diameter decrease when the blood pressure decrease is greater than about 30% of control values. Calculation of the x-intercept for this regression equation yields a value of 28.52. The slope of the regression (0.98) indicates that, for perfusion pressures less than 30% of control, essentially a one-to-one relationship exists between the artery diameter and the perfusion pressure, i.e., a 10% decrease in perfusion pressure is associated with a 10% decrease in the diameter of the small arteries.

Discussion. The results presented in this study indicate that substantial decreases in the diameter of the small arteries in the rat cremaster muscle accompany decreases in perfusion pressure in excess of about 30% of the control value. Blood pressure was measured at the femoral artery while graded occlusion of the abdominal aorta was used to reduce the pressure perfusing the cremaster muscle. It has been assumed that the femoral artery pressure is a reflection of the pressure at the major artery supplying the cremaster at the point where this artery first becomes visible in the *in vivo* preparation. The anatomical basis for this justification is that the cremasteric artery is derived from the external spermatic artery, which branches directly from the femoral (7). Bohlen *et al.* (8) measured pressures directly in the rat cremaster using a servo-null system and found that the pressures in the first order arterioles (84.5 μm diameter) were approximately one-half of the systemic pressures. Although these vessels were slightly smaller than the vessels observed in the present study (possibly due to differences in animal weight or in the area of observation within the cremaster) this data does suggest that the pressure in these vessels will be somewhat lower than systemic pressure. In any case, the percentage change in pressure within the cremaster small artery during aortic occlusion should be very similar to the percentage change in femoral pressure, and, since we have not attempted to calculate wall stresses, an exact knowledge of the absolute values of the pressures within these vessels is not necessary.

We were initially concerned that partial occlusions of the abdominal aorta might substantially alter pressures proximal to the occlusion and thus evoke other mechanisms (e.g. carotid baroreceptor) that would influence the diameter of the microvessels under study. To test this, we conducted a series of preliminary studies in which blood pressures were recorded at the femoral artery and the carotid artery before and during occlusions of the abdominal aorta. Rapid total or graded occlusion of the abdominal aorta did not alter carotid artery pressure in these studies, and, therefore, central baroreceptor mechanisms should not have contributed to the observed changes in cremaster arteriolar diameters.

The small artery diameters in this study decreased rapidly upon reductions of the intravascular pressure greater than 30% of control, and they remained essentially constant as long as the decreased pressure was

maintained. However, since flow measurements were not determined, and since this study did not involve observations at the smaller arteriolar level, one cannot conclude from this data that autoregulation is absent. In addition, responses such as those presented in Fig. 1 show no overshoot in artery diameter upon release of the occlusion, which suggests that a reactive hyperemia was not present. However, it must be emphasized again that we did not make flow measurements, and it is possible that hemodynamic changes downstream could have resulted in increases in velocity, and hence increased flow, without substantial increases in diameter beyond the control level.

Overall, the results of this study indicate that the diameters of the small arteries within the cremaster muscle can decrease directly in response to reductions in intralumenal pressure, and that this response becomes quantitatively significant when systemic pressures are reduced by amounts greater than about 30% of their control values. Studies of these vessels which involve experimental forcings which result in systemic pressure alterations of this magnitude should take this into consideration, and the data presented in this study provides a mechanism for quantitative evaluation of the magnitude of this component.

The authors would like to express their gratitude to Christina L. Tompkins for her excellent technical assistance. This study was supported by Organized Research funds from the Office of University Research, Texas A&M University. Dr. Granger is the recipient of a Research Career Development Award (HL-00409) from the National Heart, Lung and Blood Institute.

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Received July 6, 1979. P.S.E.B.M. 1980, Vol. 163.