The Microcirculation in Experimental Hypertension

State-of-the-Art Review

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SUMMARY Information concerning the microcirculation has been obtained largely by intravital microscopy of selected tissues in the rat-skeletal muscle and splanchnic viscera, as well as in the cheek pouch of the hamster. The data for the most part pertain to the spontaneous form of hypertension in the SHR strain of rats and renovascular forms of the disease involving surgical manipulation of the kidney or its blood supply. Direct measurements of pressure and flow in exteriorized skeletal muscle preparations reveal a 2- to 3-fold increase in resistance to flow. An increase in resistance appears in the terminal arterioles and their precapillary branches as early as at 4 weeks of age for SHR animals and is progressively exacerbated in older more mature animals. The increase in microvascular resistance appears to develop as a consequence of neurogenic and humoral mechanisms which act initially on larger arterioles (50-100 μm) and subsequently on the peripheral arterioles (15-25 μm in diameter). The smaller arterioles not only show an increased tone under steady state conditions but what can be referred to as a functional rarefaction in which 30% to 50% of the precapillary extensions of the arterioles are shut off for varying periods from the active muscle circulation. Structural rarefaction (a reduced number of arterioles) is seen only in the late stages of the SHR syndrome and can account for a small portion of the increase in peripheral resistance. The cause and effect relationship between the microvascular changes and the associated elevation in systemic pressure cannot be unequivocally demonstrated in intravital preparations. Hypertension induced by different experimental modalities does not have a consistent microcirculatory counterpart, leading to the conclusion that depending on the precise mechanisms involved in the initiation of the syndrome, microcirculatory derangements can arise as either initiating or secondary phenomena. Tissue pathology is uniformly associated with a reduction in arterial supply, and uneven distribution of blood and red cell hematocrit in the network proper. On the postcapillary side, there is a trend for venular tortuosity and distension.

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Key Words • microvascular resistance • microcirculation • SHR hypertension • rarefaction in hypertension • micropressure vs systemic blood pressure • microvessel reactivity hypertension • etiology • experimental hypertension

The key manifestation of hypertension by definition is a progressive elevation of systemic blood pressure that is paralleled by a comparable increase in peripheral vascular resistance. Under normal circumstances the systemic blood pressure is maintained and buffered by the action of the heart in concert with neurohumoral mechanisms that adjust the run off of blood into the various tissues. This latter function is achieved by a narrowing or widening of the arterioles just proximal to the mass of tissue that they supply. Although cardiac manifestations may be of importance initially, a key ingredient of this insidious elevation of blood pressure would appear to be the concomittant rise in vascular resistance. It can be appreciated that an uncoupling of one or more of the many different factors that can influence blood pressure would bring about a hypertensive state. An extraordinary amount of research has been directed at these basic mechanisms through the use of whole animal methods,1 organ studies,2 3 and in vitro analyses of isolated blood vessels.4 5 With refinement of the tools for quantifying microvascular behavior, detailed information through intravital microscopy has become available concerning vessels directly involved in maintaining peripheral resistance in representative tissues.6 8 No consensus exists, either as to the primacy of neurogenic as opposed to blood-borne humoral mechanisms, or more important, as to the cause and effect relationship between the elevation in systemic blood pressure and the associated microcirculatory disturbances during the evolution of the hypertensive syndrome.
The multifaceted nature of the problem is reflected by the fact that a hypertensive state can be reproduced experimentally by a whole array of models — dietary, hormonal, renal, neurogenic, gland ablation, or genetic selection. Each of these modalities in turn can be modified by a range of variables so that the extensive data on the subject in the literature are difficult not only to analyze but to distill into some generalized scheme. The lethal course of the syndrome is related to a microvascular insufficiency, which culminates in pathology in especially susceptible organs such as the heart, brain, and kidneys.  

Detailed examination of the status of the microcirculation during hypertension offers a unique opportunity for sorting out suspected etiological factors. Limited information on the microcirculation can be obtained in man by studying external structures such as the retina, bulbar conjunctiva and nailfold of the digits. More precise quantitative data concerning the operation of the microcirculation as an organic unit can only be obtained in animal preparations. Although immobilization for vital microscopy reinforces a number of uncertainties such as the effects of general anesthesia, the relevance of the model syndrome to humans, or surgical intervention for exteriorizing tissues for microscopy, the insight afforded by the intravital approach provides basic information not available by any other method. Hopefully in the future, as a background of detailed information sharpens our focus, indirect methods applicable to man can be introduced.

The majority of intravital studies dealing with microvascular behavior in hypertension have been carried out on skeletal muscle, the intestinal wall, or the skin. A variety of hypertension models have been used: spontaneous forms of hypertension in inbred strains of rats, interference with kidney blood flow, high salt intake without hormonal manipulation, or with DOCA, stimulation or ablation of centers in the brain, etc. By far the largest number of investigations have utilized the spontaneously hypertensive rat (SHR) an inbred offshoot of the Wistar-Kyoto strain. The applicability of this model to essential hypertension in humans has been questioned since the precise etiology of the syndrome in these genetically predisposed animals has not been established.

Limitations of space make it necessary to focus the present analysis on microcirculatory rearrangements associated with hypertension to the SHR animal model and particularly with microhemodynamics in skeletal muscle preparations. In view of the substantial contribution of the large mass of skeletal muscle to peripheral resistance, such constraints should not detract from the broad objectives of this overview.

Before assessing the relationship of microvascular changes to the etiology of hypertension, the term "arteriole" as it relates to peripheral resistance must be defined more precisely. Its generic implication as a small artery can include vessels ranging in size from 100 to 125 μm wide down to 8 to 10 μm precapillaries. Not only are these vessels structurally different but their dominant control mechanisms differ. Both neurogenic and humoral mechanisms have been shown to be involved in the vasomotor activities of the microcirculation, but their relative contribution varies with particular tissues. The influence of the autonomic nervous system is gradually diminished along the successively smaller segments of the microvascular tree until local factors or blood borne substances predominate and are responsible for the highly localized, autoregulatory readjustments. In an anatomical context, the innervation of the muscular microvessels becomes progressively more sparse until many of the more distal smooth muscle cells show no direct contact with nerve endings. The most peripheral precapillaries fail to respond to stimulation of the nervous system. Strength of stimulus cannot be responsible, since higher frequency electrical stimulation causes only a further narrowing of the larger arterioles.

The smaller arterioles (< 20 μm) show a more pronounced response to the various vasoactive agents that have been proposed as potential contributors to the hypertensive syndrome. It is not known whether this difference is due to the physical properties of the wall (e.g., wall:lumen ratios) and therefore, to the actual workload, or to a higher intrinsic reactivity of the muscle cells. Thus although the state of constriction or dilation of the entire hierarchy of arterioles will have an effect on the overall peripheral resistance, a distinction should be made between the contribution of the larger arterioles and that of the more distal extensions of the tree within the microcirculation proper. On the venular side the muscle cells have significantly different dose-response characteristics as well as specificities presumably due to the unique distribution of surface receptors.

**Blood Flow Relationships**

It has been fairly well established that the overall blood flow to skeletal muscle in hypertension, including the SHR rat model, is essentially the same as in normotensive controls even where systemic blood pressure has been increased by 70% to 90%. Unfortunately, few quantitative studies on flow distribution within the microcirculation of normotensive and hypertensive preparations are reported in the literature. Our own measurements on SHR muscle preparations of rats as young as 4–5 weeks of age show essentially normotensive flow levels in the arterioles and capillaries at a time when the trend toward an elevated systemic blood pressure is already manifest. Later, by Weeks 10–15, despite a further increase in systemic pressure in SHR, volumetric flow rates remain essentially within the normotensive range throughout the successive segments of the arteriolar branchings including the precapillaries proper. Evidently, in view of the substantial increase in systemic blood pressures in hypertensives, some factor or set of factors must be operating to provide the increase in vascular resistance needed to maintain flow in the normotensive range.
along the successive segments of the arteriolar-precapillary hierarchy of vessels.

The phenomenon of microvascular resistance is usually dealt with in a hydrodynamic context employing Poiseuille flow terms to describe pressure and flow relationships. Although such an approach is useful for individual vessels, the microcirculation is a complex network of in-series coupled and in-parallel coupled vessels with unusual interconnections such as artery to artery arcades, shunt pathways, and a continuously shifting pattern of hematocrit distribution. No completely acceptable method has been developed for calculating the distribution of resistance values in such complex networks.

### Blood Pressure Distribution

In weighing the etiological implications of the flow data, it is of interest to determine whether this increase in resistance in SHR is uniform or selective in its location. A useful guide in this regard is the distribution of pressure within the microcirculation during hypertension. Depending on the tissue topography, the reduction in pressure along the successive segments of the microvascular network can be gradual, as in mesentery, or show a sharp gradient in the region of the terminal arterioles (below 25 μm in diameter) in various skeletal muscle beds or in the intestinal wall. Micropressure distribution plots show the site of major resistance to be located in the arteriole-to-precapillary region of the microvascular network. Close inspection reveals that systemic pressure is lowered in two separate steps. Pressures in the small arteries entering the muscle proper have already been lowered by some 40% in normotensive WKY preparations. In SHR preparations, pressures in the large (100 to 125 μm) arterioles feeding the tissue are 50% to 55% below systemic levels. Inasmuch as these more proximal arterioles are almost exclusively under control of the nervous system, such evidence favors the participation of a neurogenically related mechanism during this early phase.

Regional differences probably exist even within different muscle preparations. Pressure measurements by Bohlen and coworkers in the cremaster muscle of 5- to 6-week-old SHR show proportionately higher levels in arterioles and precapillaries in line with the change in systemic blood pressure. Comparable pressure gradients have not been established in this muscle for more mature hypertensives.

It should be kept in mind that pressure gradients within the microcirculation can be modified by the state of neurogenic tone as shown by the effects of nerve stimulation on the small intestine. Inasmuch as exteriorized preparations of skeletal muscle and intestine have to be studied under general anesthesia, factors of this kind must have a significant influence on vascular tone and hence on pressure-flow distribution within the terminal vascular network.

Furthermore, observations on renal-clip hypertension show that the normal gradient of successively higher reactivity to changes in PO2 and PCO2 in the smaller arterioles is shifted until the constrictor response of the large arterioles is equivalent to that produced in the smaller arterioles. Such a shifting relationship makes it difficult to interpret either changes in steady state diameter or in the magnitude of the diameter change upon stimulation with respect to mechanism in hypertensive preparations.

### Branch Conditions

In setting up models of the microvascular network, consideration is given to factors such as length, diameter, branching frequencies, as well as the relative number of vessels at the successive cross sections of the network. However, not enough attention has been given to the progressively larger contribution of in-parallel coupled vessels to the branching pattern. These latter vessels are deployed as side arm offshoots so that the nature of entry conditions into them becomes a critical factor affecting pressure-flow relationships in the network (fig. 1). The branches are encircled in the junctional region by a coil of smooth muscle cells. During hypertension, the increase in smooth muscle tone throws the endothelium of these narrow vessels
into folds and creates noncircular, irregular cross sections. The contours of the entry into side branches will be distorted by a narrowing of both the parent and daughter portion of the branch so that movement of blood into the precapillaries encounters a disproportionately high resistance. This effect is strikingly illustrated by the much sharper drop in pressure from the feeding arteriole into its transverse arteriole branch in SHR.

As a consequence an almost 1.6-fold increase in arteriolar resistance in hypertensive preparations is evident across this discrete location in the microvascular network. Here, again, the change is functional in nature since local dilation with papaverine or nitroprusside equals WKY and SHR resistances in this region.31

Despite the fact that the pressure drop in the arterioles proximal to the spinotrapezius muscle is about 20% greater in hypertensives than in normotensives controls, blood pressure levels in the arteriolar arcades within the muscle proper are still about 25 to 30 mm Hg above normal. The subsequent sharp fall in pressure in the transverse arteriole-precapillary region can be accounted for by several factors. Overall tone of transverse arterioles (as measured by papaverine dilation) is about 30% to 35% higher in SHR.30 In addition, the number of the succeeding precapillary branches that remain open to the active circulation is about half that in WKY controls. After application of papaverine, the number of actively perfused precapillaries is the same as in WKY controls showing that there are no substantial structural alterations during the formative phases of the hypertensive condition.

Rarefaction Concept

The concept that arteriolar narrowing is the factor responsible for the increase in microvascular resistance in SHR has not been supported by direct observations of the microcirculation. Earlier studies in the literature on the cremaster muscle indicated that the diameter of arteriolar vessels with an active flow in hypertensive preparations on the average is not reduced.14, 18, 32 In these preparations, however, counts of A3 type arterioles (20–35 μm wide) showed some 30% to 45% fewer vessels open to the active circulation in SHR than in WKY controls. Similar reports of arteriolar rarefaction have been made for mesentery,8 skin,20 and the anterior gracilis muscle of the SHR preparations,14 but with different overall percentages and no clear distinction between functional closure of these arterioles as opposed to structural modification. Such calculations are somewhat misleading since these same studies emphasized that the number of arterioles (so-called “A3 type”) open to the circulation was reduced in hypertensives by 35% to 40%, an observation that by itself indicates that a substantial number of vessels were not only narrowed but had changed diameter sufficiently to obliterate the vessel lumen. Chen and coworkers33 found that most of these vessels could be reopened to the active circulation by local administration of a vasodilator agent, or by the section of the sympathetic nerve supply to the tissue. Inasmuch as the reduction in number involves mostly small arterioles the rarefaction phenomenon may account for only a small fraction of the rise in microvascular resistance. In fact, recent morphological studies by Gray34 were unable to confirm the structural rarefaction concept using the spinotrapezius muscle preparation.

Just what factors lead to a changeover from functional rarefaction to structural rarefaction is not well established. The latter apparently occurs well after the blood pressure has plateaued out in the hypertensive range. In some ways this concept is merely a modification of previous studies that indicated an increased sensitivity of arterioles during the hypertensive state except for the proviso that some smaller arterioles and precapillaries may be unstable in a partially narrowed state so as to close down completely. In terms of mechanisms responsible for this type of change Dusseau and Hutchins26 have suggested that β, adrenergic receptors may be involved since β, agonists such as salbutamol29 were found to stimulate the formation of additional arterioles.

Smooth Muscle Reactivity

Numerous reports in the literature, based on pharmacological and biochemical studies of vascular smooth muscle behavior, favor a heightened responsiveness of vascular smooth muscle to specific vasoactive stimulants such as norepinephrine5 angiotensin II,23 a more generalized reaction involving calcium channel blockers,22 or an ionic imbalance.22 Such intervention during hypertension could produce not only a narrowing of arteriolar vessels under steady state conditions but an exaggerated reaction to spontaneously occurring fluctuations in pressure and flow. In renovascular hypertension in the hamster,4 an increased sensitivity of the arterioles exists but is associated with little or no change in vessel lumen diameter or in wall lumen ratios during the formative stages of the hypertension.

In addition to a resetting of the intrinsic vascular smooth muscle threshold and reactivity patterns in hypertension, the suggestion has been advanced23 that small vessels in which wall hypertrophy occurs have a mechanical advantage and show a greater narrowing with identical smooth muscle responses because of more favorable wall thickness to lumen ratios.35 Conceivably, then, a structural modification at the arteriolar level could account for the apparent heightened reactivity to constrictor stimuli. In addition the physical encroachment on the vessel lumen would serve to limit the capacity of these arterioles to be opened under conditions of need. Support for wall hypertrophy has not been forthcoming from intravital studies of the microcirculation.7, 30 except for observations on renal clip hypertension where the larger feeding arterioles (75 to 150 μm wide) show a significant increase in the wall thickness to lumen ratio.49 It should be pointed out...
that these late changes occur only in vessels where the prevailing blood pressure remains some 30% to 45% above normotensive levels.

Tone

Recent evidence indicates that the arterioles in general show a significantly higher tone in hypertensives. Such tone can be demonstrated by comparing the steady state diameter (D0) with that after maximal dilation (Dmax). In hypertensive SHR preparations, vessel tone is 30% to 40% higher in the transverse arterioles than in WKY controls despite the fact that age-matched cohorts show almost identical Dmax values. Smooth muscle reactivity is increased not only to vasoactive agonists, but to elevated levels of oxygen in the suffusion fluid bathing the skeletal muscle. The shift in the pattern in a renovascular form of hypertension makes the larger arterioles much more reactive to constrictor stimuli in contrast to a reverse gradient in normotensive preparations.

Microvessel Hematocrit

Under normal conditions, blood flow is distributed uniformly throughout the microvascular network, but there is a substantial reduction in hematocrit particularly in precapillaries and capillaries where diameters of red blood cells not only approach but may exceed that of the vessels themselves. Under hypertensive conditions, an increase in vessel tone and a shutdown of many of the smaller arterioles and precapillaries, distribution of blood is uneven with striking differences in vessel hematocrit. Many of the capillary sized vessels contain almost no red blood cells so that they are difficult to identify in parenchymal tissues such as skeletal muscle.

Although many of the changes described above would by themselves be sufficient to account for the increase in microvascular resistance, a cause and effect relationship between the elevation in systemic blood pressure and particular microcirculatory arrangements is not easily demonstrated. Attempts to clarify this issue are based primarily on a comparison of the prehypertensive state in young rats with that observed during the evolution and subsequent stabilization of the hypertensive state in young rats with that observed during the evolution and subsequent stabilization of the hypertensive state. Micropressures appear to be increased in proportion to the magnitude of the systemic change, although not in absolute terms. However, some 5-6 weeks later in more mature SHR, micropressures are brought down to normotensive levels in the transverse arterioles and their branches by an increase in resistance in the smallest arterioles and precapillaries. As indicated blood flow remains within the normal range despite the further increase in resistance. In 8- to 10-month-old SHR flow distribution becomes increasingly uneven with some channels between arterioles and venules showing a rapid continuous stream while others have only an intermittent and scanty flow.

Venous Changes

Data from in vivo and in vitro experiments indicate substantial changes in the venous microvasculature in SHR. They include an increase in venular number and size. The venular location precludes a direct effect on precapillary resistance but they will have an impact on overall microvascular resistance and tissue perfusion. Resistance values can be calculated for whole organs on the basis of arterial input and venous output pressure and flow relationships. In a similar way comparable measurements can be obtained at the microvascular level involving various portions of the entire network. By comparing the resistance across the network bridged by arterioles and venules with comparable flow rates, it is possible to calculate average resistance values for progressively smaller segments of the intervening networks until only the true capillaries are bridged. Thus estimates of precapillary, postcapillary and capillary resistances have been documented.

Postcapillary and venular resistance values are substantially lower in SHR than in WKY controls. This change is much more pronounced in mature animals (above 12 weeks of age) than in the early formative stages of the hypertensive (at 4-5 weeks). In view of the comparatively poor vasomotor reactivity of venular vessels, a reduction in postcapillary resistance is most likely associated with some structural modification. In normotensive preparations, values for pre- to postcapillary resistance ratios tend to increase as the boundaries of the vessel included in the measurement are moved closer to the capillary network. In SHR, the trend is not only completely reversed but much more exaggerated, again indicating a substantial remodeling of the venular microvasculature and possibly a redistribution of circulating blood volume. This shift in the relative magnitude of venular resistance in successively larger vessels serves to lower resistance immediately distal to the capillary network and counters the trend for underperfusion of the entire arteriolar-capillary-venular complex.

General Considerations

Interpretation of microcirculatory data is clearly beset with other uncertainties because of the multiplicity of factors involved. To what extent are the phenomena documented in detail for skeletal muscle the same as in other tissues? Even intravital measurements made in the cremaster, spinotrapezius, and gracilis muscles of the rat are not in total agreement. Although a compelling case may be made for a cause and effect relationship with respect to the progressive increase in systemic blood pressure, an equally plausible explanation may be advanced on the assumption that the microcirculatory sequelae are secondary to an elevation in arterial blood pressure.

It is not possible to decide arbitrarily which of the array of seemingly disparate mechanisms is in accord with the observed behavior of the microcirculation in hypertension, particularly since differences in the pattern of microvascular response have been reported in
skeletal muscle when different models of hypertension are studied.\textsuperscript{7,21}

Once the syndrome has been initiated, however, the elevation in systemic blood pressure and local microcirculatory adjustments interact with one another in a positive feedback sequence that exacerbates the condition and eventually brings into play structural modifications that undermine tissue homeostasis.

Another aspect of the problem has been largely overlooked, one that may be of major importance in interpreting the in vivo observations of microcirculatory dynamics. With repeated subdivision of the vascular tree transport involves not only the partition of the circulating blood volume but the distribution of red blood cells. It is a well-established fact that vessel hematocrit falls sharply as the size of the red cells and the microvessels approach one another, until in the precapillaries and capillaries hematocrit levels as low as 8\% to 10\% are not uncommon. This uneven distribution of red blood cells depends not only on the diameter of the perfused vessels but on the relative velocities in each of the successive branchings. Distribution under normotensive conditions is dependent upon the topographical alignment of the microvascular network and on temporal adjustment of arteriolar caliber-called spontaneous vasomotion.

Under hypertensive conditions, this facet of tissue homeostasis can be distorted to the point where tissue perfusion is uneven and ineffective. With a narrowing of arterioles and their precapillary branches, combined with the closure of some vessels, and possibly even blood rheological changes during hypertension, the partition of the bloodstream at branching points in the microvascular network during hypertension becomes so uneven that many capillary sized branches receive essentially no red blood cells. In addition to its functional implications, many precapillary and capillary vessels are no longer visible in intravital preparations of skeletal muscle where the microscopic sized vessels are detected by the movement of the red blood cells. This difficulty in distinguishing between open versus closed microvessels, except in unusually thin tissues, may be a factor for emphasis on the magnitude of rarefaction of vessels below 20 \micrometer\textsuperscript{2} in diameter.

The severity of the microcirculatory involvement, as well as the ultimate breakdown of tissue homeostasis, are strongly dependent upon host factors that have not as yet properly evaluated. The degree of structural rarefaction of arterioles could create limits in terms of therapeutic intervention. Different facets of the adjustments in microvessel behavior are secondary to the elevation in systemic pressure and lead to a positive feedback that eventually undermines tissue perfusion and exchange.

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