Review

The microcirculation in experimental hypertension and aging

Phillip M. Hutchins *, Colleen D. Lynch, Paula T. Cooney, Kimberly A. Curseen

Department of Physiology and Pharmacology, The Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157-1083, USA

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Abstract

Objective: The purpose of this manuscript is to review the literature concerning the alterations in the microvasculature in experimental hypertension and aging. We also present new unpublished data and results where previous studies have not addressed important questions.

Methods: The new studies were performed using a chronic cranial window to allow multiple observations of the cortical surface vasculature over time. In vivo video, microscopic techniques were used to study long-term changes in microvascular caliber (vasomotion). In some studies, a chronic, in-dwelling aortic catheter allowed chaotic analysis of short-term blood pressure and heart rate variations. Results: In these new studies we demonstrated a reduction in number of small arteriolar endpoints per cortical surface area in the spontaneously hypertensive rat and in the old Brown-Norway rat. There was also a reduction in the number of arteriole-to-arteriole anastomotic connections in the older rat. These vascular changes in the old rat were revised or prevented by caloric restriction. In the old rat, there was also a reduction in the variability of blood pressure, heart rate and microvessel caliber (vasomotion). Conclusions: These studies suggest that there is an alteration in the morphology of the small arterioles in hypertension and aging, that may lead to reduced ability to perfuse cortical tissue. In addition, there appears to be a diminution of overall short-term cardiovascular and microvascular control.

Keywords: Microcirculation; Age; Hypertension; Morphology; Arterioles

1. The microcirculation in hypertension

Since the early days of Galen, Harvey, and Hales, scientists and philosophers have pondered the source of the driving force propelling blood through the microcirculation, or "porosities of the flesh". Once arterial blood pressure was discerned to be a good thing, it was left to Bright, Pickering and others to observe that too much of a good thing could be bad for individual organs and blood vessels. Based on the early work of Folkow and others, most investigators assumed that high blood pressure was a disease brought about by enhanced vasoconstriction. Only recently have scientists explored the time course and true hemodynamic mechanisms responsible for the elevated arterial pressure. A common feature among essentially all reports is a late increase in total peripheral resistance. This review will focus on potential mechanisms responsible for this sustained elevation in vascular resistance after the development of hypertension.

1.1. Vascular resistance in hypertension

The physical determinants of vascular resistance could be the subject of another separate chapter. Suffice it to summarize by declaring that peripheral resistance depends upon three vascular dimensions—vessel diameter, the number of similar vessels in parallel, and vessel length. Effecting alterations in vascular resistance by changing vessel diameter would yield the greatest change in resistance, since the relationship is parabolic. Alterations in the vascular density, or number of vessels in parallel, has been suggested as being the most accurate and efficient manner in which the circulation is regulated in the long term. Changes in length might be considered the most simple mathematically, since the relationship between length and resistance is linear.

1.1.1. Diameter and wall encroachment

Vessel narrowing by structural and functional means has been described by Folkow and others, has been the
subject of numerous reports and reviews [1–3], and will not be further documented here. Suffice it to say that with so much literature suggesting reduced diameters in hypertension, the question becomes one of where, when, to what extent, and by what mechanism.

1.1.2. Rarefaction

Prewitt and co-workers have compiled several excellent reviews on the subject of a decrease in number of blood vessels per unit volume of tissue, or rarefaction, in various hypertensive models [4–6]. The reader is referred to these reviews for a more complete discussion of this subject. This review will deal with certain issues that the authors believe unresolved or unstated.

1.1.2.1. Cerebral vascular rarefaction in hypertension.

Numerous studies have reported a definitive increase in cerebrovascular resistance in hypertension. However, controversy exists concerning the mechanism of this increased resistance. Statistically significant rarefaction of total cerebral arterioles and capillaries has been shown in fixed tissue [7]. This study has been criticized for lack of a normalization for surface area or volume of tissue. Another study found no statistically significant rarefaction of cortical surface arterioles in hypertensive rats, although a trend was clearly present [8]. The cranial window preparation used in that study was an acute preparation utilizing measurements taken from photographs. We investigated the density of arteriolar and venular endpoints on the cortical surface (points where the surface vessels enter or exit the cortical surface) using high magnification (760 × ) video techniques in a chronic cranial window preparation. This technique reports more vessels than the previously referred to study [8], since the density of arteriolar endpoints is approx. 8 × the density reported for 4A vessels in that study. The microvascularity of the cortical surface in sedated middle-aged normotensive (n = 9) and spontaneously hypertensive (n = 11) rats was mapped monthly by video micrographic and image processing techniques. Normotensive rats (7–16 months) were found to have a density of arteriolar endpoints (entry of small arterioles into the cortical parenchyma) of 15.02 ± 1.91 arterioles per square millimeter of cortical surface. Hypertensive rats (9–16 months) had a reduction in arteriolar endpoint density to 12.09 ± 1.10 arterioles per square millimeter. Venular endpoint densities were similarly different between the two groups (37.12 ± 3.27 vs. 31.51 ± 1.85). There was also a trend toward reduced surface anastomotic connections (arteriole to arteriole and venule to venule) in the spontaneously hypertensive rat. Thus, normotensive WKY rats had a 25% greater density of arteriolar endpoints compared to spontaneously hypertensive rats. The venular density was also 18% larger in WKY rats. No differences were seen in the average arteriolar or venular diameters between normotensive and hypertensive rats.

Fifty-three percent of the spontaneously hypertensive rats in the previous study had documented balloonings or sausage formations visible within the cranial window. Only 14% of the middle-aged, normotensive WKY rats had similar malformations.

1.1.2.2. Microvascular reserve in hypertension.

Between the ages of 3 and 30 weeks, the normotensive Wistar Kyoto rat experiences a decrease in the total number of arterioles per area of cremaster skeletal muscle [9]. Although the total number of arterioles that could potentially open to flow is reduced to one-half, the number actually open in the resting muscle is approximately the same. This indicates that there is an age-related decrease in the available vascular reserve during maximal vasodilation. In other words, the microcirculation is not capable of expanding to accommodate large increases in blood flow due to the decrease in the total number of arterioles (those not normally open in skeletal muscle). The SHR, on the other hand, maintain a more constant fraction of vessels open from 3 to 30 weeks of age, but have a reduced total number of arterioles. This is due to the reduced growth and spread of the vascular system in skeletal muscle of the hypertensive rat. These data suggest that microvascular changes occur very early in the hypertensive process, between 6 and 12 months in skeletal muscle.

1.1.2.3. Length and tortuosity.

Moody et al. [10] observed an elongation and increased tortuosity in cerebral vessels of hypertensive and elderly normotensive humans. The increased tortuosity and length of the cerebral vessels would increase vascular resistance. As a result, there would be a tendency for diminished cerebral perfusion capacity and a resultant decline in cerebral blood flow with hypertension and age. In all of our studies on the microcirculation in hypertension, we have never seen an increase in average, individual vessel length in the hypertensive patient or animal model, when age effects were controlled. Nevertheless, an increase in tortuosity would, by itself, be a source for elevated vascular resistance, all other factors being equal.

2. The microcirculation as an endocrine organ

2.1. Growth hormone and hypertension

Growth hormone has long been implicated in the repair and maintenance of tissue. Growth hormone injections of three per week to elderly patients increased muscle mass by 9%, reduced fatty tissue by 14% and increased skin thickness by 7% [11]. Sonnag et al. [12] have presented evidence that there is an age-related decline in growth hormone pulses in rats. Florini et al. [13,14] have demonstrated a reduction in circulating insulin-like growth factor-1 (IGF-1) levels with age in both rats and humans, which is subsequent to the decrease in plasma GH pulses. Son-
Hormone and lymphocytes have been shown by many investigators to be necessary in order for proper repair and maintenance of body structures, including blood vessels. The implication is that levels of IGF-1 expression similar to that observed in young animals are necessary in order to properly effect structural and functional plasticity in vascular tissues. In fact, Folkow et al. [19] reported that growth hormone supplementation is required in hypophysectomized animals in order for structural remodeling to occur in response to hypertension. Florint’s group also has evidence that the IGFs are a major factor in cell differentiation and presumably in cell remodeling, like that reported to occur in hypertension [20].

2.2. Leukocytes and growth hormone

Lymphoid cells have been reported to synthesize growth hormone [21] and lymphocytes have been shown by many investigators to have receptors for growth hormone [22]. Weigent and Blalock [23] have shown an immunoreactive growth hormone-releasing hormone in rat leukocytes. This group of investigators has also reported that leukocyte growth hormone is bioactive and capable of being blocked with specific antibodies to rat pituitary growth hormone [24]. They also conclude that the ability to produce growth hormone is heterogeneous among different lymphocytes.

2.3. Leukocytes and hypertension

Schmid-Schönbein et al. [25] have reported that the total leukocyte count is elevated in hypertensive rats. Granger’s group has confirmed that the circulating polymorphonuclear leukocyte count is increased in hypertensive rats [26]. However, the adhesive interactions brought about by platelet activating factor or leukotriene B4 were decreased significantly in the hypertensive rat. There was also a reduced surface expression of CD11b/CD18 on leukocytes from hypertensive rats. This is consistent with reduced production of basic proteins (and likely growth hormone) by leukocytes from hypertensive rats.

2.4. IGF-1 and vascularity

Growth hormone exerts its actions through its growth promoting peptide, insulin-like growth factor-1 (IGF-1). The maintenance, repair and restoration of vascular tissue may be due to the effects of growth hormone on the local production of IGF-1 or on circulating IGF-1 produced mainly in the liver. Supporting a role for locally produced IGF-1, Delafontaine et al. [27] have found mRNA for IGF-1 in vascular tissue. They report that mRNA levels and secretion of IGF-1 are both increased with exposure of cultured rat smooth muscle cells to two dimers of PDGF (AB and BB). Re-exposure of quiescent cells (serum deprived) to serum also increased IGF-1 mRNA levels. Hansson et al. [28] observed that the amount of IGF-1 immunoreactivity increased with the amount of wall tension existing in the vessel. This group has also reported significant amounts of IGF-1 immunoreactivity in regenerating blood vessels [29]. They conclude that IGF-1 functions partly as a parahormone exerting its effect by paracrine/autocrine mechanisms. Jialal et al. [30] found large numbers of receptors for IGF-1 and IGF-II on endothelial cells from large and small blood vessels. The fact that IGF receptors are found on these vessels could support either an autocrine/paracrine action or an endocrine effect of IGF-1 produced by the liver and circulating in the blood.

2.5. Conclusions

Ample evidence exists that there are alterations in the vasculature of the hypertensive rat which is principally evidenced by vessel narrowing, a reduced vascuarity and increased vascular malformations. These findings indicate that the repair and maintenance of vessels is diminished. Recently, reductions in growth hormone and IGF-1 levels have been implicated in repair and growth processes. IGF-1 has been shown to have a definite effect on blood vessel growth, function, and viability. However, the role of growth hormone and IGF-1 in the maintenance and support of the microcirculation, and the specific role of plasma levels of growth hormone versus local production of IGF-1, have yet to be investigated. Fig. 1 illustrates the role of growth hormone and IGF-1 on blood vessel development, maintenance and repair, in the normal rat. Fig. 2 depicts the suggested reduction in growth hormone and IGF-1 support of vascular growth and repair functions in hypertension.

3. The microcirculation in aging

A common observation in all species, including man, is a reduction in the repair and maintenance of most bodily...
tissues [31]. Much conjecture has led to the competing hypotheses of "use it or lose it" and "wear and tear" [31]. For whatever reason, these age- and stress-related phenomena occur in most organ structures, particularly in skeletal muscle, bone, neural, connective and vascular tissues. The elastin and smooth muscle elements of vascular structures are especially observed to diminish [32-34]. In this manner, the vasculature is rendered less compliant and less able to withstand distending pressures. Connective tissue with collagen cross-linkages has been shown to expand in scope within walls of the heart and blood vessels of older animals [35]. The vasculature is thus made less compliant and contraction and relaxation become more difficult. The overall regulation of the cardiovascular system is also altered. Our data, and those of others, would suggest that the normal association of specific hemodynamic (heart rate and mean arterial pressure) setpoints or attractors with behavior-specific activities is changed with age. In the past decade, several investigators have reported age-related changes in cardiovascular control, indicating a reduced ability to regulate individual organ blood flow, particularly in response to stressors, and a diminished capacity to effect ordinary central control of the cardiovascular system [36-38].

The effect of aging on the microcirculation, or, of the microcirculation on aging, has received little attention from the community of microvascular researchers. Sobin et al. [39,40] have shown important histochemical changes in the extracellular matrix of the microvasculature with age. Other investigators have reported for many years that organ blood flow diminishes with age. For some time, geriatricians have described an age-related decline in almost all physiological functions and body tissue structural integrity. Altered microvascular structure and function would be expected to be associated with age. This review will attempt to summarize the most recent data on the decline in vascular and microvascular repair and maintenance with age.

3.1. Vascular patterns

In preliminary studies, we have observed a reduced cortical surface vascularity with age (see Table 1). The cortical surface microvasculature was mapped using video micrographic and image processing methods similarly to that reported in Section 1. Vessel endpoints (entrance into or exit from the cortical parenchyma) were identified at high magnification (760×), tabulated, and normalized per cortical surface area.

The observation that the cortical surface arteriolar density, more than the venular density, is reduced with age, is indeed a most interesting finding (see Table 1). Additionally, the arteriole-to-arteriole anastomoses are even more reduced than the arterioles in the old rats. Young rats have five [5] arterioles for each anastomotic connection, whereas the old rats have only one anastomotic connection per every 6.6 arteriolar endpoints. Yamaguchi et al. [41] have also reported indirect evidence for a diminished arteriolar anastomotic connection in the old rat. No difference was seen in the venule-to-venule anastomotic connections. Thus, in the old rat, the venous vasculature seems to undergo more repair and maintenance activities than the arteriolar vessels.

While investigating the alterations in the microcirculation of spontaneously hypertensive rats, we found age-related changes in the number of small arterioles in the normotensive control rats. The total number of small arterioles (latex injected-no tone) in the cremaster muscle of these normotensive rats declined by approx. 80% from 15 to 30 weeks of age. In spite of this decrease in total number of arterioles available for perfusion, the number of open small arterioles (in vivo perfused with tone) remained relatively constant. Thus the number of arterioles with blood flow, as a percentage of the total available (all vessels with and without blood flow) rose from 37% at 3 weeks to 59% at 30 weeks. This finding indicates an age-related decrease in functional reserve capacity for increasing blood flow (by increasing the percentage of open arterioles) during times of increased need.

An age-related increase in the arteriolar vessel segment length between branches has also been described in skeletal
tal muscle by Cook et al. [42]. Hachamovitch et al. [43] report that the coronary blood flow reserve in Fischer 344 rats is reduced at 12 and 20 months to approx. 45% of that observed at 4 months of age. These data suggest that microvascular changes occur very early in the aging process, between 6 and 12 months in skeletal and cardiac muscles in the rat.

The age-related decrease in vascular density suggests an increase in vascular resistance and a reduction in blood flow. Several investigators have previously reported a decrease in organ blood flow with age. As a result of the decrease in blood vessel density and the reduction in blood flow, the remaining flow would be more heterogeneous and the diffusion distances between vessels (for nutrient dispersion and metabolite uptake) would be greater. For example the concentration of CO$_2$ and hydrogen ions in the cerebral circulation would be greater in locations between supplying vessels. The cerebral circulation is most exquisitely sensitive to these vasoactive factors. These factors are generally maintained within certain limits by the control systems contained with the cerebral vasculature. Therefore, the hypothesis that this control is diminished with age must be entertained seriously.

The contribution of convolutions, twists, tangles and loops in the cerebral capillary bed to the age-related decrement in cerebral blood flow has been noted by Ravens [44]. He suggests that these would yield a "constant and progressive alteration in the dynamics of cerebral circulation". A proliferation of glial fibrillar and astrocytic cells around blood vessels and adventitial proliferation of connective tissue fibers was also observed with age [44]. The implied alterations in the microcirculation would have the same effect as increasing the intercapillary distance by impeding the transport of substances between the capillary and parenchyma. Akima et al. [45] describe an intertwining of the small arteries and arterioles in the cerebral cortex into rope-like twisted formations. These observations would relegate responsibility for a portion of the increased cerebrovascular resistance to an increase in arteriolar length and tortuosity.

To study the mechanism of the age-related reduction in the ability of the body to maintain blood vessel morphology and function, we investigated the effect of caloric restriction on old rats. The rats were 44 months of age, restricted to 60% of ad lib calories from weaning, with vitamin and mineral supplementation. In this study, we observed a cortical arteriolar density in the calorically restricted rats equal to that of the young ad lib fed rats (14.99 ± 0.88 vs. 15.42 ± 1.19 arteriolar endpoints per square millimeter of cortical surface, respectively). Likewise, the number of arteriole-to-arteriole anastomotic connections in the caloric restricted rats was also seen to be equal to that observed in the young ad lib fed rats (3.07 ± 0.49 vs. 3.05 ± 0.21 per millimeter, respectively).

The usual age-related decline in growth hormone in older rats is reversed in the calorically restricted rats. However, with caloric restriction there is not a reversal of the usual fall in IGF-1 with age. Thus, the caloric restriction model separates the effects of growth hormone and IGF-1. Measurements of the plasma values of IGF-1 were found to positively correlate with cortical arteriolar density, except in old calorically restricted rats. In the old calorically restricted rats, arteriolar densities were high (similar to young rats) while plasma IGF-1 values were low (similar to old control rats). Since growth hormone has been shown to be maintained in these old calorically restricted rats, this would indicate that arteriolar density is more closely associated with growth hormone than IGF-1. The actions of locally expressed growth hormone and IGF-1, however, must also be taken into account. We know that lymphocytes produce immunoreactive growth hormone which has similar biologic activity and structure to pituitary-derived growth hormone [21,24]. The more complete repair and maintenance of the venous microcirculation during aging may be the result of the well-known, close association of white cells (and their locally active growth hormone) and the venous vessel wall. Locally secreted growth hormone may still act through locally produced IGF-1. Cultures of vascular endothelial and smooth muscle cells have been shown capable of producing IGF-1, and both have receptors for IGF-1. Immunoreactive IGF-1 has been shown to be greatly increased in areas of angiogenic activity [28,29].

Elastin gene expression and the resultant elasticity in the rat aorta have also been shown to decrease with age. Likewise, these changes could be reversed by administering insulin-like growth factor-1 (IGF-1) [46]. In line with this generalized age-related reduction in distensibility, Ha~jdu et al. [54] have demonstrated an atrophy and decreased distensibility of cerebral arterioles in 24–27-month-old Fischer 344 rats. This group has also shown a decline in EDRF mediated cortical surface vasodilation in aged rats [47].

3.2. Vascular plasticity

In young rats, a significant growth of new blood vessels has been seen in the cerebellar cortex in response to the increased neural activity associated with forced exercise [48]. A lack of growth of new cerebral microvessels in support of increased neural structure and activity has been reported in the aged rat [49]. Podhajsky and Meyers [50] observed new blood vessel growth preceding neurite regrowth after neuron damage, supporting the hypothesis that angiogenesis is crucial to the metabolic support of new neuronal tissue. In the aged rat, a reduced ability to form new anastomotic connections after middle cerebral artery occlusion has also been reported [41]. This would suggest that, in addition to the previously noted decrement in blood flow with age, there is also a diminished ability to maintain homogeneous flow during periods of localized occlusions.
Fig. 3. Chaos plot of arterial blood pressure and pulse interval (inverse of heart rate) in a 1-month-old Wistar Kyoto rat over a 24 h period. Note three distinct peaks corresponding to (1) sleep, with the lowest pressure and highest pulse interval, (2) awake resting and (3) activity, with the highest pressure and lowest pulse interval.

4. Hemodynamic and vasomotor changes with aging

To complement our techniques for assessment of the long-term microvascular alterations, we have also developed a methodology to analyze the acute spontaneous fluctuations in microvascular and hemodynamic variables. These oscillations are easily monitored in chronic experiments. The correlation obtained between microvascular and hemodynamic minute-to-minute behavior, and how this behavior changes in response to various interventions, has been reported to provide information concerning the mechanisms involved in cardiovascular regulation [51,52].

We have found that the young adult rat exhibits three cardiovascular setpoints (major attractors in Chaotic Theory analysis) of blood pressure and pulse interval (inverse of heart rate) during a 24 h period (Fig. 3). These setpoints correspond to sleep, awake resting, and activity (grooming, eating, etc.). With aging, these setpoints are not nearly so distinct, indicating a loss of flexibility of cardiovascular adjustments attendant to everyday stressors. In normotensive Wistar-Kyoto rats, we have found that the heart rate peaks at approx. 6 weeks of age and that blood pressure declines after 12 weeks. All of this denotes a less chaotic, more stationary circulatory system with less ability to respond necessarily to external challenges.

Intrinsic rhythmic changes in the diameter of cerebral arterioles in sedated young, middle-aged, and old Brown-Norway rats were also assessed in vivo. All diameter measurements were analyzed using a traditional graphic analysis technique. Graphic analysis of the data revealed
that the vasomotion frequency was markedly reduced with age from over 2 cycles/min in the young Brown-Norway rat to less than 1 cycle/min in the old rat (Fig. 4). Furthermore, the young cerebral arterioles exhibited a significantly greater amplitude of vasomotion when compared to the old cerebral arterioles of the same diameter (Fig. 5). Slaaf et al. [53] contend that flow motion (vasomotion) is necessary to increase perfusion and to reduce the capillary diffusion distances, and thereby maximize transport for a particular vascular geometry. Diameter measurements revealed that there was no difference in the size of the arterioles on the cortical surface of any of the three age groups of Brown-Norway rats (Fig. 6).

These data suggest that a fundamental defect of intrinsic tone develops in the old rat that may contribute to the age-related reduction in microvascular and cardiovascular regulation. We hypothesize that this defect, affecting the short-term regulation of the microvascular system, is brought about by long-term structural regulatory alterations.

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References


