Ocular and regional cerebral blood flow in aging Fischer-344 rats

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Salter, Jennifer M., Vincent M. Cassone, M. Keith Wilkerson, and Michael D. Delp. Ocular and regional cerebral blood flow in aging Fischer-344 rats. J. Appl. Physiol. 85(3): 1024–1029, 1998.—Vascular remodeling and changes in vascular responsiveness occur in the rat cerebrum with old age. This includes reductions in cerebral arteriolar numerical density, cross-sectional area, distensibility, the relative proportion of distensible elements in the cerebral arteriolar wall, and reduced endothelium-dependent relaxation. The purpose of this study was to test the hypothesis that old age results in an increase in vascular resistance and, correspondingly, a decrease in blood flow to ocular, regional cerebral, and spinal tissue in the rat. Blood flow was measured in the eye, occipital bulb, left and right cerebrum, pituitary gland, midbrain, pons, cerebellum, medulla, and spinal cord of juvenile (2-mo-old, n = 6), adult (6-mo-old, n = 7), and aged (24-mo-old, n = 7) male Fischer-344 rats. Arterial pressure and blood flow were used to calculate vascular resistance. Vascular resistance in the eye of aged rats (6.03 ± 1.08 mmHg·ml⁻¹·min⁻¹·100 g) was higher than that in juvenile (3.83 ± 0.38 mmHg·ml⁻¹·min⁻¹·100 g) and adult rats (3.12 ± 0.24 mmHg·ml⁻¹·min⁻¹·100 g). Similarly, resistance in the pons of older rats (2.42 ± 0.55 mmHg·ml⁻¹·min⁻¹·100 g) was greater than in juvenile (0.66 ± 0.06 mmHg·ml⁻¹·min⁻¹·100 g) and adult rats (0.80 ± 0.11 mmHg·ml⁻¹·min⁻¹·100 g). In contrast, vascular resistance in the pituitary gland was lower in the aged rats (juvenile, 3.09 ± 0.22; adult, 2.79 ± 0.42; aged, 1.73 ± 0.32 mmHg·ml⁻¹·min⁻¹·100 g, respectively). Vascular resistance was not different in other cerebral tissues or in the spinal cord in the aged rats. These data suggest that regional cerebral and spinal blood flow and vascular resistance remain largely unchanged in conscious aged rats at rest but that elevations in ocular vascular resistance and, correspondingly, decreases in ocular perfusion with advanced age could have serious adverse effects on visual function.

eye; maturation; microsphere; spinal cord

REGIONAL CEREBRAL AND OCULAR BLOOD FLOW and its regulation are vital to normal brain and visual function; therefore, its perturbation, either as a result of aging itself or as a result of a disease, can cause severe deterioration of function (3, 30). For example, changes in cerebral blood flow have been implicated in several common diseases associated with aging, such as hypertension, arteriosclerosis, Parkinson's disease, Huntington's disease, and Alzheimer's-type dementia, as being either a cause or a symptom of the disease (3, 4). Thus, to ascertain the effects of normal aging from those induced by clinical or subclinical disease, investigators have used the aged Fischer-344 rat model, which is relatively free of atherosclerotic lesions on the cerebral vasculature (11, 12).

Several studies investigating the effects of aging on the mechanics, composition, vasodilatory responsiveness, and numerical density of rat cerebral arterioles suggest that cerebral vascular resistance is increased by old age. For example, Hajdu et al. (14) reported that with aging there are reductions in the cross-sectional area and distensibility of cerebral arterioles. The decrease in distensibility was associated with reductions in the relative proportion of distensible elements elastin and smooth muscle in the arteriolar wall. Mayhan and co-workers (23) also found that the dilator responses induced by acetylcholine, bradykinin, ADP, and serotonin were impaired in cerebral arterioles from aged rats. Finally, Sonntag et al. (32) reported an old age-induced rarefaction of cerebral arterioles that resulted from a decline in growth hormone and insulin-like growth factor-I. Collectively, these studies indicate that cerebral vascular resistance is likely to be elevated in older animals. Although several studies have indicated that regional cerebral blood flow in the aged rat is either unaltered (1) or reduced in a few regions (27, 34), there are currently no studies available documenting the effects of aging on regional cerebral vascular resistance. Furthermore, there are currently no data in the literature reporting the effects of old age on blood flow and vascular resistance in the rat eye or spinal cord. Therefore, the purpose of this study was to test the hypothesis that old age results in an increase in ocular, regional cerebral, and spinal vascular resistance in the rat.

MATERIALS AND METHODS

The methods employed in this study were approved by the Texas A&M University Institutional Animal Care and Use Committee. The investigation conforms with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892].

Animals and surgical procedures. Juvenile (2-mo-old, n = 6), adult (6-mo-old, n = 7), and aged (24-mo-old, n = 7) male Fischer-344 rats (National Institutes of Aging Colony) were used. The animals were individually housed in an environmentally controlled room (23 ± 2°C) with a 12:12-h light-dark cycle, and were given food (commercial rat chow) and water ad libitum.

Under methoxyflurane anesthesia (Metofane), a catheter (Dow Corning, Silastic; ID 0.6 mm, OD 1.0 mm) filled with

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heparinized (200 U/ml) saline and connected to a pressure transducer and chart recorder was advanced into the left ventricle of the heart via the right carotid artery as previously described (7). This catheter was subsequently used for the infusion of radiolabeled microspheres to measure tissue blood flow and for recording intraventricular pressure. A second polyurethane catheter (Braininex Scientific, Micro-renathane; ID 0.36 mm, OD 0.84 mm), used for the withdrawal of a reference blood sample and measurement of arterial blood pressure, was implanted in the caudal artery of the tail and filled with heparinized saline as previously described (5). Both catheters were externalized and secured on the dorsal cervical region.

Experimental protocol. After 2 days of recovery from the surgical procedure, all catheters and instrumentation were connected while the animals remained in their cages. The unanesthetized and unrestrained rats were allowed to stabilize for 20 min from the instrumentation procedure before the microsphere infusion was performed. Pulsatile intraventricular pressures (and, hence, heart rates) were monitored during this period, which was sufficient for the heart rate to stabilize. When the microsphere infusion and reference withdrawal sampling were completed, pentobarbital sodium (35 mg/kg) was infused through the carotid catheter to anesthetize the animals before euthanasia by exsanguination. The eyes, skull, and spinal cord (cervical through lumbar portion) were removed from the carcass. The brain was removed from the skull, detaching cranial nerves, and was further sectioned into distinct regions (Table 1). The samples were weighed and placed in counting vials for blood flow determination.

Blood flow determination. Radiolabeled ($^{99m}$Tc or $^{103}$Ru) microspheres (New England Nuclear) with a 15.5 ± 0.2-µm diameter were used for blood flow measurements as previously described (7, 18). Microspheres were suspended before infusion by 10 min of sonication followed by 1–2 min of agitation on a vortex mixer. Approximately 1.0 million spheres were infused into the juvenile rats, and 1.5 million spheres were infused into the adult and aged rats over a 15- to 20-s period. After dissection, tissue samples were counted in a gamma counter (Packard Auto-Gamma 5780) and flows computed (IBM personal computer) from counts per minute and tissue wet weights. By comparing tissue counts with counts from standards containing known numbers of microspheres, it was determined that the infusion of 1.0 million spheres into the juvenile rats and 1.5 million spheres into the adult and aged animals was sufficient for each tissue sample to receive at least 200 microspheres. Microsphere mixing with the blood was assessed by comparing bilateral kidney flows. Mixing was considered adequate if bilateral flows were within 15% of each other. No data were discarded in this study because of inadequate mixing.

Typically, in a 350-g rat, blood flow distribution is measured by infusing 500,000 microspheres per condition, and up to three measurements or conditions can be made in one animal (e.g., Refs. 5, 7). In the present study, the infusion of 1.0 million microspheres in juvenile rats or 1.5 million microspheres in the two groups of adult rats for a single blood flow measurement was done to ensure that small-mass tissue samples contained sufficient numbers of spheres for accurate flow determinations. To determine whether the infusion of 1.0 million microspheres in juvenile rats or 1.5 million microspheres in adult rats disturbed the cardiovascular system or altered the distribution of blood flow in the brain and other tissues, we made two sequential blood flow determinations using two different radiolabeled microspheres in a preliminary group of juvenile (n = 6) and 6-mo-old adult (n = 6) animals. In the juvenile rats, blood flow was sequentially measured with two infusions of 500,000 microspheres/infusion. In the adult rats, flow was measured with one infusion containing 1.0 million microspheres and a second containing 500,000 microspheres. In both the juvenile and adult animals, tissue blood flows, heart rates, and arterial pressures were similar between the first and second infusions. In addition, heart rates and blood pressures were similar before and after the microsphere infusions, indicating there were no hemodynamic disturbances induced by the infusion of 1.0 and 1.5 million microspheres in juvenile and adult rats, respectively. These results are similar to those of Stanek et al. (33).

Arterial pressure, heart rate, and vascular resistance determination. Mean arterial pressure was electronically averaged from pulsatile pressure measurements from the caudal catheter. Heart rate was estimated from pulsatile left intraventricular pressure tracings. Pressure recordings, made with pressure transducers (Electromedical) and recorded on a polygraph (Gould 2800), were made immediately before and after the microsphere infusion and averaged, since simultaneous pressure measurements and blood withdrawal or microsphere infusion were not possible. Ocular, regional cerebral, and spinal vascular resistances (mmHg·ml$^{-1}$·min$^{-1}$·100 g$^{-1}$) were calculated by dividing mean arterial pressure (mmHg) by the tissue flows (ml·min$^{-1}$·100 g$^{-1}$).

Statistical analysis. For each variable (body and tissue mass, arterial pressure, heart rate, tissue blood flow, and vascular resistance) a one-way analysis of variance was used to compare means across groups. Pairwise multiple-comparison procedures (Tukey's test) were used to determine the significance of differences among means. For all analyses, the 0.05 level was used to indicate statistical significance.

### RESULTS

Body and tissue mass. Body mass increased as a function of age (Table 1). Similarly, the eye and most structures in the brain of the adult rat were larger than those in the juvenile rat (Table 1). Further increases in mass occurred with old age in the eye, olfactory bulb, pituitary gland, and spinal cord.

**Heart rate and arterial pressure.** Resting heart rate (juvenile, 395 ± 5; adult, 396 ± 8; aged, 368 ± 15 beats/min, respectively) and mean arterial pressure (juvenile, 115 ± 3; adult, 117 ± 5; aged, 115 ± 3 mmHg, respectively) were not different among groups.

<table>
<thead>
<tr>
<th>Mass</th>
<th>Juvenile</th>
<th>Adult</th>
<th>Aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body, g</td>
<td>187 ± 3</td>
<td>379 ± 8†</td>
<td>438 ± 9*</td>
</tr>
<tr>
<td>Eye, mg</td>
<td>101 ± 7</td>
<td>164 ± 6†</td>
<td>208 ± 3*</td>
</tr>
<tr>
<td>Olfactory bulb, mg</td>
<td>60 ± 5</td>
<td>51 ± 5</td>
<td>76 ± 4*</td>
</tr>
<tr>
<td>Left cerebrum, mg</td>
<td>472 ± 15</td>
<td>559 ± 19†</td>
<td>574 ± 17†</td>
</tr>
<tr>
<td>Right cerebrum, mg</td>
<td>454 ± 30</td>
<td>537 ± 17†</td>
<td>557 ± 15†</td>
</tr>
<tr>
<td>Pituitary gland, mg</td>
<td>33 ± 1</td>
<td>39 ± 3</td>
<td>46 ± 2*</td>
</tr>
<tr>
<td>Midbrain, mg</td>
<td>249 ± 35</td>
<td>327 ± 20</td>
<td>357 ± 19†</td>
</tr>
<tr>
<td>Pons, mg</td>
<td>6 ± 1</td>
<td>11 ± 4</td>
<td>20 ± 5†</td>
</tr>
<tr>
<td>Cerebellum, mg</td>
<td>243 ± 11</td>
<td>314 ± 10†</td>
<td>339 ± 10†</td>
</tr>
<tr>
<td>Medulla, mg</td>
<td>213 ± 11</td>
<td>282 ± 16</td>
<td>342 ± 31†</td>
</tr>
<tr>
<td>Spinal cord, mg</td>
<td>441 ± 14</td>
<td>742 ± 29†</td>
<td>942 ± 33†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Aged group mean is different from juvenile and adult group means (P < 0.05). †Group mean is different from juvenile group mean (P < 0.05).
Blood flow and vascular resistance. Ocular, regional cerebral, and spinal blood flows are summarized in Table 2. There were no differences in perfusion rates to any tissues measured between juvenile and adult rats. However, flow to the eyes was 35 and 42% lower in the aged animals relative to that in juvenile and adult rats, respectively. Perfusion rates in most parts of the brain and spinal cord were not affected by old age. Exceptions to this were blood flows to the pons and pituitary gland. Pontine blood flow in the aged rats was 62% lower than that in juvenile rats and 58% lower than that in adult animals. In contrast, pituitary gland blood flow increased with old age. Flow to the pituitary gland was 100% greater in old rats than juvenile rats and 64% higher than in adult animals.

Calculated vascular resistance was not different among groups in the left cerebrum (juvenile, 0.92 ± 0.04; adult, 0.85 ± 0.12; aged, 0.83 ± 0.11 mmHg·ml⁻¹·min⁻¹·100 g, respectively), right cerebrum (juvenile, 0.94 ± 0.07; adult, 0.86 ± 0.11; aged, 0.98 ± 0.16 mmHg·ml⁻¹·min⁻¹·100 g, respectively), midbrain (juvenile, 1.29 ± 0.07; adult, 1.15 ± 0.12; aged, 1.08 ± 0.15 mmHg·ml⁻¹·min⁻¹·100 g, respectively), cerebellum (juvenile, 1.09 ± 0.07; adult, 1.16 ± 0.12; aged, 1.29 ± 0.24 mmHg·ml⁻¹·min⁻¹·100 g, respectively), medulla (juvenile, 1.53 ± 0.13; adult, 1.68 ± 0.29; aged, 2.16 ± 0.56 mmHg·ml⁻¹·min⁻¹·100 g, respectively), and spinal cord (juvenile, 2.99 ± 0.21; adult, 3.48 ± 0.35; aged, 3.54 ± 0.71 mmHg·ml⁻¹·min⁻¹·100 g, respectively). Olfactory bulb vascular resistance was 44% higher in juvenile rats than in adult rats (Fig. 1); no differences existed in olfactory bulb vascular resistance between juvenile and old rats or adult and old rats. Vascular resistance in the pituitary gland was 44 and 38% lower in aged rats relative to juvenile and adult rats, respectively (Fig. 1). In contrast, pontine vascular resistance was 239 and 180% higher in old animals than in juvenile and adult rats, respectively (Fig. 1). Ocular vascular resistance in old animals was 57 and 93% higher, compared with juvenile and adult rats, respectively (Fig. 2).

**DISCUSSION**

The purpose of this study was to test the hypothesis that old age increases ocular, regional cerebral, and spinal vascular resistance in conscious unrestrained rats. The results support the hypothesis that advanced age is associated with an increase in ocular vascular resistance but do not support the notion that there is a generalized increase in vascular resistance in cerebral and spinal tissues.

Perhaps the most significant finding of the present investigation is that blood flow to the eye is diminished by old age. To our knowledge, this is the first study to demonstrate this phenomenon in an animal model.
This observation corroborates a recent study, which reported a decrease in human ocular blood flow with increasing age (30). These investigators measured ocular blood flow (Langham ocular blood flow system) in apparently healthy subjects ranging in age from 10 to 80 yr, who were screened for heart disease, arrhythmias, hypertension, hemopathy, diabetes, and other systemic vascular pathologies. When using multiple-regression analysis, a significant inverse relationship between ocular blood flow and age was found. Thus results from this single human study (30) and the present investigation (Fig. 2) support the notion that vascular resistance in the eye is elevated with advanced age.

The mechanism for the increase in ocular vascular resistance is currently unknown. One possibility is an age-related increase in intraocular pressure. In humans, for example, elevations in intraocular pressure have been shown to occur with increasing age (17, 29), and this could serve to increase vascular resistance in the eye. However, in the above-mentioned study of Ravalico et al. (30), the decrease in ocular blood flow with old age was not accompanied by an increase in intraocular pressure. Thus old-age-related decreases in ocular blood flow can occur in the absence of corresponding increases in intraocular pressure. A second potential mechanism for the increase in ocular vascular resistance could be a diminished release of endothelium-derived relaxing factors. Aging has been shown to diminish endothelium-dependent relaxation in the abdominal aorta (6), mesenteric conduit and resistance arteries (9, 25), carotid arteries (15), and cerebral arteries (23) of the rat. Whether this seemingly global impairment of endothelium-mediated dilation similarly affects the resistance vasculature of the eye requires further investigation.

In the aged-rat model, regional cerebral blood flow has been shown to remain unchanged with advanced age (1) or to undergo slight modification (27, 34). In the present study, olfactory bulb vascular resistance was significantly lower in adult rats than in juvenile animals, and, correspondingly, blood flow tended to be higher in the adult rats, although this difference was not significant. A similar nonsignificant pattern of olfactory bulb perfusion has been previously reported in juvenile, adult, and aged rats (27). The decrease in pontine blood flow found in the present study corroborates previous observations that aging lowers pontine perfusion in normotensive (27) as well as hypertensive (34) rats, although in the latter case the pons and medulla from the hypertensive animals were reported as a single structure. To our knowledge, the present study is the first to report the effects of maturation and aging on pituitary gland and spinal perfusion. There was a higher pituitary gland blood flow in the old rats, whereas aging did not alter the perfusion rate of spinal tissue. The lack of significant changes in perfusion of most other brain regions with advancing age in rats is similar to that reported in humans when rigorous screening for vascular disease risk factors is employed (3, 16, 19, 20).

The present study also demonstrates that regional cerebral and spinal vascular resistances are largely unaffected by old age. This appears counterintuitive in light of the numerous cerebral vascular alterations that occur in the aging rat (21). For example, aging has been shown to diminish cerebral arteriolar numerical density (32), endothelium-dependent relaxation (23), cross-sectional area, distensibility and the relative proportion of distensible elements in the cerebral arteriolar wall (14). With no change in arterial perfusion pressure, these alterations imply that cerebral vascular resistance is elevated in the aged rat. However, it is possible that these apparent age-related vascular deficits may not influence cerebral perfusion or vascular resistance when the animal is at rest but could diminish the ability of the cerebral circulation to redistribute flow within the brain during periods of altered metabolism, such as during mental stress or physical activity. In support of this hypothesis, Haining and co-workers (13) reported that, whereas blood flows to the frontal cortex and cerebellum were similar in 6- and 24-mo-old rats, alterations in flow in response to hypoxia and hypercapnia were markedly slower in the older animals. Thus the potentially deleterious functional and structural alterations of cerebral arterioles that occur with advancing age may only become manifest during periods of cardiovascular and orthostatic stress.

The effects of aging on regional cerebral blood flow in humans have been pursued by many researchers attempting to answer the question of whether regional cerebral blood flow decreases in normal aging or whether it decreases only in a disease state. Inconsistent and contradictory results and conclusions abound in the literature, and this has made it difficult to answer the question. The highly variable experimental designs used by researchers in the field appear to be a major cause for the confusion. First, several different techniques have been used to measure regional cerebral blood flow. These include 15O-labeled inhalation with positron emission tomography (16, 19, 20, 22, 28), 18F]altanserin with positron emission tomography (31), and 133Xe inhalation (24, 26). These methods measure slightly different variables and all have different resolving capabilities. Second, what are defined as regions of interest are often arbitrary and can include several different brain structures within a single region of interest. Finally, and perhaps most importantly, criteria for selection of suitable aged human subjects, with regard to health, age, and gender, appear highly variable and many times do not incorporate rigorous exclusion criteria.

Many of these problems are avoided by using a well-characterized animal model, such as the Fischer-344 rat strain. Fischer-344 rats have several advantages. The genetic background is known and kept constant. Water and nutrient intakes, lighting conditions, and housing can be easily controlled. And the normal life span and sexual development of the rat are well defined; 1.5- to 2-mo-old rats are juvenile, 3- to 6-mo-old rats are young mature adults, and 24-mo-old or older rats are considered aged (8). Their life expen-
tancy is ~29 mo, with a maximal survival time of ~36 mo (2). In addition, this strain is relatively free from atherosclerotic lesions (11, 12). All of these properties make Fischer-344 rats an excellent animal model for aging in humans (1, 8, 11). Furthermore, the Fischer-344 rat is a useful model to study the effects of age on ocular, cerebral, and spinal blood flows and vascular resistance, because much of the variability associated with studies using human subjects can be avoided. The use of the radiolabeled-microsphere technique in the rat has a high resolution in that discrete neural structures can be carefully dissected for analysis of regional alterations in blood flow and vascular resistance. However, one technical concern in measuring regional cerebral blood flow with the present methodology is the effect of left ventricular cannulation via right carotid artery occlusion. For example, Tuma et al. (35) found that blood flow to the right side of the brain was lower than to the left side in adult anesthetized rats with the right carotid artery occluded. However, these authors reported that when brain perfusion was measured in the conscious state, there was no difference between left and right brain blood flow in adult or aged animals. Flaim et al. (10) also reported that right and left cerebral blood flows were not different either at rest or during exercise in conscious rats having the right carotid artery occluded. Finally, in the present study, right carotid arterial occlusion did not affect bilateral flow distribution to the cerebrum or eyes. Collectively, these studies indicate that blood flow to the right side of the brain is not compromised in the conscious state by right carotid artery occlusion.

In summary, normal function of vascular and higher order processes is critically dependent on sufficient blood flow. The present study demonstrates that vascular resistance is elevated and, correspondingly, that blood flow is decreased in the eye with advanced age. However, regional cerebral and spinal blood flow and vascular resistance remain largely unchanged in conscious aged rats at rest.

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