Cerebral vascular changes associated with hemorrhagic stroke in hypertension[1]

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There are a number of alterations that protect the cerebrovasculature from hemorrhagic stroke development during hypertension. The upper limit of cerebral blood flow autoregulation is shifted to higher blood pressure levels; this allows a constant blood flow to be maintained during hypertension. Studies we have performed have indicated that the middle cerebral arteries (MCA) of Wistar–Kyoto stroke-prone spontaneously hypertensive rats (spSHR) lose their ability to constrict in response to elevations in transmural pressure. The decline in such function precedes stroke development and totally disappears at an age where there is a 100% mortality from stroke. Prior to stroke development, spSHR also develop uremic conditions and signs of renal failure. The induction of uremia in stroke-resistant SHR (srSHR) via nephrectomy induces these animals to develop stroke. Like prestroke spSHR, prestroke uremic srSHR also have MCA with attenuated pressure-dependent myogenic function. It is hypothesized that the inability to increase vascular resistance in response to elevations in pressure might promote overperfusion of the more distal vasculature leading to cerebral hemorrhage formation. Since uremia promotes bleeding tendencies, such alterations along with the loss of cerebrovascular myogenic function could initiate or aggrevate hemorrhage formation.

Key words: stroke-prone SHR, stroke, cerebral vasculature, myogenic response, autoregulation.

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Certaines altérations protègent le système cérébrovasculaire contre le développement d'un choc hémorragique durant l'hypertension. La limite supérieure de l'autorégulation du débit sanguin cérébral est alors déplacé vers des taux plus élevés de pression sanguine, permettant ainsi de maintenir un débit sanguin constant durant l'hypertension. Nos études ont indiqué que les artères cérébrales moyennes (ACM) de rats Wistar–Kyoto spontanément hypertensifs, sujets au choc hémorrhagique (RSHsc), perdent leur capacité de constriction en réponse à des élévations de pression transmurale. La diminution d'une telle fonction précède le développement du choc, et celle-ci disparaît totalement à un âge où le taux de mortalité due au choc atteint 100%. Avant le développement du choc, les RSHsc développent aussi des conditions d'urémie ainsi que des signes d'insuffisance rénale. L'induction d'urémie chez les RSH résistants au choc (RSHrc), par suite d'une néphrectomie, entraîne le développement d'un choc chez ces animaux. Tout comme les RSHsc, les RSHrc urémiques en pré-choc ont des ACM dont la fonction myogénique dépendante de la pression est atténuée. On émet l'hypothèse que l'incapacité d'augmenter la résistance vasculaire en réponse à des élévations de pression pourrait favoriser la surperfusion du système vasculaire le plus éloigné et encourager la formation d'une hémorragie cérébrale. Comme l'urémie augmente les tendances au saignement, de telles altérations, ainsi que la perte de fonction myogénique cérébrovasculaire, pourraient provoquer l'hémorragie ou l'aggraver.

Mots clés : RSH sujets au choc hémorrhagique, système cérébrovasculaire, réponse myogène, autorégulation.

[Traduit par la rédaction]

General review

There are very few animal models of stroke. Researchers have tried to mimic stroke by occluding the major blood vessels of the brain (Bederson et al. 1986; Grabowski et al. 1988) or by injecting blood into the brain ventricles (Batton and Nardis 1987; Watanabe et al. 1988). Such studies provide information as to the types of secondary changes that occur after stroke but do not clarify the mechanisms involved in initiating stroke. In view of this, there was great interest generated when Okamoto and his colleagues (Okamoto et al. 1974; Nagaoka et al. 1976) developed a strain of hypertensive rats with a genetic predisposition to develop stroke spontaneously (called Wistar–Kyoto stroke-prone spontaneously hypertensive rats (spSHR)). However, in North America, enthusiasm in this model decreased, owing to the fact that researchers failed to achieve the high incidence of stroke development within spSHR that was reported in Japan (Wexler 1983; Yamori et al. 1984). This frustration was expressed in early articles. For example, Wexler (1983) wrote "this author expended six years of investigation on these stroke-prone animals and found although they did indeed develop an accelerated severe high blood pressure (BP) but not a single spontaneously occurring incidence of stroke was encountered." Subsequently, studies performed by Yamori et al. (1984) indicated that when spSHR were fed a Purina Rat Chow type diet, they developed a very low incidence of stroke; however, the incidence of stroke was increased from 30 to 88% at 9 months of age when rats were fed a Japanese diet produced by Funahashi Farm (Funahashi City, Chiba, Prefecture, Japan; subsequently called a Funahashi-SP diet). An exhaustive analysis of the two diets failed to indicate major compositional differences with respect to electrolytes, minerals, fats, fibre, or amino acid composition of the proteins. However, the protein content of the Funahashi-SP diet was slightly less than that present in the Purina diet (Wexler 1983; Yamori et al. 1984) and researchers have suggested that the decreased protein levels as well as the origin of the protein (fish meal in the Funahashi-SP diet versus animal and plant meal in the Purina diet) are important factors enhancing stroke development in spSHR (Wexler 1983). Such a discovery did not greatly help the situation since there was no North American distributor for the Funahashi-SP diet. Furthermore, the Funahashi-SP diet spoiled easily and had to be shipped by air,

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thus making the diet prohibitively expensive to import from Japan in the quantities necessary to carry out most studies.

Recently, through communications with Dr. Y. Yamori (Shimane Institute of Health Sciences, Isumo, Japan) and Dr. J. Knapka (National Institutes of Health, Bethesda, MD, U.S.A.), I was told of a North American reproduction of the Funahashi diet (termed a Japanese-style diet) that was produced by Zeigler Bros. (Gardners, PA, U.S.A.) (Smeda 1989). The balance of this article will discuss the characteristics of stroke development in spSHR fed a Japanese or Japanese-style diet and the important mechanisms that might be involved in initiating stroke development in these animals.

Characteristics of stroke development in spSHR

When spSHR are fed a Japanese style diet containing 4% NaCl, a rapid onset of stroke development occurs. The characteristics of stroke development have been previously outlined (Smeda 1989). In summary, the first behavioural symptoms of stroke consist of repetitive convulsive movement of the forelimbs or head. These symptoms disappear within days and the rats subsequently become immobile, lethargic, and poorly groomed. During this latter period, the rats often adopt a "Kangaroo" type posture where they will remain motionless with their legs hyperextended below their body. Often during this latter period, the face of the animal will have a puffy edematous "hamster-like" appearance with microhemorrhages developing around the eyes. On average, the animals live 1.6 weeks after the first symptoms of stroke are observed. The above Japanese-style diet did not initiate stroke development in regular Wistar–Kyoto spontaneously hypertensive rats (subsequently termed srSHR to differentiate these animals from spSHR).

Stroke development can be altered by modifying the diet of the animals. Elevations in dietary Na^+ accelerate stroke development (Nagaoka et al. 1976; Yamori 1981), whereas increases in dietary protein, fibre (Wexler 1983; Yamori et al. 1978; Yamori 1981), and surprisingly fat and cholesterol (Yamori 1981) retard stroke development. Stroke development in spSHR can also be retarded by elevating the dietary levels of K^+ (Tobian 1986; Smeda 1989).

Animals sacrificed after the first symptoms of stroke primarily indicate the presence of brain hemorrhages. Within the literature, various researchers have demonstrated the presence of thromboemboli and ateriosclerotic plaques within the cerebrovasculature of spSHR (Fredriksson et al. 1988). In my view, the development of ischemic brain damage as a result of the development of arterio- or athero-sclerotic lesions that trap emboli and promote the formation of infarcts is not the primary event initiating stroke within spSHR. In this regard, when spSHR are fed a high fat, high cholesterol diet (20% suet, 5% cholesterol, 2% cholic acid), sudan staining indicates that fat is extensively deposited within the cerebrovasculature and foam cell containing intimal plaques develop (Yamori et al. 1976), yet the same diet reduces the incidence of stroke in the animals (Yamori et al. 1978; Yamori 1981). Likewise, long-term treatment of spSHR with thromboxane synthetase inhibitors, which are of benefit in acute incidences of thrombosis, does not alter the incidence of stroke development (Stier et al. 1988).

The hypothesis that most accurately fits the research evidence is that the primary event leading to stroke development in spSHR results from a breakdown in the blood barrier, which allows the extravascular movement of plasma (Shibota et al. 1978; Tamaki et al. 1984; Baumbach and Heistad 1988) and blood cells thus leading to hemorrhage development. In this regard, factors that initiate hyperperfusion or promote an increase in the arterial tangential stress will aggravate stroke in spSHR. This would explain why vascular occlusive diseases such as atherosclerosis, which increase cerebrovascular resistance to flow and decrease the distensibility of the vasculature to pressure, exert a protective effect and inhibit stroke development in spSHR. Ischemic areas do develop in the brain of spSHR with stroke; however, it is likely that these result from vasospasm secondary to the presence of hemorrhage or are due to the presence of microvascular compression caused by edema.

The importance of hypertension in the initiation of stroke in spSHR

Various studies have indicated that the normalization or near normalization of blood pressure (BP) with antihypertensive treatment severely retards stroke development in spSHR (Nagaoka 1986; Gries et al. 1989). However, blood pressure is not the only factor governing stroke development in these animals. Studies have indicated that the Ca^{2+} antagonist CV-4093 has very little effect on the BP of spSHR but totally retards stroke over an 8-week treatment period during which there is a 100% incidence of stroke in untreated control animals (Nagaoka 1986). Likewise, at the correct dosage, idebenone (Nagaoka 1986), a drug that improves cellular metabolism by activation of mitochondrial function and enalapril (Stier et al. 1989), an angiotensin converting enzyme inhibitor, also markedly retard stroke development without altering BP in spSHR. Of particular interest are studies carried out by Nagaoka et al. (1979), in which it was demonstrated that both dexamethasone or thyroxine treatment elevated the mean systolic blood pressures of spSHR from approximately 230 (in untreated spSHR) to, respectively, 280 and 296 mmHg (1 mmHg = 133.3 Pa). Despite elevating BP, both of these treatments virtually totally prevented stroke development over a 6-week treatment period during which 100% incidence of death occurred in untreated control animals.

The above experiments indicate that although a minimal level of hypertension is necessary for the development of stroke, it is not the only factor governing stroke development. Since pharmacological treatments can modify the incidence of stroke development in spSHR under conditions whereby BP is either unchanged or elevated, it would appear that the mechanisms initiating stroke in spSHR are potentially modifiable via drug intervention.

Mechanism that protects the cerebrovasculature from hemorrhagic stroke development

The cerebrovasculature of hypertensive humans and animals has various safeguards that help prevent hemorrhagic stroke development. The primary goal of these safeguards is to maintain a situation in which overdistention of the cerebral blood vessels under conditions of high BP is prevented. Cerebral blood vessels forced into a dilated state might be expected to be more prone to rupture, since the increase in lumen radius would create a situation where the tangential stress on the artery, per given transmural pressure (TMP), would be increased (Cox 1979). The forced dilation of the arteries

would increase the downstream blood flow to the smaller blood vessels and could cause these vessels to rupture, creating hemorrhagic stroke.

Alterations in the neurogenic, myogenic, and structural properties of the cerebral vasculature play a role in preventing forced dilation of the blood vessels during hypertension. There are various examples of how the above mechanisms are altered during hypertension and protect the cerebral vasculature from overdistention. It has been observed that in renal hypertensive rats, baboons, srSHR, and hypertensive humans, the lower (Barry et al. 1982; Strandgaard 1976) and upper limit (Strandgaard et al. 1975; Sadoshima et al. 1985) of cerebral blood flow (CBF) autoregulation (i.e., the arterial BP range over which CBF remains constant) was shifted to higher BPs. A shift in the upper limit of CBF autoregulation during hypertension could have a beneficial effect, in that it would prevent the overperfusion of the brain and counteract vascular distension. Consistent with this view, hypertensive encephalopathy occurs at lower BPs in previously normotensive than chronic hypertensive patients (Dinsdale 1982). Likewise, young WKY develop venule hemorrhages and loss of arterial tone when the cerebral vasculature of these animals is exposed to BPs comparable to that present in age-matched SHR (Bohen 1987). It was hypothesized that the shift in CBF autoregulation was caused by a thickened vascular wall that could occlude the vessel lumen, produce hyperreactivity to contractile stimuli, and distend to a lesser degree in response to elevated BP (Strandgaard et al. 1975; Sadoshima et al. 1985). Supporting this hypothesis is the observation that the cerebral arteries of srSHR exhibit a thicker vascular wall and a smaller lumen diameter, and are less distensible than cerebral arteries sampled from WKY (Brayden et al. 1983; Osol and Halpern 1985). When pressurized, arteries sampled from srSHR also exhibit slightly enhanced degrees of myogenic depolarization and spontaneous action potentials with faster rates of rise and fall and a greater amplitude than cerebral arteries sampled from WKY (Harder et al. 1985).

Alterations in sympathetic nerve activity could also play an important role in the development of stroke. Studies have shown that an elevated sympathetic nerve activity protects the blood brain barrier during acute elevations in BP. In these studies, hypertension was found to augment the degree of disruption, while stimulation of the sympathetic nervous system (SNS) prevented such disruption under both hypertensive and normotensive conditions (Bill and Linder 1976; Edvinsson et al. 1978). Some researchers have hypothesized that even under normotensive conditions, stroke in humans could be caused by a deficiency in the SNS (James 1977). Previous studies I have performed indicate that the density of the SNS does not differ between srSHR and spSHR (Smeda 1990). However, other studies involving spSHR, srSHR, and WKY have indicated that the cervical sympathetic ganglion (SCG) of srSHR (whose fibers innervate the cerebrovasculature) exhibits an elevated firing activity at rest and during hemorrhage when compared with spSHR or WKY, whereas such activity in spSHR is comparable to that present in WKY (Mueller and Black 1975).

The exact mechanisms through which the SNS acts to protect the cerebrovasculature are not fully understood. Studies have indicated that the SNS exerts a trophic effect on the cerebrovasculature (Bevan et al. 1983; Sadoshima et al. 1983; Mayhan et al. 1987) and that sympathectomy, when performed at an early age, produces a reduction in the wall mass of the cerebral arteries (Bevan et al. 1983) and a thinning of the blood vessel wall. Studies by Sadoshima et al. (1983) indicate that when the SCG is unilaterally removed from spSHR at 1 month of age, the cerebral arteries in the contralateral brain hemisphere lose their sympathetic innervation and the pial arteries subsequently exhibit a thinner wall. In this instance, of the spSHR that developed stroke, 79% did so in the sympathetically denervated side of the brain. It was thought that stroke developed because the thinner walled vessels were less capable of resisting mechanical distension during hypertension. During acute hypertension, Mayhan et al. (1987) suggested that disruption of the blood brain barrier occurs in venules and that activation of the sympathetic nerves protects this barrier by attenuating the increases in venous pressure.

Alterations in renal function and their implications in the development of stroke

Researchers have observed the presence of renal lesions (Tobian 1986; Volpe et al. 1990), which can be reduced by elevations in dietary K^+ (Tobian 1986), and proteinuria (Nagaoka et al. 1981; Stier et al. 1989) at a time when stroke onset occurs in spSHR. The importance of kidney function in relation to stroke development in spSHR is further emphasized by the observation that renal transplants of spSHR kidneys into srSHR enable srSHR to develop stroke (Shibota et al. 1979).

Studies we have performed (J. S. Smeda, unpublished results) indicate that spSHR with stroke exhibit signs of renal failure. Poststroke spSHR have a reduced glomerular filtration rate (as estimated by the creatinine clearance), elevated plasma urea and creatinine, lower plasma albumin levels, blood within the urine, and 10- to 13-fold higher urinary protein excretion rates than age-matched srSHR without stroke. Some of these alterations precede stroke development, in that 13- to 15-week-old prestroke spSHR exhibit significantly elevated serum urea and creatinine levels, lower serum albumin, and proteinuria.

The above changes may have significant implications with regard to stroke development in spSHR. Uremic conditions, such as those observed in spSHR prior to and after stroke development, have been associated with bleeding tendencies, hemorrhage formation in the gut, as well as the occurrence of intracranial hematomas (Remuzzi 1989). In part, the bleeding tendencies of uremic patients are thought to result from altered platelet adhesive properties (Remuzzi 1989) promoted by the accumulation of uremic toxins such as urea (Eknoyan et al. 1969), guanidinosuccinic acid (Stein et al. 1969) and phenolic acid (Rabiner et al. 1970) owing to reduced glomerular filtration by the kidneys.

The initial stages of cerebral hemorrhage formation in spSHR have been documented to be a breakdown of the blood brain barrier followed by edema formation at the site of disruption, but not the extravascular movement of blood cells (Tamaki et al. 1984). The presence of decreased serum albumin prior to stroke development in spSHR would reduce the colloidal osmotic pressure of the plasma and could facilitate edema formation. The presence of bleeding tendencies secondary to a uremic condition could potentiate the extravascular movement of blood and hemorrhage formation within the brain.

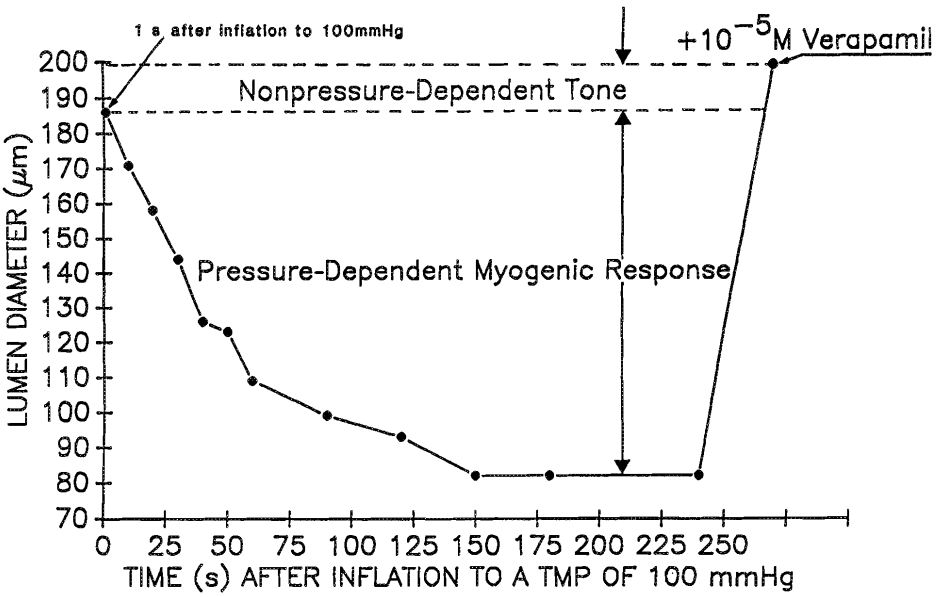

FIG. 1. Pressure-dependent myogenic constrictor response and nonpressure-dependent tone in the middle cerebral artery (MCA) of srSHR. The MCA was allowed to equilibrate to a transmural pressure (TMP) 0 mmHg for 6 min, causing the smooth muscle to dilate. The TMP was then increased to 100 mmHg. Initially, 1 s after pressurization, the artery expanded to a large lumen diameter and constricted within 4 min to a diameter appropriate to the applied pressure (100 mmHg). The change in lumen diameter between 1 s and 4 min after the application of a 100-mmHg TMP step has been termed the pressure-dependent myogenic response. The difference between the lumen diameter at the start of the myogenic response (1 s after pressurization) and maximal relaxation (produced by 10^{-5} M verapamil) has been termed the nonpressure-dependent tone present in the artery.

New Results

Alterations in cerebrovascular pressure-dependent myogenic function in spSHR and uremic srSHR

Introduction

A key mechanism allowing the maintenance of a normal constant blood flow under differing blood pressure conditions is the ability of the cerebral arteries to constrict under conditions where TMPs are elevated (Harder 1984; Osol and Halpern 1985; Johnson 1986). Under such circumstances, the potential increases in blood flow produced by elevations in transmural pressure are counteracted by an increased vascular resistance to flow. This mechanism is particularly taxed under conditions of hypertension in which the elevated blood pressure can potentially produce forced dilation and overperfusion of the cerebrovasculature and initiate hemorrhage formation in the brain.

Studies we have done tested the hypothesis that a breakdown in pressure-dependent myogenic function in spSHR might lead to an overperfusion of the brain and the initiation of hemorrhagic stroke. The myogenic function of the middle (MCA) and posterior (PCA) cerebral arteries was studied in srSHR and in spSHR prior to and after stroke development. In addition, the cerebral arteries of spSHR fed a low K^+ diet versus high K^+ diets were compared to determine if elevations in dietary K^+ also alter pressure-dependent myogenic function in a manner that might be conducive to retarding stroke development.

In view of the potential importance of alterations in renal function in the initiation of stroke, a second study was undertaken in which srSHR were made uremic via partial nephrectomy. The pressure-dependent myogenic function of the MCA was studied in 10- to 11-week-old uremic srSHR without stroke. The purpose of the study was to determine if the production of uremia via nephrectomy was associated with a compromise in pressure-dependent myogenic function in the MCA and stroke development in the animals.

Methods

At weaning (5 weeks of age), male spSHR from each litter were divided equally; half were fed a Japanese-style diet containing 4% NaCl + 0.75% K^+ (Zeigler Bros., Gardners, PA, U.S.A.), whereas the other half were fed the same diet with a K^+ content of 2.11%. All srSHR used in the study were fed the 0.75% K^+ version of the above diet from 5 weeks of age.

To study the effects of uremia on pressure-dependent myogenic function, a subgroup of srSHR were subjected to partial nephrectomy. Six-week-old srSHR were anesthetized with a combination of rompun (16 mg/kg) plus ketamine (83 mg/kg) given as a single intramuscular injection. An incision was made in the dorsal side of the animal exposing one kidney. A temporary tourniquet was placed around the main renal artery and vein to restrict blood flow. Subsequently, one third to two thirds of the kidney was surgically amputated in a manner in which the adrenal gland and its circulation remained undamaged. During this amputation, the cut areas were cauterized to prevent bleeding and the tourniquet was removed. The kidney was replaced in the body cavity and the external wound was sutured. After a 10-day-recovery period, the other kidney of the animal was totally removed in such a way that the adrenal gland and its circulation were left intact. The net result of the operation was the removal of two thirds to five sixths of the total renal mass of each animal.

The right or left MCA and (or) PCA sampled from the above animal groups was mounted in a pressure myograph comparable to that previously described by Osol and Halpern (1985). The arteries were tied on a small hollow glass pipette with 10.0 thread and the end

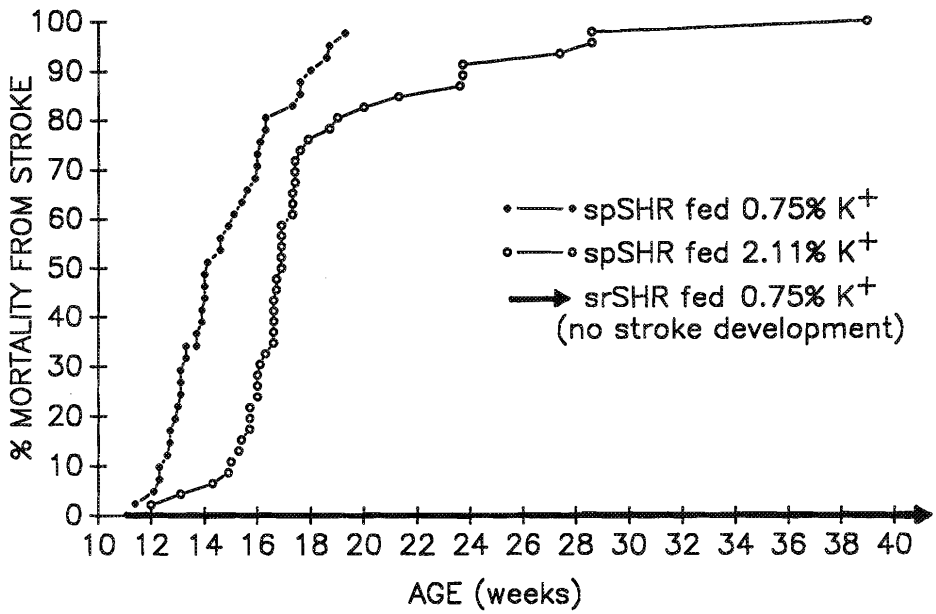

FIG. 2. Mortality from stroke in spSHR fed a Japanese-style diet containing 4% NaCl and either 0.75 or 2.11% K^+. srSHR fed the 0.75% K^+ version of the above diet failed to develop stroke over the time period shown.

of the artery was tied off forming a blind sac. The lumen of the artery as well as the pipette were filled with Krebs saline solution and were connected to a pressure reservoir connected to a 95% O_2 – 5% CO_2 gas cylinder. The system allowed the artery to be pressurized to any given TMP instantaneously via a valve system. The outside of the artery was suffused with Krebs' solution bubbled with 95% O_2 – 5% CO_2 at a pH of 7.4. The artery was viewed with a microscope and was observed as a transparent image with the inside lumen diameter visible. Alterations in lumen diameter were recorded via a VCR and played back on a TV monitor at 322× magnification. The alterations in lumen diameter with pressure were measured from the TV monitor.

The experiment was started by equilibrating the artery to a TMP of 100 mmHg for 30 min, which caused the arteries to constrict (30–40% reduction in lumen diameter). Subsequently, the TMP was decreased to 0 mmHg for 6 min. Under such conditions, the artery perceives a situation analogous to that which might be present under massive bleeding conditions and attempts to relax. Subsequent to equilibration for 6 min to 0 mmHg, the artery was reinflated to a TMP of 100 mmHg for 4 min. Since the artery was initially equilibrated to a TMP of 0 mmHg (causing the smooth muscle to relax), reinflation to a TMP of 100 mmHg initially caused the lumen to expand to large diameter, with the artery subsequently recontracting to a level appropriate for a TMP of 100 mmHg.

Figure 1 demonstrates the change in lumen diameter with time occurring in a MCA from 1 s to 4 min after inflation to a TMP of 100 mmHg subsequent to an equilibration to 0 mmHg for 6 min. The lumen diameter at 1 s after inflation represents the diameter that would be present prior to significant engagement of pressure myogenic constriction; the change in diameter between 1 s and 4 min after pressurization represents the amplitude of pressure-dependent myogenic constriction in response to a 100 mmHg pressure step. Within MCA and PCA, 10^{-5} M verapamil maximally relaxed the arteries to a level equivalent to that produced by Ca^{2+}-free Krebs' solution containing 2 mM EGTA.

From Fig. 1, it can be noticed that the start of the pressure-dependent myogenic response (1 s after inflation to a TMP of 100 mmHg) never occurs from maximal relaxation (produced by 10^{-5} M verapamil). The difference in lumen diameter observed at 1 s after inflation to a TMP of 100 mmHg versus that present at a TMP of 100 mmHg in the presence of verapamil has been termed the nonpressure-dependent tone in the artery. This latter measurement is important in that the pressure-dependent myogenic constrictor response can be abolished by inhibiting mechanisms that allow the artery to constrict to pressure and (or) by elevating the level of nonpressure-dependent tone to a level at which the artery remains locked in a constricted state and fails to relax when the TMP is decreased. Such a situation is analogous to that which may occur if pressure-dependent myogenic function is measured in an artery subjected to contraction with a strong agonist.

Statistical analysis was performed using a Minitab computer program (Minitab Inc., State College, PA, U.S.A.). In the case of multiple comparisons of three or more groups of data, a one-way analysis of variance was used to test if a significant group effect was present. Subsequently, an unpaired Student *t*-test was used to determine which of the individual groups differed from each other. Results were considered significant at $p < 0.05$. All values in the Result section are expressed as the mean ± one standard error.

Results

As shown in Fig. 2, spSHR fed a low (0.75%) K^+ Japanese-style diet typically start dying from stroke after 11.5 weeks of age and an 80% mortality occurs by 16 weeks of age; the average life-span is 14.8 ± 0.3 weeks ($n = 41$). Elevating dietary K^+ to 2.11% increased the average life-span of spSHR to 18.3 ± 0.7 weeks ($n = 46$, $p < 0.0001$ versus 0.75% K^+ diet). SrSHR fed the 0.75% K^+ version of the diet did not develop stroke. The average life-span of these animals was approximately 65 weeks of age and within our colony such animals usually die from respiratory as opposed to stroke-related complications.

Hemorrhages were not randomly distributed within the brain. They virtually only occur in the cerebrum, which is perfused by the anterior (ACA), middle, and posterior cerebral arteries and not in the cerebellum or brain stem, which in the rat is virtually exclusively perfused by the vertebral basilar arterial systems. As shown in Table 1, the number of hemorrhagic lesions in the cerebrum, their distribution between the right and left cerebral hemispheres, and their occurrence within the perfusion domains of the ACA, MCA, and PCA did not significantly differ in poststroke spSHR fed 0.75 or 2.11%

K^+ diets. Within each group, lesions were equally distributed between the right and left cerebral hemispheres.

Some trends were observed in that within the left cerebral hemisphere hemorrhage predominantly occurred in the perfusion domain of the MCA, whereas in the right hemisphere the mean incidence of hemorrhage is greatest in the perfusion domain of the PCA. However, the above trends were not statistically significant ($p > 0.05$, one-way analysis of variance; ACA versus MCA versus PCA). An interesting observation (last column, Table 1) was that approximately 50% of all hemorrhages overlap an area where the perfusion domain of the MCA intersects that of the ACA or PCA, an area where in the rat the MCA anastamose and form collateral circulation with the ACA and PCA (Coyle and Jokelainen 1982; Coyle and Heistad 1986).

Pressure-dependent and -independent myogenic tones were studied in four groups of animals. The ages, strains, diets, blood pressures, and stroke status of the rats are outlined in Table 2. One group of spSHR consisted of animals 11.0–12.2 weeks of age without stroke and was fed low or high K^+ versions of the diet (respectively, called young prestroke LK and HK spSHR in Figs. 3 to 6). From Fig. 2, it can be observed that these animals are sampled at an age when the incidence of stroke is low. A second group of spSHR was sampled between 12.3 and 15.5 weeks of age (respectively, called old prestroke LK and HK spSHR in Figs. 3 to 6). As the above group, these spSHR exhibited no evidence of hemorrhagic stroke but had brothers that had already developed stroke. Therefore, the probability of stroke development in the near future was very high within this group, particularly in spSHR fed the low versus the high K^+ version of the diet. The third group of spSHR sampled was made up of 12.3- to 15.5-week-old spSHR fed high or low K^+ diets and showed physical evidence of hemorrhage within the brain. The fourth group was srSHR fed a low K^+ diet and was sampled between 11.0 to 15.5 weeks of age. As previously mentioned, these SHR never develop stroke at any age when fed a low K^+ diet.

The above groups enabled us to study myogenic function within the cerebral arteries of spSHR with stroke and in prestroke spSHR prior to and during time periods when the risk of stroke was high. The myogenic function of the arteries was compared between spSHR and srSHR, which are resistant to stroke, and between spSHR fed low and high K^+ versions of the diet.

Middle cerebral arteries of spSHR

Figure 3 outlines the lumen diameters present 1 s and 4 min after the MCA were subjected to a 100-mmHg TMP step (subsequent to the artery being equilibrated to 0 mmHg for 6 min). The MCA were sampled from the various groups of SHR outlined in Table 2. The arrows indicate the amplitude (in μm) of constriction observed in response to 100-mmHg pressure step.

Young 11.0- to 12.2-week-old prestroke spSHR fed high or low K^+ diets exhibited pressure-dependent myogenic responses of comparable amplitude to those present in srSHR fed a low K^+ diet. The lumen diameters present at the start and finish of the pressure-dependent myogenic response were not significantly different between the three groups. In older prestroke spSHR between 12.3 and 15.5 weeks of age, the amplitude of the myogenic response was significantly reduced over that present in srSHR and younger prestroke spSHR. In this instance, the lumen diameter of the MCA at the start (1 s after

TABLE 1. Mean number and percent distribution of hemorrhagic lesions between the right and left cerebral hemispheres and between the perfusion domains of the anterior (ACA), middle (MCA), and posterior (PCA) cerebral arteries of spSHR with stroke

Diet	Age (weeks)	Mean no. of lesions per brain	% lesions in left hemisphere	% lesions in right hemisphere	Distribution of lesions (%) in relation to arterial perfusion domain						% of total lesions lying in the MCA–ACA or MCA–PCA perfusion overlap
					Left hemisphere			Right hemisphere			
					ACA	MCA	PCA	ACA	MCA	PCA	
spSHR											
(n = 20) 0.75% K	13.9±0.3	3.1±0.5	46.8±8.8	53.2±8.8	18.9±5.6	16.4±4.0	10.4±4.3	9.0±3.0	20.6±5.4	24.7±6.0	47.7±8.3
spSHR											
(n = 20) 2.11% K	15.8±0.5	2.6±0.3	56.2±8.8	43.8±8.8	14.0±3.8	28.2±5.0	13.2±4.0	5.2±2.1	14.1±5.4	25.3±7.5	54.5±9.6
p* values	0.003	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NOTE: Values are means ± one standard error.
*Unpaired Student t-test; NS, $p > 0.05$.

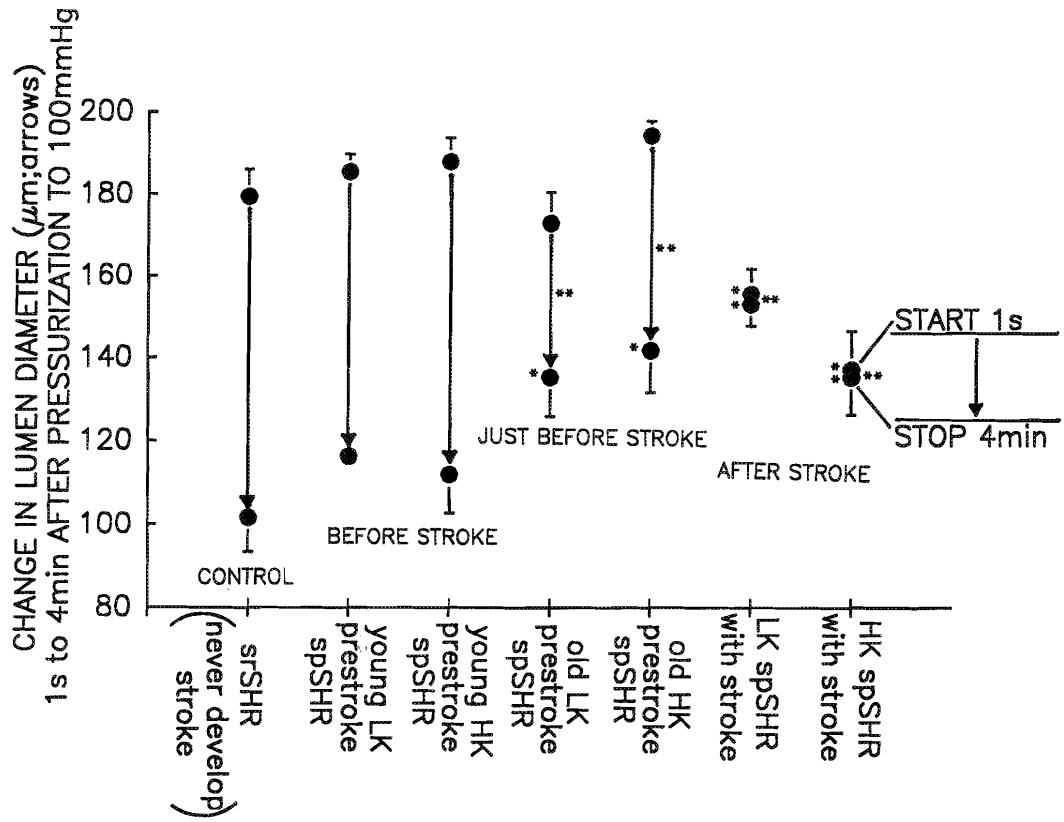

FIG. 3. The pressure-dependent myogenic response in the middle cerebral arteries of srSHR fed a 0.75% K^+ diet and pre- and post-stroke spSHR fed 0.75 or 2.11% K^+ diets. The physical characteristics of each subgroup of SHR are described in Table 2. LK, 0.75% K^+ diet; HK, 2.11% K^+ diet. *Significant difference ($p < 0.05$) in lumen diameter when compared with control; **significant ($p < 0.05$) difference in amplitude (change in lumen diameter between 1 s and 4 min after pressurization) when compared with control amplitude (unpaired Student t-test; means ± one standard error shown).

TABLE 2. Physical characteristics of spSHR and srSHR used within the study

Animal groups	Age (weeks)	n	Diet	Systolic blood pressure (mmHg)	Stroke status
Young prestroke spSHR	11.0–12.2	7	0.75% K^+	211±6*	No hemorrhage
		7	2.11% K^+	217±12	
Old prestroke spSHR	12.3–15.5	7	0.75% K^+	231±18*	No hemorrhage
		6	2.11% K^+	246±14*	
spSHR with stroke	12.3–15.5	9	0.75% K^+	243±12*	All with hemorrhagic stroke
		12	2.11% K^+	243±19	
Stroke-resistant srSHR	11.0–15.5	10	0.75% K^+	187±4	No hemorrhage

NOTE: Values are means ± one standard error.
*$p < 0.05$ compared with srSHR of the same age range (unpaired t-test).

pressurization) of the myogenic response was comparable in spSHR to that present in srSHR, but the arteries maintain a significantly larger lumen diameter 4 min after pressurization. After stroke development, the myogenic response was totally abolished in spSHR fed high or low K^+ diets. Such MCA not only failed to achieve a level of luminal constriction in response to a 100 mmHg TMP step that was comparable to that present in the MCA of srSHR but also maintained a smaller lumen diameter at the start of the pressure-dependent myogenic response. This latter observation suggested that after stroke, MCA are not only less able to constrict to pressure but also lose the ability to relax when equilibrated to 0 mmHg for a period of 6 min. This is further demonstrated in Fig. 4 in which the level of nonpressure-dependent tone present in the MCA of the various groups is outlined. Here the lumen diameter at the start of the pressure-dependent myogenic response (1 s after pressurization) is compared with that present under maximally relaxed conditions. It can be observed that the differences in lumen diameter between relaxed conditions and the start of the myogenic response are small and comparable between srSHR and both groups of prestroke spSHR, whereas the level of nonpressure-dependent tone present in MCA is significantly larger in both high and low K^+ fed spSHR after stroke than in prestroke spSHR or srSHR.

The ability of altered dietary K^+ to modify myogenic function was also assessed in the various groups of spSHR studied.

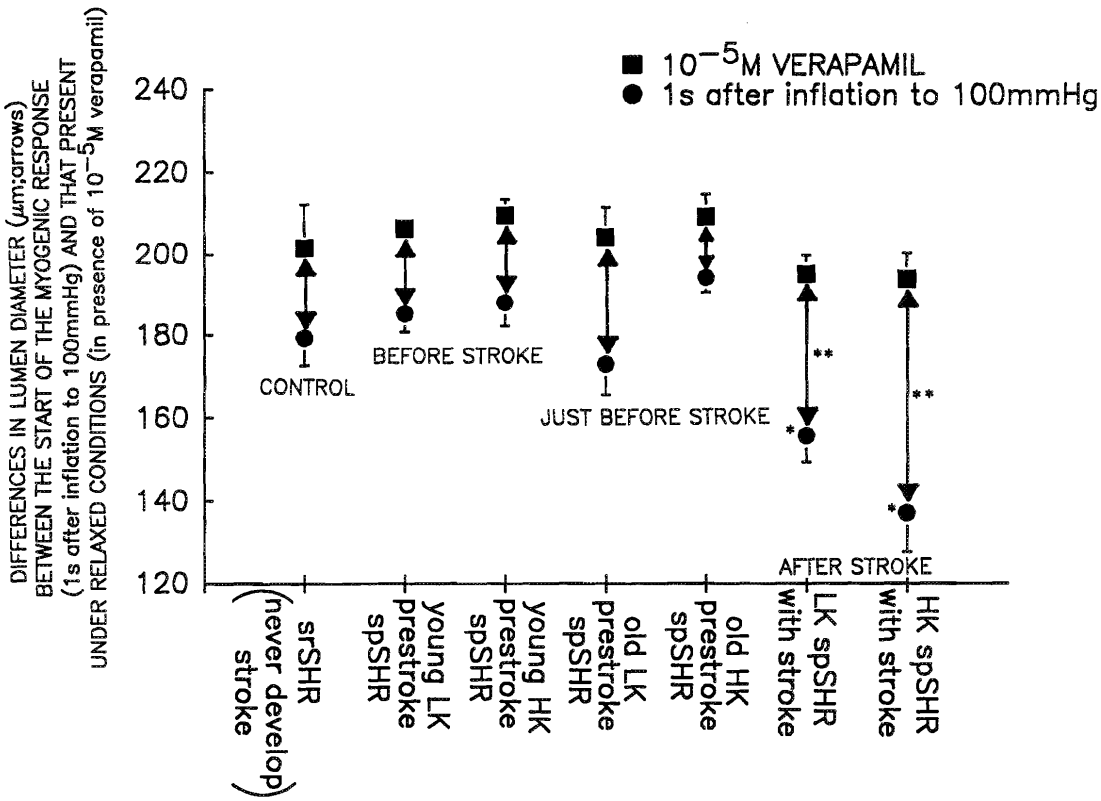

FIG. 4. The nonpressure-dependent tone present in the middle cerebral arteries of srSHR fed a 0.75% K^+ diet and pre- and post-stroke spSHR fed a 0.75 or 2.11% K^+ diet. The physical characteristics of each subgroup of SHR are described in Table 2. LK, 0.75% K^+ diet; HK, 2.11% K^+ diet. *Significant difference ($p < 0.05$) in lumen diameter when compared with control; **significant difference ($p < 0.05$) in amplitude of pressure-dependent tone (lumen diameter in presence of verapamil minus lumen diameter after 1 s of inflation to 100 mmHg) when compared with control (unpaired Student t-test, means ± one standard error shown).

It was observed that the amplitude of pressure-dependent myogenic constriction to a 100 mmHg TMP step (Fig. 3) as well as the levels of nonpressure dependent tone (Fig. 4) were not significantly different in MCA when high versus low K^+ fed spSHR were compared within each group.

Posterior cerebral arteries of spSHR

The pressure myogenic response of PCA to a 100 mmHg TMP step is outlined in Fig. 5. The amplitude of myogenic constriction (arrows) that occurred in response to a 100 mmHg pressure step, and the lumen diameters present at the start and finish of the constrictor response, were comparable in srSHR fed a low K^+ diet and 11.0 to 12.2-week-old prestroke spSHR. Low K^+ but not high K^+ fed 12.3- to 15.5-week-old prestroke spSHR exhibited smaller amplitudes of pressure-dependent myogenic constriction than either low K^+ fed srSHR or younger spSHR. Unlike the situation present in the MCA, pressure-dependent myogenic responsiveness, although reduced in amplitude when compared with srSHR, was still maintained in the PCA after stroke development.

The levels of nonpressure-dependent tone present in the PCA of the various groups of SHR are outlined in Fig. 6. The differences in lumen diameter observed under relaxed conditions and those present at the start of the pressure-dependent myogenic response were comparable in all the groups of SHR studied; however, the lumen diameters of the PCA under relaxed conditions were smaller in 12.3- to 15.5-week-old pre- and post-stroke spSHR than the PCA of srSHR. Hence, the somewhat smaller pressure-dependent myogenic constrictor responses observed in 12.3- to 15.5-week-old pre- and post-stroke spSHR achieved the same final lumen diameter as that present in srSHR after a 4-min equilibration to a TMP of 100 mmHg (see Fig. 5).

Within the various groups of spSHR, the levels of nonpressure-dependent tone present and the amplitude of constriction observed in response to a 100 mmHg pressure step were not significantly different in PCA sampled from high versus low K^+ fed spSHR. However, the PCA of 12.3- to 15.5-week-old low K^+ fed but not high K^+ fed prestroke spSHR exhibited a significant decline in the amplitude of the pressure-dependent myogenic constrictor response when compared with 11.0- to 12.2-week-old prestroke spSHR fed, respectively, low and high K^+ diets.

Middle cerebral arteries of uremic, nephrectomized srSHR

As previously described, in our colony srSHR fed a low K^+ Japanese-style diet normally never develop hemorrhagic stroke and live on average to about 65 weeks of age. Nephrectomized uremic srSHR that survived for a period greater than 10 days postoperatively exhibited a 45% incidence of cerebral hemorrhage (10 out of 22 animals). An example of a brain sampled from one of these animals is shown in Fig. 7.

A group of 10- to 11-week-old uremic nephrectomized srSHR that had not yet developed cerebral hemorrhages were sampled. The pressure-dependent constriction of the MCA of these animals in response to a 100-mmHg pressure step is outlined in Fig. 8. Since many nephrectomized srSHR exhibited not only uremia but also a further elevation in blood pressure,

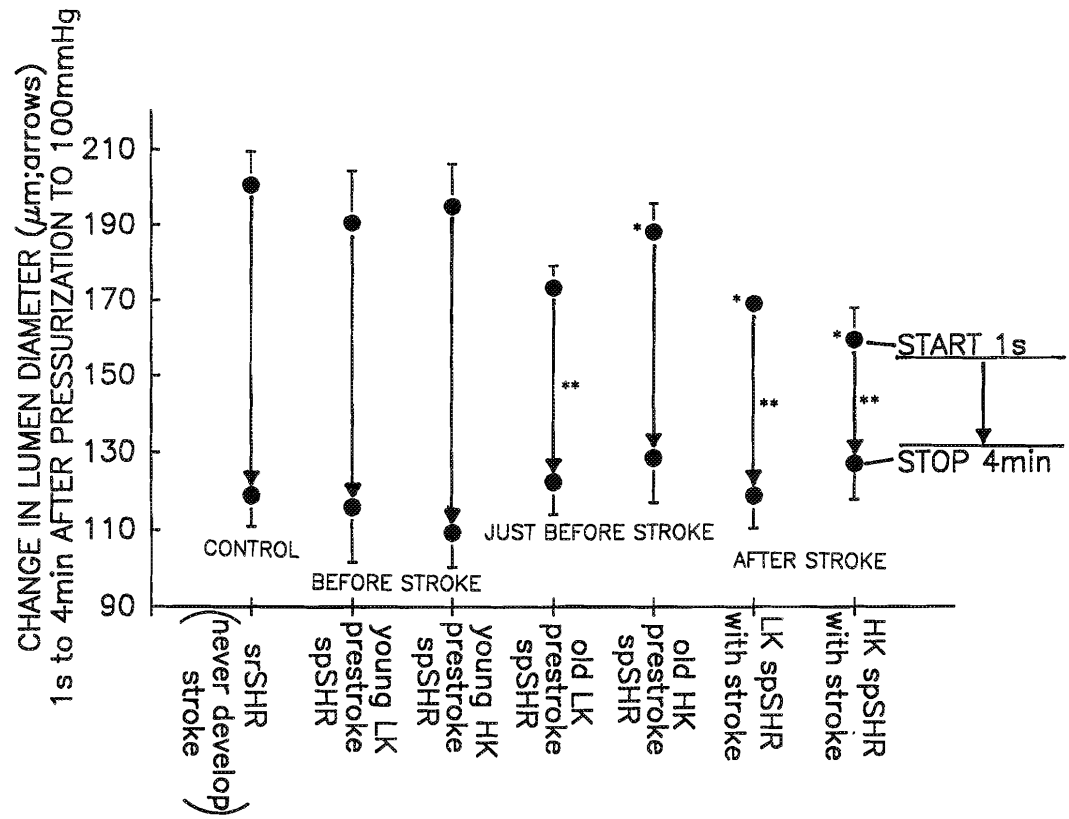

FIG. 5. Pressure-dependent myogenic response in posterior cerebral arteries of srSHR fed a 0.75% K^+ diet and pre- and post-stroke spSHR fed a 0.75 or 2.11% K^+ diet. (For further details, see caption for Fig. 3.)

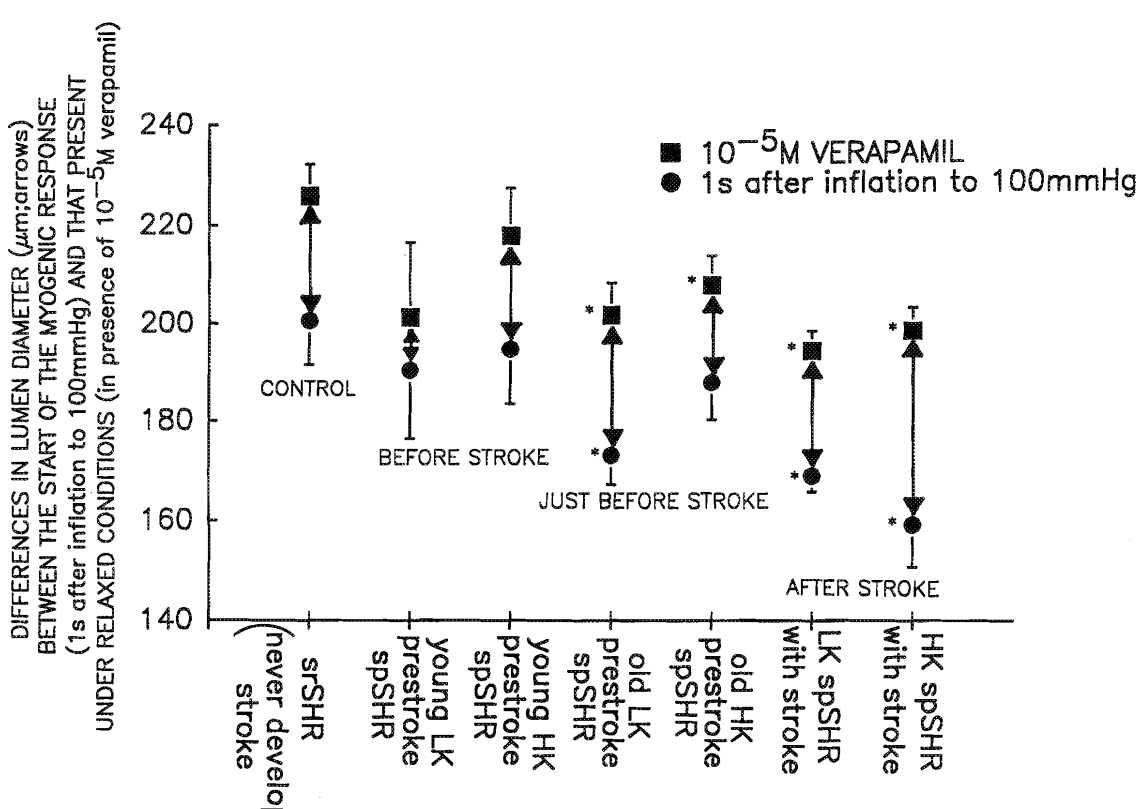

FIG. 6. The nonpressure-dependent tone present in the posterior cerebral arteries of srSHR fed a 0.75% K^+ diet and pre- and post-stroke spSHR fed a 0.75 or 2.11% K^+ diet. (For further details, see caption for Fig. 4.)

FIG. 7. An example of a hemorrhagic lesion (arrow) in a uremic srSHR. Under normal conditions, srSHR never develop stroke. A high incidence of stroke occurs in srSHR made uremic via nephrectomy.

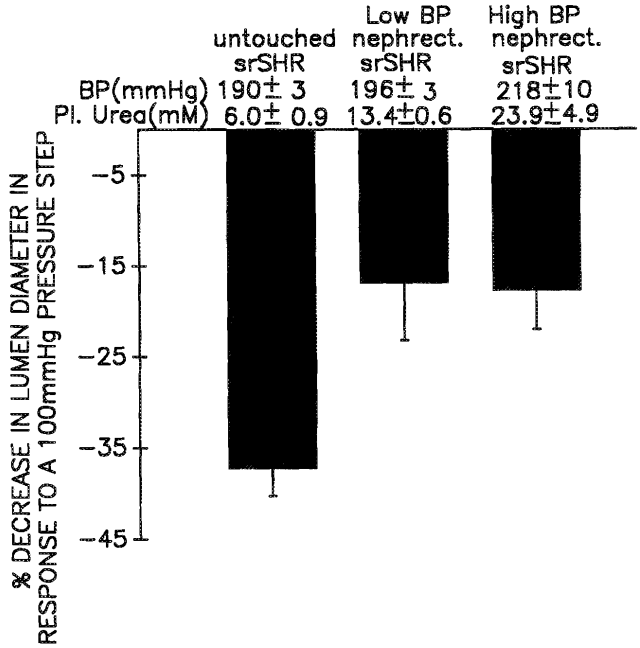

FIG. 8. The pressure-dependent myogenic response to a 100-mmHg pressure step (see Fig. 1) expressed as a percent change in lumen diameter in two groups of two-thirds to five-sixths nephrectomized, uremic srSHR having elevated ($n = 8$) and unaltered ($n = 4$) systolic blood pressures and in non-nephrectomized srSHR ($n = 5$). ($p < 0.05$, both uremic vs. non-uremic srSHR, means ± one standard error shown.)

the above group of srSHR was divided into uremic srSHR having hyper-elevated blood pressure and blood pressures comparable to those present in non-uremic, non-nephrectomized srSHR. As shown in Fig. 8, the MCA of both groups of uremic srSHR exhibited compromised pressure-dependent myogenic function. The amplitude of the constrictor response was approximately half of that present in non-nephrectomized srSHR.

Discussion

The results of the study indicate that the ability of MCA and PCA to constrict in response to elevations in TMP declines prior to stroke development. Such alterations are particularly pronounced in the MCA of spSHR that exhibit a significant reduction in myogenic function prior to stroke at an age when the onset of stroke is likely. After stroke development, the ability of the MCA to elicit pressure-dependent myogenic constrictor responses is lost. The net effect of this is that prior to and after stroke the MCA of spSHR maintain a larger lumen diameter at 100 mmHg and as well at TMPs up to 200 mmHg (not shown) when compared with srSHR. In spSHR, the loss of pressure-dependent myogenic function in MCA after stroke was not dependent upon the location or size of the hemorrhage present in the brain. In this regard, even spSHR with small pinpoint hemorrhages in areas far from the perfusion domain of the MCA had MCA with compromised pressure-dependent myogenic function. Such defects were not the result of a generalized defect in the ability of the MCA to constrict. Many MCA from poststroke spSHR that lack the ability to constrict to pressure were still capable of constricting to 10^{-6} M serotonin (5-HT); MCA preconstricted with 10^{-6} M 5-HT readily relaxed in response to 100 μM sodium nitroprusside (J. S. Smeda, unpublished results). The above observations suggest that the contractile apparatus as well as the cGMP relaxation mechanisms of the MCA remain functional after stroke development.

A compromise in myogenic constrictor function to pressure and an enlargement of the MCA lumen (when compared with srSHR) prior to stroke development in spSHR could be important in the initiation of stroke. Such an alteration in the intermediate sized segments of the MCA might increase the downstream blood flow and pressure within the arterial system. The above effects could lead to increased shear on the endothelium and an increase in tangential wall stress in the smaller downstream vessels. This could increase permeability and might promote a breakdown of the smaller downstream arterioles leading to hemorrhage.

When compared with srSHR, prestroke spSHR fed a low K^+ diet and both low and high K^+ fed poststroke spSHR also have PCA with compromised pressure-dependent myogenic function. However, the decline in such function was not as large as that observed in the MCA. Furthermore, unlike the MCA of poststroke spSHR, the PCA were still capable of eliciting appreciable myogenic constrictor responses to pressure. In addition, despite some decline in the ability of these arteries to constrict to pressure prior to and after stroke at equal pressures, the lumen diameters of the PCA remain comparable to those observed in srSHR. This is largely due to the observation that at ages when there is a decline in the myogenic function, such arteries have structurally smaller lumen diameters. Hence, a smaller myogenic constriction achieves

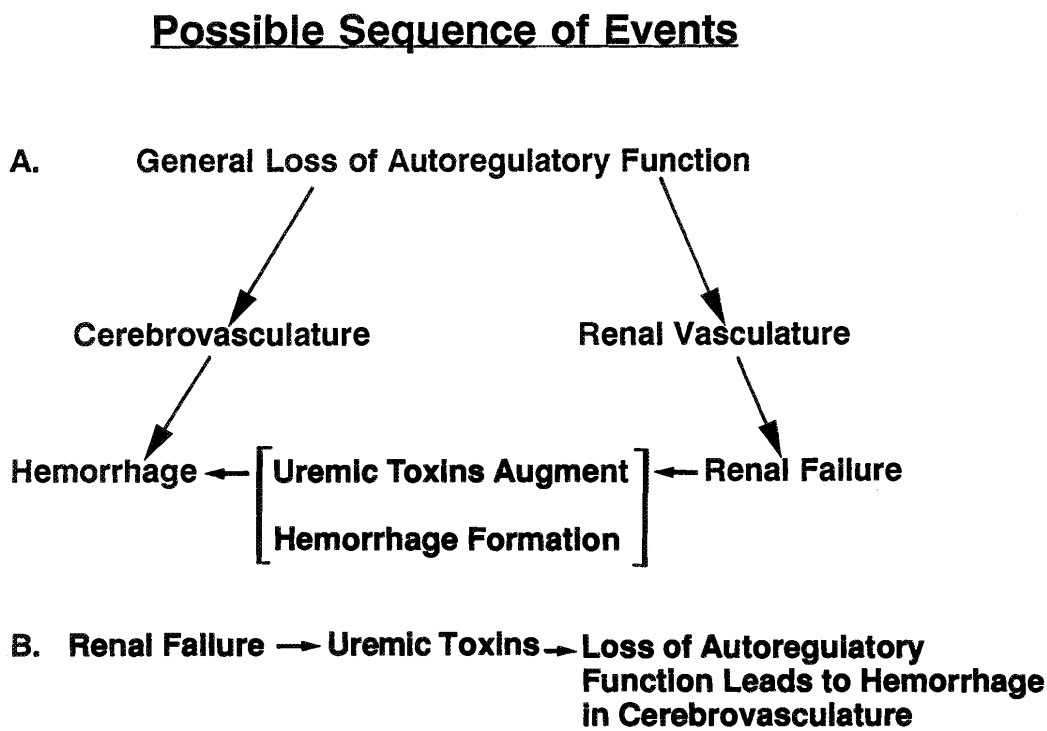

FIG. 9. Two hypothetical sequences of events outlining how alterations in kidney function might be involved in stroke development in spSHR. (A) There could be a general breakdown in the ability of autoregulating blood vessels to exhibit pressure-dependent vasoconstriction. Since both cerebral as well as renal vessels carry out such functions, such alterations may lead to cerebral hemorrhage and renal failure simultaneously. In this instance, the development of cerebral hemorrhage and renal failure might be coincidental; however, the development of uremia might augment or complicate cerebral hemorrhage formation. In sequence B, it could be possible that renal failure precedes cerebral hemorrhage formation and the presence of uremia may compromise pressure-dependent myogenic function in the cerebrovasculature, thus resulting in an overperfusion of the brain leading to hemorrhage.

the same final lumen diameter in the PCA of spSHR as that present in the PCA of srSHR.

In spSHR prior to stroke, the presence of a MCA with an expanded lumen and a PCA having a more normal lumen could have implications with respect to the development of hemorrhagic stroke. Since both the MCA and PCA are in competition for blood entering the circle of Willis via the internal carotid, it might be expected that having a more constricted PCA and a dilated MCA would result in even more blood being shunted to the MCA than if both arteries had enlarged lumina. Such an alteration could amplify the pressure and shear stress experienced by the MCAs and hence potentiate stroke development.

In addition to lacking the ability to constrict to pressure, poststroke spSHR also had MCA that maintained elevated levels of nonpressure-dependent tone and failed to relax when pressure was decreased. Since such changes occurred only in poststroke spSHR, it would appear that the alterations were secondary to the presence of hemorrhagic stroke. Such an alteration could be of physiological significance. If the downstream pressure decreased in the MCA due to the presence of blood seepage in more proximal regions of the MCA, the inability of the artery to relax in response to a decrease in pressure might amplify the development of ischemia in the downstream portion of the artery after hemorrhage has occurred.

The differences in myogenic function observed in high versus low K^+ fed spSHR were very small. Using linear regression analysis, I have observed that the amplitude of pressure-dependent constriction to a TMP step of 100 mmHg exhibits a significant linear decline with age in both low K^+ fed ($r = 0.786$, $p < 0.001$) and high K^+ fed ($r = 0.663$; $p < 0.05$) spSHR after 11 weeks of age, and that the rate of decline in such function is slightly steeper in the low K^+ versus the high K^+ fed groups of spSHR. It is of interest to note that extrapolation of the best fitting lines of the above analysis to a point where myogenic function is totally lost coincides nearly perfectly with an age at which 100% mortality from stroke occurs in spSHR fed high or low K^+ diets. The lack of a large difference between the high versus the low K^+ fed spSHR with respect to myogenic function could reside in the fact that in absolute terms, elevations in dietary K^+ produce only a modest retardation in stroke development, and that techniques used in the present study may be limited in their ability to detect the potentially small differences in myogenic function that may exist. It could also be possible that elevation in dietary K^+ alters some other mechanism in spSHR, which exerts a protective effect against hemorrhagic stroke development despite the presence of compromised pressure-dependent myogenic function in these animals.

The loss of myogenic function in the MCA prior to and after stroke development in spSHR may be related to systemic changes taking place as a result of an alteration in kidney function in spSHR. We have observed that the kidneys of spSHR with cerebral hemorrhage exhibit ischemic unperfused areas. It is possible that the presence of uremia also decreases pressure-dependent myogenic constriction in the MCA of spSHR and therefore makes these animals susceptible to hemorrhagic stroke. Consistent with this observation, srSHR

made uremic via nephrectomy have MCA with compromised pressure-dependent myogenic function when compared with non-uremic srSHR. The decline in such function does not appear to be related to the further elevation in blood pressure produced by nephrectomy. Uremic nephrectomized srSHR also develop hemorrhagic stroke. In this latter instance, however, the relative contributions of uremia versus a further elevation in blood pressure produced by the nephrectomy are unclear. In this regard, it is worth noting that the only two drug treatments that have been demonstrated to retard stroke development in spSHR without altering BP (enalapril (Stier et al. 1989) and CV-4093 (Nagaoka 1986)) also alter renal function. Enalapril treatment of spSHR was associated with an increase in glomerular filtration (Stier et al. 1989) and if the action of CV-4093 is to dilate the preglomerular vasculature as suggested (Nagaoka 1986), it might also increase glomerular filtration. It is possible that the antistroke action of enalapril and CV-4093 could reside in the ability of these agents to increase glomerular filtration and hence decrease the accumulation of uremic toxins. An alternative suggestion that has been proposed is that activation of the renin–angiotensin system is important in the initiation of stroke development in spSHR (Volpe et al. 1990). The relationship of such activation of stroke development is unclear; however, converting enzyme inhibitors as well as drugs that increase glomerular filtration via an increase in renal blood flow would, respectively, decrease angiotensin II formation by decreasing renal renin secretion.

Conclusion

Figure 9 summarized two hypothetical sequences of events that may occur in spSHR. (A) There could be a general breakdown in the ability of autoregulating blood vessels to exhibit pressure-dependent vasoconstriction. Since both cerebral as well as renal vessels carry out such functions, such alterations may lead to cerebral hemorrhage and renal failure simultaneously. In this instance, the development of cerebral hemorrhage and renal failure might be coincidental; however, the development of uremia might complicate cerebral hemorrhage formation. In sequence B, it could be possible that renal failure precedes cerebral hemorrhage formation and the presence of uremia may compromise pressure-dependent myogenic formation in the cerebrovasculature, thus resulting in an overperfusion of the brain leading to hemorrhage.

Acknowledgement

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