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Spironolactone Improves Structure and Increases Tone in the Cerebral Vasculature of Male Spontaneously Hypertensive Stroke-Prone Rats

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Abstract

Background: Previous studies show that ischemic cerebral infarct size is related to cerebral vessel structure. Spironolactone, a mineralocorticoid receptor antagonist, decreases ischemic cerebral infarct size in male spontaneously hypertensive stroke-prone rats (SHRSP). Therefore, we hypothesized that chronic spironolactone treatment would improve cerebral artery structure in the SHRSP.

Methods: Six-week-old male SHRSP were treated with spironolactone (2.5 mg/day) for six weeks and were compared to untreated control SHRSP and normotensive Wistar Kyoto (WKY) rats. Using a pressurized arteriograph, structural measurements of the middle cerebral artery (MCA) were taken under passive (calcium-free), zero-flow conditions. Myogenic tone was calculated from active and passive measurements taken at 75 and 125 mmHg. Mean arterial pressure was measured using radiotelemetry.

Results: Myogenic tone was increased only at 75 mmHg in the spironolactone-treated SHRSP compared to control rats. The MCA lumen and outer diameters were increased in the spironolactone-treated SHRSP compared to control SHRSP, but were not different from WKY rats, indicating a decrease in vascular remodeling. There was no effect of spironolactone on blood pressure, suggesting this is a blood pressure independent effect.

Conclusion: Increased myogenic tone and lumen diameter in the spironolactone-treated SHRSP may be responsible for the protective role of spironolactone in ischemic stroke.

Keywords

aldosterone; hypertension; SHRSP; spironolactone; vascular remodeling; arteriograph; middle cerebral artery

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Introduction

Stroke is the third leading cause of death in the United States and 88 percent of all strokes are ischemic, caused by a blockage in a cerebral blood vessel, which impairs blood flow in the brain (Thom et al., 2006). Hypertension is a known risk factor for stroke (Kannel, 1979). Studying the combined effects of hypertension and stroke in the spontaneously hypertensive stroke prone rat (SHRSP) has proven to be a useful tool, due to the similarity of this model to human cerebrovascular disease (Yamori et al., 1976). Normotensive Wistar Kyoto (WKY) rats have smaller cerebral infarcts after experimental induction of ischemia when compared to SHRSP (Coyle and Jokelainen, 1983), and have traditionally been utilized as the control strain for SHRSP. Previous studies of the cerebral vessel collaterals in SHRSP and WKY rats have shown that the density of these vessels does not differ between the two strains (Coyle, 1987), but that the luminal widths of these vessels are a major determinant of the amount of tissue protected from infarction (Coyle and Feng, 1993; Coyle and Heistad, 1991). These studies link cerebral vessel lumen diameters and the damage caused by an ischemic stroke in the SHRSP.

Increased plasma aldosterone has also been identified as a risk factor for stroke (Brunner et al., 1972) and there is increasing evidence that aldosterone has a direct effect on the vascular pathology of essential hypertension (Connell et al., 2003; Connell and Davies, 2005). Blockade of components of the renin-angiotensin-aldosterone system (RAAS) has been shown by many studies to prevent or reduce injury from stroke and cardiovascular remodeling, usually through pressure dependent mechanisms (Schiffrin and Touyz, 2004). While most studies of the RAAS focus on the effects of angiotensin II, investigators are now beginning to show that aldosterone is also a key mediator of deleterious cardiovascular events, mostly through activation of the mineralocorticoid receptor (Funder, 2004; Rocha and Funder, 2002; Struthers and MacDonald, 2004). Human studies using spironolactone (Pitt et al., 1999) and eplerenone (Pitt et al., 2003) have shown a beneficial effect of mineralocorticoid receptor antagonism in patients with cardiovascular disease. Chronic treatment with captopril, an angiotensin converting enzyme inhibitor, afforded the SHRSP fed a high sodium diet protection from hemorrhagic stroke, but this protection was lost when aldosterone was given back (MacLeod et al., 1997), suggesting aldosterone may be mediating the effects of angiotensin II. Interestingly, chronic treatment with spironolactone in SHRSP fed a stroke-prone (or high sodium) diet reduces mortality and the incidence of spontaneous hemorrhagic strokes (Rocha et al., 1998). Studies from our laboratory show that chronic treatment with spironolactone causes a reduction in ischemic cerebral infarct size in both male SHRSP and male WKY rats by approximately 50% (Dorrance et al., 2000; Dorrance et al., 2001). Similar to the studies by Rocha, *et al* (Rocha et al., 1998), these changes occurred without a reduction in blood pressure. The opposite effect has also recently been shown by our laboratory; whereby, intact, male Wistar rats that were treated with the mineralocorticoid, deoxycorticosterone acetate (DOCA), had increased ischemic cerebral infarct sizes compared to non-treated rats (Dorrance et al., 2006b).

Since ischemic infarct size is directly related to blood flow in the cerebral blood vessels (Coyle and Heistad, 1987) and blood flow through a vessel is proportional to the lumen diameter of the vessel, it is possible that an alteration in cerebral blood vessel structure accounts for the decreased ischemic infarct size with spironolactone treatment. Alternatively, activation of the mineralocorticoid receptor, using DOCA, has been shown by our laboratory to cause hypertrophic remodeling of the MCA (middle cerebral artery) and increased ischemic infarct size in intact male Wistar rats on a normal salt diet (Dorrance et al., 2006b). Similarly, when cerebral ischemia is induced experimentally, using MCA occlusion (Longa et al., 1989), the resultant ischemic infarct is greater in SHRSP compared to normotensive rats (Coyle and Jokelainen, 1983). The difference in ischemic infarct size may be due to SHRSP having smaller cerebral blood vessel lumens and an impaired ability to dilate in response to cerebral ischemia (Coyle and Heistad, 1987). A reduction in the external diameter of cerebral arterioles was also

found to play an important role in the impaired ability of these arterioles to dilate (Baumbach and Heistad, 1989). Increasing both the outer and inner blood vessel diameters are important factors for reducing the damage caused by an ischemic stroke.

In the current study, we investigated the effects of chronic mineralocorticoid receptor antagonism in the cerebral vasculature of the SHRSP. Our hypothesis was that cerebral vascular structure is improved, via an increase in cerebral vessel lumen diameter, by chronic administration of spironolactone and that these changes would occur without a reduction in blood pressure.

Material and methods

Animals

Male SHRSP were obtained from the breeding colony maintained at the Medical College of Georgia by our laboratory. Male WKY rats were purchased from Harlan (Indianapolis, IN). WKY rats were chosen to serve as normotensive controls for the SHRSP. All rats were maintained on a 12-hour light/dark cycle and were allowed access to food (regular rat chow with normal sodium content (0.25%, diet #8656, Harlan Teklad) and tap water *ad libitum*. All experiments were approved by The Medical College of Georgia Institutional Animal Care & Use Committee and followed protocols outlined by the American Physiological Society.

Blood Pressure Measurement

In separate groups of rats, telemetry transmitters (Data Sciences, St. Paul MN) were implanted in the abdominal aorta and treated as described below. Mean arterial pressure was collected as described previously (Sasser et al., 2002). Briefly, measurements were recorded every 10 minutes over a 24 hour cycle for six weeks. The average of the day and night measurements was calculated for each day. In additional groups of rats, blood pressure was measured during the last week of treatment using tail-cuff plethysmography with a CODA 6 VPR sensor rat-tail blood pressure system (Kent Scientific; Torrington, CT). An average of at least 15 measurements taken each day for 3 days was used in the final average for systolic, diastolic, and mean arterial pressure.

Treatments and Vessel Preparation

Six-week-old rats were anesthetized with ketamine/xylazine (67/6.6 mg/kg, IM) and time-release spironolactone (2.5 mg/day) pellets (Innovative Research of America, Sarasota, FL) were implanted subcutaneously in the nape of the neck, as described previously (Dorrance et al., 2001). At 12 weeks of age, rats were anesthetized with sodium pentobarbital (100 mg/kg, IP) and the brain was removed. Body weight measurements and blood, collected via cardiac puncture, were taken at time of death. Heparanized blood samples were centrifuged and the plasma was collected for analysis of electrolytes. The first branch-free segment of the MCA most proximal to the Circle of Willis was dissected and placed in cold physiological salt solution (PSS) (in mmol/L: 141.9 NaCl, 4.7 KCl, 1.7 MgSO₄, 0.5 EDTA, 2.8 CaCl₂, 10.0 HEPES, 1.2 KH₂PO₄, and 5.0 glucose). The vessel was mounted on two glass micropipettes (outer diameter, 125 - 150 μ m) and secured with silk thread (diameter, 20 μ m) in a small vessel arteriograph (Living Systems Instrumentation, Burlington, VT). The distal pipette was closed off, creating a zero-flow or blind-sac experiment; any vessels possessing leaks were discarded. All experiments performed for this study were done in a no-flow situation. The vessel was then pressurized to 75 mmHg and the arteriograph was placed atop an inverted microscope connected to a camera and monitor. To assure vessel integrity, the time between animal sacrifice and vessel mounting was kept within 45 minutes. The vessel was visualized by videomicroscopy and a calibrated video dimension analyzer was used to obtain lumen diameter and wall thickness measurements in micrometers.

Experimental Protocol

Vessels were allowed to equilibrate for one hour at 75 mmHg and 37°C in PSS at a pH of 7.4. 75 mmHg was chosen based on previous experiments performed in the rat MCA (Cipolla et al., 2000). This pressure also lies within the autoregulatory range of the rat MCA and is close to the pressure normally experienced by the MCA (Coulson et al., 2002). Initially, lumen diameter measurements were taken at 75 mmHg and 125 mmHg to represent active tone. Cumulative dose responses to serotonin were performed with the intraluminal pressure maintained at 75 mmHg. Serotonin was added to the arteriograph bath in increasing concentrations (10^{-9} to 10^{-5} mol/L); measurements were made of lumen diameter and wall thickness after a five-minute equilibration at each concentration. After dose responses were performed, passive structure was assessed in calcium-free PSS containing 2.0 mmol/L EGTA over a range of pressures from 40 mmHg to 180 mmHg in 20 mmHg increments; measurements of lumen diameter and wall thickness were taken at each pressure increment. At the completion of the experiment, sodium nitroprusside (10^{-3} mol/L) was added to the bath and similar measurements were taken for passive tone.

Calculations and Statistics

Equations used to assess vessel structure are as follows:

$$\text{Wall Thickness (WT)} = \text{Left WT} + \text{Right WT}$$

$$\text{Outer Diameter (OD)} = \text{Lumen Diameter (LD)} + \text{WT}$$

$$\text{Wall / Lumen ratio} = \text{WT} / \text{LD}$$

$$\text{Area of Vessel} = \pi(\text{OD}/2)^2$$

$$\text{Area of Lumen} = \pi(\text{LD}/2)^2$$

$$\text{Cross-Sectional Wall Area (CSA)} = \text{Area of Vessel} - \text{Area of Lumen}$$

$$\text{Circumferential Wall Stress} = (\text{Intraluminal Pressure} * \text{LD}) / (2 * \text{WT})$$

$$\text{Circumferential Wall Strain} = (\text{LD} - \text{LD at 0 Pressure}) / \text{LD at 0 Pressure}$$

$$\text{Remodeled LD} = \sqrt{(\text{Hypertensive OD}^2 - 4 * \text{Normotensive CSA} / \pi)}$$

$$\text{Remodeling Index} = 100 * ((\text{Normotensive LD} - \text{Remodeled LD}) / (\text{Normotensive LD} - \text{Hypertensive LD}))$$

$$\text{Growth Index} = 100 * ((\text{Hypertensive CSA} - \text{Normotensive CSA}) / \text{Normotensive CSA})$$

Remodeling and growth indices were calculated as described previously (Heagerty et al., 1993). Statistical analysis was not performed on the remodeling and growth indices because the vessels were not studied in a paired fashion. Percent tone was calculated using the following formula: $1 - (\text{active diameter/passive diameter}) * 100$ (Cipolla et al., 2000). Beta-coefficients were calculated by performing exponential regression on individual stress/strain curves using KaleidaGraph software (version 4.0).

Statistical analysis was performed using GraphPad Prism software, version 3.02. Statistical significance was determined using ANOVA, with Newman-Keuls Multiple Comparison post-test for all dose and pressure responses. T-tests were performed on physiological measurements, myogenic tone, LogEC₅₀ data, and beta-coefficients; the Bonferroni correction was utilized when appropriate. A p-value of 0.05 or below was considered significant. All values presented are mean $\pm$ SEM.

Results

Physiological Parameters and Blood Pressure

Spirolactone treatment had no effect on body weight in SHRSP. Control WKY rats had higher body weights compared to both groups of SHRSP (Table 1, $p < 0.05$). Plasma potassium and sodium levels were not different between groups (Table 1). Spirolactone treatment had no effect on mean arterial pressure measured using telemetry in the SHRSP. Mean arterial pressure was lower in the control WKY rats compared to both SHRSP groups (Figure 1). Systolic and diastolic pressures followed the same pattern as mean arterial pressure and were not affected by spirolactone treatment (data not shown). Mean arterial pressure measured using tail-cuff plethysmography was also not altered by spirolactone treatment in SHRSP, as there was no difference between spirolactone-treated SHRSP (162.3 ± 3.0 mmHg, $n=6$) and control SHRSP (157.7 ± 5.0 mmHg, $n=5$) ($p > 0.05$). Systolic and diastolic pressures were also not altered by spirolactone treatment in SHRSP (data not shown).

Cerebral Vessel Tone

Spirolactone treatment increased percent tone in the MCA by over 6.7% at 75 mmHg in the SHRSP compared to control groups ($p < 0.05$) (Figure 2A). There was no difference in percent tone in the MCA between groups at 125 mmHg ($p > 0.05$) (Figure 2A).

Cerebral Vessel Reactivity

MCA reactivity to serotonin is presented as the change in lumen diameter in microns. Constriction to 10^{-6} M and 10^{-5} M serotonin was blunted in the spirolactone-treated SHRSP compared to control SHRSP and WKY rats, as evidenced by a smaller change in lumen diameter (ANOVA $p < 0.05$) (Figure 2B). There was no difference in the LogEC_{50} between groups, as calculated from the change in lumen diameter (ANOVA $p > 0.05$; -6.60 ± 0.07 spirolactone-treated SHRSP vs. -6.37 ± 0.24 control SHRSP vs. -6.47 ± 0.11 control WKY rats).

Passive Cerebral Vessel Structure

The MCA lumen and outer diameters were larger in the spirolactone-treated SHRSP compared to control SHRSP over the range of pressures (ANOVA $p < 0.05$) (Figure 3A, 3B). Over the range of pressures, vessel wall thickness was increased in the MCA of both SHRSP groups compared to control WKY rats (ANOVA $p < 0.05$) (Figure 3C). Spirolactone treatment had no effect on MCA wall thickness in the SHRSP.

Although, there is a trend towards increased cross-sectional wall area in the MCA of spirolactone-treated SHRSP, statistical analysis showed that there was no difference between groups (ANOVA $p > 0.05$) (Figure 4A). The MCA wall-to-lumen ratio of the spirolactone-treated SHRSP was decreased compared to control SHRSP and increased compared to control WKY rats (ANOVA $p < 0.05$) (Figure 4B). In the control SHRSP group, the MCA wall-to-lumen ratio was increased compared to the control WKY rat group (ANOVA $p < 0.05$) (Figure 4B).

The presence of eutrophic remodeling in the MCA of SHRSP is indicated by the high remodeling index and small growth index, as was calculated at 80 mmHg (Figure 5). These indices were similar across the range of pressures (data not shown). Spirolactone treatment reduced the remodeling index by greater than 70% and only slightly increased the growth index in the MCA of SHRSP at 80 mmHg (Figure 5).

There was a rightward shift in the stress/strain curve for spirolactone-treated SHRSP, indicating an increase in vessel compliance (Figure 6A). The β -coefficient, calculated from

the individual stress/strain curves, was used as a measure of vessel stiffness. The β -coefficient was significantly decreased in spironolactone-treated SHRSP compared to control SHRSP and WKY rats (t-test with Bonferroni correction; control SHRSP vs. spironolactone-treated SHRSP, $p=0.004$) (Figure 6B).

Discussion

There are two novel findings of this study: 1) Spironolactone treatment increases tone in the MCA of SHRSP and 2) Spironolactone treatment alters vascular structure of the MCA by increasing the lumen and outer diameters, without a change in blood pressure. When combined, these changes may explain the protective effects of spironolactone on the outcome of ischemic stroke, as observed previously by our laboratory (Dorrance et al., 2000; Dorrance et al., 2001).

The effects of spironolactone on cerebral vessel structure in the SHRSP are not due to a reduction in blood pressure. Consistent with the current study, previous studies performed in the male salt-loaded SHRSP fed a stroke-prone diet indicated that there was no effect on blood pressure with spironolactone or eplerenone treatment (Rocha et al., 1998; Rocha and Stier, 2001). Few studies have assessed blood pressure in the spironolactone-treated SHRSP on a normal salt regimen for comparison. However, we have previously shown no difference in systolic blood pressure, measured by tail-cuff plethysmography, between control and spironolactone-treated SHRSP on normal salt diets (Dorrance et al., 2001). Hypertension is influenced by many genetic factors in the SHRSP, but mineralocorticoid receptor antagonism using spironolactone does not lower blood pressure in this model. Measurement of blood pressure using telemetry has been shown to have significant advantages over using other methods requiring restraint, such as tail-cuff plethysmography (Irvine et al., 1997). The differences in vessel structure also do not appear to be related to overall growth, as body weight was not changed by treatment with spironolactone. Other circulating factors, such as plasma potassium (Dorrance et al., 2006a), can also have effects on the outcome of ischemic stroke. However, plasma potassium is not significantly elevated in the spironolactone-treated SHRSP, indicating that the effects of spironolactone are most likely through the inhibition of the mineralocorticoid receptor and not due to increased plasma potassium.

Maintenance of vascular tone is one of the mechanisms that contribute to autoregulation of cerebral blood flow (Izzard et al., 2003). Cipolla, *et al* found that after induction of ischemic stroke in rats, the amount of myogenic tone in the cerebral arteries decreased as the duration of ischemia increased (Cipolla et al., 2001). These studies highlight the importance of maintaining myogenic tone in the cerebral arteries in the face of an ischemic event. 75 mmHg is in the middle of the autoregulatory range of the rat (Coulson et al., 2002), which is where we observed our differences. However, previous studies have shown that the SHRSP have little or no autoregulatory response (Izzard et al., 2003). The increase in percent tone at 75 mmHg in the spironolactone-treated SHRSP may serve to enhance the autoregulatory behavior of the middle cerebral artery, therefore helping to protect the brain in the event of an ischemic stroke. An alteration in vascular reactivity can also have an impact on the outcome to ischemic stroke (Baumbach and Heistad, 1989). Constriction to serotonin at the highest doses was blunted in the spironolactone-treated SHRSP, but there was no difference in the sensitivity to serotonin, as indicated by similar LogEC₅₀ values. In light of the fact that tone was increased in the spironolactone-treated SHRSP, it is possible that the blunted constriction to serotonin was due to the higher level of basal tone already present in the spironolactone-treated SHRSP.

Many studies have shown that mineralocorticoid receptor antagonism can reduce cardiac fibrosis and ventricular remodeling (Rocha and Funder, 2002), but few have focused on the effects of mineralocorticoid receptor antagonism on vascular structure. To the best of the

authors' knowledge, our study is the first to assess the effect of spironolactone on the cerebral vasculature in the SHRSP. Previous studies of vascular structure using mineralocorticoid receptor antagonism, with either spironolactone or eplerenone, have been completed in the aorta (Benetos et al., 1997) and the mesentery (Endemann et al., 2004; Viridis et al., 2002). Benetos, *et al* found that chronic spironolactone treatment could prevent aortic fibrosis in the spontaneously hypertensive rat, by preventing aortic collagen accumulation. Other studies that were carried out by Viridis, *et al* and Endemann, *et al* were performed in mesenteric resistance vessels. These studies found that both spironolactone (Viridis et al., 2002) and eplerenone (Endemann et al., 2004) could reduce the media-to-lumen ratio of the vessels, where a decrease in the ratio correlates to decreased vascular remodeling, i.e. increased lumen diameter (Mulvany et al., 1996). Taken together, these studies suggest that mineralocorticoid receptor activation is important in regulating vascular structure.

Inward eutrophic remodeling is particularly important in the cerebral vasculature as it can affect the autoregulation of blood flow to the brain (Izzard et al., 2003), which is an important determinant for the outcome of ischemic stroke (Coyle and Heistad, 1987). Eutrophic remodeling in the MCA of SHRSP is indicated by the high remodeling index combined with the low growth index. Hypertrophic remodeling would have been indicated by a high growth index. Spironolactone treatment prevented the eutrophic remodeling in the MCA of SHRSP, as evidenced by the increase in lumen and outer diameter, without a change in wall thickness, combined with the dramatic decrease in the remodeling index. The MCA from spironolactone-treated SHRSP may still be hypertrophic, as indicated by the increased trend in cross-sectional wall area compared to control WKY rats. This may be partially explained by a lack of reduction in blood pressure, as increased blood pressure is known to cause hypertrophy in blood vessels (Baumbach and Heistad, 1989). Although the wall-to-lumen ratio in the MCA of spironolactone-treated SHRSP was increased compared to control WKY rats, there was a trend towards a decreased ratio with spironolactone treatment.

The MCAs from spironolactone-treated SHRSP are more compliant and less stiff, as was indicated by the rightward shift in the stress/strain curve and decreased beta-coefficient. The stress/strain curve from WKY rats was not rightward shifted compared to control SHRSP, similar to previous studies performed in cerebral vessels from WKY rats (Baumbach and Heistad, 1989). Increased compliance and decreased cerebral vessel stiffness has been shown by our laboratory to be associated with a reduction in ischemic infarct size (Dorrance et al., 2005). These findings signify that the same effects observed in the spironolactone-treated SHRSP in this study may have played a role in the ischemic stroke protection afforded by spironolactone treatment found in our previous study (Dorrance et al., 2001).

In summary, we have shown that chronic treatment with spironolactone can prevent eutrophic remodeling in the cerebral vasculature of male SHRSP, without reducing blood pressure. Along with the alteration in structure, the vessels from spironolactone-treated SHRSP are more compliant and less stiff. The effects of spironolactone on the cerebral vasculature may explain its protective effect in reducing ischemic infarct size (Dorrance et al., 2001). These novel studies add to the increasing literature demonstrating the favorable effects of mineralocorticoid receptor antagonism on the cardiovascular system, independent of changes in blood pressure.

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Figure 1.

Telemetry Blood Pressure. Mean arterial pressure is presented as the average of the day and night measurements. Spironolactone treatment had no effect on mean arterial pressure in the SHRSP ($n = 3$ rats per group); mean arterial pressure was lower in control WKY rats ($n = 4$) compared to both groups of SHRSP. Abbreviations: SHRSP – spontaneously hypertensive stroke-prone rats, WKY – Wistar Kyoto

Figure 2.

Cerebral Vessel Function. A) Percent tone is calculated as: $1 - (\text{active diameter} / \text{passive diameter}) \times 100$. Spironolactone treatment increased tone at 75 mmHg compared to control groups (* $p < 0.05$ vs. control SHRSP and WKY rats). Percent tone was not different between groups at 125 mmHg. B) Reactivity to serotonin is presented as the change in lumen diameter in microns. Constriction to serotonin was blunted in the spironolactone-treated SHRSP at the highest doses (* $p < 0.05$ vs. control SHRSP and WKY rats, † $p < 0.05$ vs. WKY rats). $n = 8$ for spironolactone-treated SHRSP, $n = 6$ for control SHRSP, $n = 4$ for control WKY rats. Abbreviations: MCA – middle cerebral artery, SHRSP – spontaneously hypertensive stroke-prone rats, WKY – Wistar Kyoto

Figure 3.

Vessel structure was assessed under calcium-free conditions. A) Lumen Diameter. Over the range of pressures, the lumen diameter of the MCA was larger in the spironolactone-treated SHRSP compared to control SHRSP ($*p<0.05$). B) Outer Diameter. Over the range of pressures, the outer diameter of the MCA was smaller in the control SHRSP compared to spironolactone-treated SHRSP and control WKY rat groups ($*p<0.05$). C) Wall Thickness. Over the range of pressures, wall thickness was increased in the control and spironolactone-treated SHRSP groups compared to the control WKY rat group ($*p<0.05$). $n = 8$ for spironolactone-treated SHRSP, $n = 6$ for control SHRSP, $n = 4$ for control WKY rats

Abbreviations: MCA – middle cerebral artery, SHRSP – spontaneously hypertensive stroke-prone rats, WKY – Wistar Kyoto

Figure 4.

Vessel structure was assessed under calcium-free conditions. A) Cross-Sectional Wall Area. Over the range of pressures, there was no difference in cross-sectional wall area in the MCA between groups. B) Wall-to-Lumen Ratio. The wall-to-lumen ratio of the spironolactone-treated SHRSP was decreased compared to control SHRSP and increased compared to control WKY rats (* $p < 0.05$). $n = 8$ for spironolactone-treated SHRSP, $n = 6$ for control SHRSP, $n = 4$ for control WKY rats. Abbreviations: MCA – middle cerebral artery, SHRSP – spontaneously hypertensive stroke-prone rats, WKY – Wistar Kyoto

Figure 5.

Remodeling and growth indices as calculated from structural measurements at 80 mmHg. Spironolactone treatment greatly reduced the remodeling index and only slightly increased the growth index in the MCA of SHRSP. n = 8 for spironolactone-treated SHRSP, n = 6 for control SHRSP, n = 4 for control WKY rats Abbreviations: MCA – middle cerebral artery, SHRSP – spontaneously hypertensive stroke-prone rats, WKY – Wistar Kyoto

Figure 6.

Circumferential wall stress and strain were calculated from measurements made over a range of pressures in passive, calcium-free conditions. A) There was a rightward shift in the stress/strain curve of the spironolactone-treated SHRSP, indicating an increase in MCA compliance. B) Beta-coefficients, a measure of vessel stiffness, were calculated by performing exponential regression on individual stress/strain curves. Beta-coefficients were significantly decreased in the MCAs from spironolactone-treated SHRSP (* $p < 0.05$). $n = 5$ for spironolactone-treated SHRSP, $n = 5$ for control SHRSP, $n = 5$ for control WKY rats. Abbreviations: MCA – middle cerebral artery, SHRSP – spontaneously hypertensive stroke-prone rats, WKY – Wistar Kyoto

Table 1

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Experimental Group	Body Weight (grams)	Plasma Potassium (mmol/L)	Plasma Sodium (mmol/L)
SHRSP	272 ± 6.5 *	4.9 ± 0.49	145.0 ± 0.79
spironolactone			
SHRSP	282 ± 6.0 *	5.0 ± 0.21	142.6 ± 4.16
control			
WKY	303 ± 3.5	5.4 ± 0.25	144.9 ± 0.67
control			

Table 1. Physiological Parameters. Values are mean ± SEM.

* p<0.05 vs. control WKY rats. n = 4 rats per SHRSP group, n = 3 control WKY rats Abbreviations: SHRSP – spontaneously hypertensive stroke-prone rats, WKY – Wistar Kyoto