Local Renin-Angiotensin System in the Microcirculation of Spontaneously Hypertensive Rats

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Abstract We studied the local renin-angiotensin system in the microcirculation of cremaster muscle in spontaneously hypertensive rats (SHR) and their normotensive Wistar-Kyoto (WKY) controls. We used intravital microscopy in an original preparation of cremaster isolated from its normal blood supply and externally perfused with physiological solution, thus allowing the exclusion of circulating converting enzyme, circulating renin, and circulating angiotensinogen. We classified arterioles studied as second-, third-, and fourth-order, with mean diameters, respectively, of 67±6, 35±2, and 17±1 μm in WKY controls and 61±5, 34±2, and 16±1 μm in SHR. No difference between WKY controls and SHR was found for arteriolar vasoconstrictions in response to topical administration of 0.01 to 1 nmol/mL angiotensin II. Conversely, in response to 0.01 to 1 nmol/mL angiotensin I, significantly more arteriolar vasoconstriction was found in SHR cremaster muscle. In both strains, responses to angiotensin I were significantly inhibited by 10 nmol/mL of the angiotensin-converting enzyme inhibitor lisinopril. When angiotensinogen-rich, renin-free plasma containing 2.3 nmol/mL angiotensinogen was administered, almost no vasoconstriction was found in WKY controls, but significant constrictions were observed in SHR (23±4%, 30±5%, and 41±4% for second-, third-, and fourth-order arterioles, respectively). In SHR, vasoconstriction in response to angiotensinogen-rich, renin-free plasma was dose dependent, was inhibited by lisinopril, and was not found 24 hours after bilateral nephrectomy. Topical administration of 1.2 μg/mL renin did not induce arteriolar vasoconstriction in either WKY or SHR cremaster muscle. We conclude that in the skeletal muscle microcirculation, (1) local angiotensin-converting enzyme activity in arterioles is higher in SHR than in their normotensive controls and is consequently an important target site for angiotensin-converting enzyme inhibitors; (2) the enzymatic cascade constituted by local renin and local angiotensin-converting enzyme is able to produce significant vasoconstriction in the presence of angiotensinogen in SHR but not in their normotensive controls; (3) the bulk of vascular renin responsible for this vasoconstriction in response to angiotensinogen in SHR is likely derived from the uptake of circulating renin of renal origin; and (4) no evidence exists in either WKY rats or SHR for the presence of local angiotensinogen, which can induce arteriolar constriction in the presence of renin. (Hypertension. 1994;24:70-76.)

Key Words • microcirculation • renin-angiotensin system • renin • angiotensin • kininase II • angiotensin-converting enzyme inhibitors

It is now well recognized that the renin-angiotensin system (RAS) is not only an endocrine system but that some of its components are generated or activated in several tissues.1,2 By studying isolated large arteries and endothelial cell cultures as well as by whole-organ perfusion, several authors have shown the presence of different components of the local RAS in the vasculature. The possibility that angiotensin II (Ang II) may be locally formed within the vasculature led some authors to test the hypothesis that local RAS and especially local angiotensin-converting enzyme (ACE) activity can be different in hypertensive states. Indeed, an increase in vascular ACE concentration or activity was found in both experimental and genetic hypertensive rat models3-9 as well as an increase in aortic ACE mRNA levels in two-kidney, one clip hypertensive rats.10

All these studies involved RAS or ACE activity in large arteries. However, it is well known that the microcirculation is an important site for pressure regulation (see Reference 11 for review) and that the terminal part of the microvascular network is part of the vascular system, which is the most sensitive to several vasopressive substances, including Ang II.12-14 Consequently, we thought it important to establish whether modifications of local microvascular RAS are associated with hypertension.

To test this hypothesis, we used an original recently published model based on intravital microscopy of externally perfused rat cremaster.15 This model allowed us to evaluate directly the capacity of local ACE, local renin, and local angiotensinogen in the microcirculation to induce effective vasoconstriction in the presence of their associated substrate or enzyme. In the present study, we compared the activity of the different components of the local microvascular RAS of spontaneously hypertensive rats (SHR) and their normotensive Wistar-Kyoto (WKY) controls by testing the arteriolar vasoconstrictions in response to Ang I and Ang II, angiotensinogen-rich plasma from binephrectomized rats, and renin.

Methods

Male SHR and their normotensive WKY controls (Iffa-Credo) weighing 24±2 g were anesthetized with 50 mg/kg IP sodium pentobarbital (Nesdonal). A patent airway was maintained with a tracheotomy tube. The carotid artery was cannulated for measurement of systemic mean arterial blood...
pressure with a Statham P23DB transducer. All animals whose mean pressure fell to less than 90 mm Hg were excluded.

Preparation of Rat Cremaster Muscle

After anesthesia, the right cremaster muscle was surgically prepared for in vivo visualization by a technique described in detail elsewhere. Briefly, the muscle was detached from the scrotum, then a transverse buttonhole slit approximately 5 mm long was made at the proximal part of the cremaster pouch. The testicle and epididymis and the cremaster itself were then drawn out through the buttonhole. The small pedicle that attaches the cremaster to the testicle was ligated with two stitches and cut between them so as to separate the cremaster completely from the testicle, which was reincorporated into the abdominal cavity. To prepare the cremaster muscle for transillumination microscopy, a flexible extendible ovoid ring 0.1 mm in diameter was made of metal wire covered by silicone elastomer rubber and introduced longitudinally into the cremaster pouch. When the clamp was removed, the ring expanded gently, spreading out the cremaster, which acquired a funnel shape. This procedure involved minimal incision of the cremaster and consequently reduces considerably the risk of hemorrhage and of lesions to the muscle and its microcirculation.

The muscle chamber was filled with Krebs’ solution at a flow rate of 2 mL/min and temperature of 34.5°C in the cremaster chamber. Before perfusing the solution and the solution with a 6% CO₂, 94% N₂ gas mixture, the pH, P O₂, and PCO₂ of this solution were fixed at 7.42±0.04, 17±2.0 mm Hg, and 42±1 mm Hg, respectively. The chamber was covered with a Plexiglas plate to isolate it from the atmosphere.

To allow external perfusion of the cremaster muscle, a catheter was introduced into the femoral artery homolateral to the cremaster studied. A ligature was placed on the common iliac artery just upstream of the bifurcation with the pudic epigastric trunk (from which runs the feeding artery of the cremaster muscle). At this time the ligature was not secured tightly. To reduce the duration of surgery as much as possible, we did not ligate all the arteries issued from the common iliac artery and pudic epigastric trunk and did not modify the normal venous return of the muscle. Consequently, the external perfusion not only perfused the cremaster muscle but also the muscles depending on these arteries. To exclude collateral perfusion of the cremaster muscle, several ligatures were made in the lateral fasciae of the muscle that connect it with the pelvic wall. Then, the ligature on the common iliac artery was tightened and the muscle perfused at a controlled constant perfusion pressure using a controlled pump (Fluid Metering Inc) with a pulse shock absorber. Perfusion pressure was recorded with a Statham pressure transducer (model P23ID, Spectramed) placed as close as possible to the artery. The perfusion medium was Krebs’ solution containing 0.5% bovine serum albumin (wt/vol) gassed at 5% CO₂, 95% O₂ and kept at 37°C. Depending on the muscle, the flow rate at 100 mm Hg of perfusion pressure ranged between 150 and 250 μL/min. Under these conditions we checked that the pressure drop between the pressure transducer and artery was less than 3 mm Hg. Each experiment, the muscle was generally perfused externally three times, and a washout period of 45 minutes of perfusion was allowed before any drug was administered. For almost all experiments, the response of WKY rats and SHR were compared using a similar perfusion pressure of 100 mm Hg to exclude the possibility that differences in responses could be due to differences in perfusion pressure. However in one complementary group we also studied the response of SHR perfused at 180 mm Hg.

Arteriolar Network Visualization

For visualization of the microcirculation, the chamber was placed on the moveable stage of a modified Leitz microscope, and the cremaster muscle was transilluminated using a 100-W tungsten-halogen lamp. The image, magnified by a ×20 or ×10 objective and ×10 oculars, was projected into a CCD camera (Sony 101) connected to a videotape recorder (Sony VP 3600) and a video monitor. Total magnification from tissue to video monitor was ×1210 or ×605. The image was positioned by an optical system in such a way that the images of most of the arterioles were vertical. The field studied was usually centered on an arteriolar bifurcation, after identification of the order of the arterioles by their relative locations in the network according to the morphological criteria derived from Zweifach. The arterioles studied in the present experiments were second-, third-, and fourth-order arterioles (A2, A3, and A4, respectively). Measurements were made at the site of maximal arteriolar constriction in the field considered and during the period of maximal response. To be sure that a constriction was observed even if its kinetics were slow, observation time after any intervention was at least 60 minutes (however, all contractions reported in the present study were maximal within less than 15 minutes). Arteriolar internal diameters were measured by playback analysis of the video record using a videodimension analyzer (IPM 303 or 908). Depending on the geometry of the network studied, two to four arterioles were measured in each experiment. Results are expressed as percent vasoconstriction, calculated as (d₁—d₂)/d₁, where d₁ is the basal inner diameter and d₂ the inner diameter at peak vasoconstriction.

Experimental Protocols

Administration of Ang II

We compared the reactivity to Ang II of the cremaster isolated from its normal blood supply and perfused with Krebs’ solution in WKY controls (n=12 rats) and SHR (n=10 rats). Three Ang II doses were studied: 0.01, 0.1, and 1 nmol/mL. These doses were chosen because they induced vasoconstrictions in a range similar to that observed with the other compounds used in the subsequent protocols described below.

Administration of Ang I

The activity of local microvascular ACE was studied by topical administration of 0.01, 0.1, and 1 nmol/mL Ang I to cremaster from which circulating RAS (and therefore circulating ACE) had been excluded by the experimental procedure described above. Ten minutes before Ang I administration, WKY rats (n=16) and SHR (n=16) were randomly divided into two groups for pretreatment with Krebs’ buffer or ACE inhibitor (10 nmol/mL lisinopriltopically administered, respectively). In a complementary group of SHR (n=5), we also measured the responses to Ang I of muscles perfused at 180 mm Hg.

Administration of Angiotensinogen-Rich, Renin-Free Plasma

The effect of topical administration of plasma from nephrectomized rats containing 2.3±0.1 nmol/mL angiotensinogen and no renin (for details on dosage see References 19 and 20) was studied in four SHR by administration of 0.01, 0.1, and 1 nmol/mL plasma containing 1.6 nmol/mL angiotensinogen to SHR (n=4) 24 hours after bilateral nephrectomy.

Administration of Renin

To test the presence of local angiotensinogen, we also examined the effect of topical administration of 1.2 ng/mL
vasoconstriction was greatest for the smallest arterioles. Vasoconstriction was significantly greater in SHR than in WKY for average values in A2, A3, and A4 arterioles, see Fig 2), but this difference between the vasoconstrictions in SHR and WKY was predominant for one arteriolar order. Similar differences in responses were found when we compared the Ang I-induced vasoconstriction in SHR and WKY muscle with the circulating renin-angiotensin system had been excluded (see text for details). Hatched columns refer to results obtained in spontaneously hypertensive rats. No significant differences were found between WKY controls and spontaneously hypertensive rats.

Results

Mean systemic blood pressure was 115±2 and 180±3 mm Hg for WKY controls and SHR, respectively. Mean basal diameters (±SEM) of A2, A3, and A4 arterioles, respectively, were 67±6, 35±2, and 17±1 μm in WKY controls and 61±5, 34±2, and 16±1 μm in SHR. Regarding the basal diameter for each arteriolar order, no significant differences were found either between the different series of experiments or between WKY rats and SHR.

Effect of Ang II on Krebs'-Perfused Cremaster Muscle From WKY Rats and SHR

As shown in Fig 1, in the three arteriolar orders and for the three Ang II concentrations studied, no significant differences were found between vasoconstrictions observed in Krebs'-perfused cremaster from WKY controls or SHR.

Effect of Ang I on Krebs'-Perfused Cremaster Muscle From WKY Rats and SHR

Administration of Ang I induced marked arteriolar constriction in both SHR and WKY controls (for average values in A2, A3, and A4 arterioles, see Fig 2), but this vasoconstriction was significantly greater in SHR (P<.001). In both normotensive and hypertensive animals, vasoconstriction was greatest for the smallest arterioles (P<.05). No significant interaction was found between the factors "strain" and "arteriolar order," showing that the difference between the vasoconstrictions in SHR and WKY muscle was not predominant for one arteriolar order. Similar differences in responses were found when we compared the Ang I-induced vasoconstriction in SHR muscle perfused at a pressure of 180 mm Hg with the vasoconstriction in WKY muscle (Fig 2).

Lisinopril had no significant effect on basal diameter, but as shown in Fig 3 for all three arteriolar orders studied it greatly inhibited Ang I-induced vasoconstriction when administered 10 minutes before Ang I in both normotensive (top) and hypertensive (bottom) rats for the three Ang I doses tested (P<.001 in all cases). In
Effects of Plasma From Binephrectomized Rats on Krebs'-Perfused Cremaster Muscle From WKY Controls, SHR, and Binephrectomized SHR

In cremaster muscle isolated from its blood supply and perfused with Krebs' solution, very limited vasoconstriction was seen in arterioles from WKY rats after topical administration of plasma from binephrectomized rats (Fig 4, hatched columns). Conversely, significant vasoconstriction was observed in SHR (Fig 4, black columns). As shown in Fig 5, this vasoconstriction was dose dependent (hatched columns) and significantly inhibited by lisinopril (P<.001, black columns). In 24-hour binephrectomized SHR, this vasoconstriction was totally blunted (Fig 5).

Effects of Renin on WKY Controls and SHR

No vasoconstriction was observed after administration of renin to Krebs'-perfused cremaster muscle from both WKY controls and SHR, no significant interaction was found between the factor “presence or absence of lisinopril” and the factor “arteriolar order,” thus showing that the effect of lisinopril was not significantly different in the three arteriolar orders.
either WKY controls or SHR, despite the high dose applied (1.2 μg/mL).

Discussion
This study was carried out to compare the different components of local microvascular RAS in SHR and WKY controls. As regards ACE activity, the possibility of differences between SHR and WKY controls was suggested by the findings of high vascular ACE activity in several models of hypertension, including the SHR. 3-8 However, all the studies cited concerned large arteries that do not play a crucial role in regulating pressure and tissue perfusion. On the other hand, the effects of Ang I and II on the arteriolar network have been studied in normotensive animals22 and renovascular hypertension.23 However, the authors of these two studies did not distinguish between the local and circulating RAS. In contrast, in a recent investigation of normotensive Sprague-Dawley rats,16 we evaluated the capacity of the different components of the local RAS of skeletal muscle to induce acute vasomotor effects in the presence of their associated substrates or enzymes, and consequently, it became possible and seemed to us important to establish whether or not this local microvascular ACE activity may be modified in hypertension.

In the present study, we used an original preparation of cremaster muscle isolated from its normal blood supply and perfused with Krebs' solution. This preparation has been largely discussed in a previous work,15 but to permit interpretation of the present results, the following main features of this experimental model must be kept in mind. (1) Because the muscle was isolated from its normal blood supply and perfused with a physiological buffer at a constant pressure, the circulating RAS was excluded, and the vasomotor responses were limited to the effects of the local RAS. (2) No vessels with a diameter of more than 120 μm were present in the area where substances were topically administered, thus excluding any possible action by the local microvascular ACE activity in the network. These possible interactions complicate the interpretation of the observed segmental differences.

One of the main findings of the present study was a greater response to Ang I in SHR than in WKY controls (see Fig 2). It is well known that intravascular pressure may affect the arteriolar response to vasopressive drugs, but the observed difference between the response to Ang I of WKY controls and SHR cannot be explained by such mechanisms because the muscles from both groups were perfused at the same pressure. In addition, in a complementary group we checked that this difference in response was also present when muscles from SHR were perfused at 180 mm Hg. Because the response to Ang II was similar in SHR and WKY rats (see Fig 1), it can be concluded that local microvascular ACE activity was greater in SHR than WKY rats. This conclusion is in accordance with the results of several studies reporting high ACE activity in hypertensive rats13-15 and with findings by quantitative autoradiography showing higher ACE concentration in arteries from SHR than from WKY rats.9 Conversely, our results contrast with the low ACE activity found in the brain microvessels24 and in a mesenteric artery25 in young SHR. These discrepant findings may be explained by a certain degree of heterogeneity in the ACE activity of the different vessels studied as well as by the possibility that a significant increase in ACE activity only appeared at a certain age. This possibility is supported by the concomitant occurrence of the increase in aortic ACE activity and the elevation of pressure found in SHR.4 It should be noted that, despite the higher ACE activity in SHR, the vasoconstrictions induced here by Ang I were greatly inhibited by pretreatment with lisinopril in both SHR and WKY controls. The remaining vasoconstrictions were indeed very limited in both strains, even at the highest dose of Ang I tested, thus demonstrating the effectiveness of lisinopril in inhibiting local microvascular ACE (see Fig 3).

As a complement, we used plasma from binephrectomized rats in preference to synthetic tetradecapeptide substrate because several authors have suggested that Ang II may be formed from tetradecapeptide independently of renin and ACE activity.28-31 In addition, in a previous study we found very different results when tetradecapeptide or plasma from binephrectomized rats was used, thus demonstrating that the vasoconstriction induced by tetradecapeptide cannot be unequivocally related to the presence of active renin.15 In the present study, effective dose-dependent vasoconstrictions were observed in SHR cremaster when angiotensinogen-rich, renin-free plasma was administered (see Figs 4 and 5). Because these vasoconstric-
vations were inhibited by pretreatment with lisinopril, it is likely that they were caused by the formation of Ang II from angiotensinogen. In contrast, under the same conditions very limited vasoconstrictions were observed in WKY controls, indicating that the production of Ang II obtained was either null or too low to be detected by our model (Fig 4). Indeed, some authors have reported high renin activity in arteries from SHR, but others support the opinion that local ACE activity, rather than local renin or angiotensinogen, plays a key role in the increase in pressure. Our results demonstrated that in SHR, the enzymatic cascade constituted by local renin plus local ACE induced the formation of more Ang II than in WKY rats. However, we also found that local microvascular ACE activity was higher in SHR, so the differences between the responses of SHR and WKY rats to angiotensinogen do not allow us to conclude unequivocally whether renin activity was also different in SHR and WKY rats or whether it was in fact similar but was amplified in SHR by the high ACE activity.

The origin of vascular renin is also controversial. Some authors have reported findings suggesting the possibility of local synthesis of renin in vascular tissue. Conversely, others reported a decrease in vascular renin activity after bilateral nephrectomy, thus suggesting that the bulk of vascular renin is derived from the uptake of circulating renin of renal origin. The present results strongly support the hypothesis that microvascular renin is of renal origin because in our study, no renin activity was found in SHR 24 hours after bilateral nephrectomy (Fig 5).

Regarding angiotensinogen, no vasoconstriction was found when 1.2 µg/mL renin was administered to the microvascular preparation isolated from either WKY rats or SHR. In a previous study of Sprague-Dawley rats and in preliminary experiments with WKY controls, we found that this high dose of renin induced large arteriolar constrictions when normal cremaster blood flow was left in place and therefore circulating angiotensinogen was present. Consequently, the present results showed that, in both SHR and WKY rats, the potential effect of local angiotensinogen at the microvascular level was either totally absent or considerably smaller than that of circulating angiotensinogen and too low to be detectable. Because renin has been found to produce an effective increase in pressure when perfused in an isolated rat hindlimb, it may be hypothesized that local angiotensinogen is unequally distributed in the vascular network and that the increase in pressure reported was due to Ang I production from angiotensinogen in large vessels.

In summary, we found that local conversion of Ang I to Ang II in the microvascular network of skeletal muscle was greater in SHR than WKY controls. We also found that local renin activity produced detectable vasoconstriction in the presence of its natural substrate in SHR but not in WKY rats. Our findings strongly suggest that in the arterioles this local renin is of renal origin. Finally, we did not find that in arterioles in either SHR or WKY controls, local angiotensinogen could produce arteriolar constriction in the presence of renin.

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