Effect of Stress on the Control of Renin Release in Spontaneously Hypertensive Rats

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Recent reports suggest that centrally induced increases in sympathetic outflow to the kidney have the potential to enhance the sensitivity of pressure-dependent renin release. In the present study, the possibility was investigated that spontaneously hypertensive rats (SHR), which are thought to have increased tonic sympathetic outflow to the kidney, exhibit enhanced renin release in response to reduced renal perfusion pressure. The increase in plasma renin activity in response to a graded suprarenal aortic constriction was determined in conscious young (6–9 weeks of age) and adult (14–16 weeks of age) SHR and age-matched Wistar-Kyoto (WKY) control rats. Under conditions of relatively little stress, the renin response to reduced renal perfusion pressure was not enhanced in young or adult SHR when compared with age-matched WKY rats. That is, this regulatory mechanism was not "reset" in the hypertensive animals. When challenged with an acute stress (air to the face) both age groups of SHR exhibited a significantly enhanced response. Neither age group of WKY rats was affected by the acute air stress. These data suggest that, under unstressed conditions, pressure-dependent renin release probably does not contribute to the elevation of arterial pressure in the SHR. However, under stressful conditions, the contribution of this system may be significant. Intermittent increases in sympathetic outflow to the kidney that can occur in the SHR in response to daily stresses have the potential to render it more sensitive to spontaneous reductions in perfusion pressure. Occasional exaggerated release of renin could then contribute to the hypertensive process.

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The sympathetic nervous system is one factor that is known to contribute to the regulation of renin secretion. Recent evidence suggests that a modest increase in renal sympathetic nerve activity may render the renin-secreting juxtaglomerular cells more sensitive to certain nonneural stimuli for renin secretion. By using conscious normotensive Sprague-Dawley rats, I recently showed that activation of sympathetic outflow by electrical stimulation of the hypothalamus or by application of an air stress enhanced the sensitivity of the renin response to graded reductions in renal perfusion pressure. Spontaneously hypertensive rats (SHR) have been reported to exhibit increased renal sympathetic activity during the development of hypertension. Could this sympathetic hyperactivity increase the sensitivity of nonneural mechanisms of renin secretion? Ehmlke et al have suggested that pressure-dependent release of renin is an important determinant of long-term arterial pressure. They hypothesized that a modest "resetting" of this regulatory system could lead to a sustained hypertension.

In the present investigation, I sought to determine if the increase in tonic sympathetic outflow in the SHR was sufficient to enhance the sensitivity of pressure-dependent renin release. Exaggerated increases in renin secretion in response to normal nonneural stimuli could lead to periods when plasma levels of angiotensin II are inappropriately elevated. Because the SHR is also known to respond to various stressful situations with an exaggerated increase in renal sympathetic activity, the ability of an acute stress to modulate the responsiveness of the system was also investigated. Experiments were performed with animals that were in the developmental phase of hypertension and animals that had established hypertension.

The experiments showed that, under baseline conditions, neither the young nor the old SHR exhibited an increased sensitivity of the renin response to reduced renal perfusion pressure when compared with age-matched control Wistar-Kyoto (WKY) rats.
However, the application of an acute stress enhanced the response in both groups of SHR but not in the WKY control rats.

**Methods**

All experiments were performed with conscious young (6–9 weeks, 120–175 g) or adult (14–16 weeks, 210–320 g) SHR (Harlan, Indianapolis, Indiana) or age-matched WKY control rats. Animals were prepared 1–3 days before experimentation as follows. Anesthesia was induced by using a mixture of ketamine (140 mg/kg) and acepromazine (1.4 mg/kg i.p.). Supplemental doses of the anesthetic were given as required during the surgery. Through a midline abdominal incision, the aorta proximal to the renal arteries was isolated and an occluding cuff was placed around the vessel. Commercially available cuffs (In Vivometrics, Healdsburg, California) or handmade cuffs (according to Gellai and Valtin) were used interchangeably. In the adult rats, a Doppler flow probe was placed around the right renal artery and sutured in place with 6-0 silk. The lead wires from the flow probe and the tubing from the occluding cuff were exteriorized through a small hole in the abdominal wall and then tunneled subcutaneously to exit at the back of the neck. The young animals did not receive flow probes. In all rats, a catheter (PE-10 heat fused to PE-50) was inserted into the lower abdominal aorta via a femoral artery so that its tip ended distal to the renal arteries. This catheter was also exteriorized at the back of the neck. The renal perfusion pressure, measured with the aortic catheter, could be reduced to any desired level by inflating the occluding cuff. After surgery, all rats were given penicillin G (30,000 units/100 g i.m.) and 3 ml 0.9% saline (s.c.). Surgical procedures were approved by the institute Animal Care and Use Committee.

**Experimental Protocols**

On the experimental day, the rats were brought from the animal care facility to a quiet room in the laboratory. Some rats were allowed to move freely in their cage and some rats were restrained in a cylindrical Plexiglas cage. In either case, the arterial catheter was connected to a pressure transducer (Century Technology, Inglewood, California), the flow probe lead wires were connected to a Doppler flowmeter (University of Iowa Biomegineering, Iowa City, Iowa), and the occluding cuff tubing was connected to a manual microdrive (Stoelting, Chicago, Illinois). Blood pressure, heart rate, and renal blood flow were monitored throughout a 60-minute equilibration period with the use of Grass Model 7 polygraph (Grass Instr. Co., Quincy, Massachusetts). At the end of the stabilization period, the rats underwent one of the following experimental protocols. Each rat was used only once.

**Young rats, restrained.** In initial experiments, rats were used 1 day after the preparatory surgery (SHR, n=5; WKY rats, n=5). At the end of the 1-hour stabilization period, a small blood sample (0.25 ml) was collected from the arterial catheter for subsequent assay for plasma renin activity (PRA). For this and subsequent blood sampling periods, an equal volume of 0.9% saline was used to replace the withdrawn blood. The renal perfusion pressure was then reduced to 90 mm Hg for 5 minutes, and a second blood sample was withdrawn. Renal perfusion pressure was then reduced to 50 mm Hg for an additional 5 minutes, and then a final blood sample was collected. The constriction was released, and the rat was given 1 hour to recover. A jet of air (140 mm Hg through a nozzle, i.d. 3 mm) was then directed at the rat's face through a hole in the front of the cage. Five minutes later a blood sample was withdrawn, and the renal perfusion pressure was reduced again to 90 and 50 mm Hg for 5-minute periods. Blood samples were collected as before at the end of each period. The air was stopped, the constriction was released, and the experiment was terminated. The order of treatment was randomized so that some rats received the air during the first occlusion period. Each rat had six blood samples totaling 1.5 ml removed over the 2-hour period. Hematocrit was monitored in initial experiments and did not change significantly during the entire procedure.

Because of the potential for the rats to still be affected by surgical stress 1 day postoperatively, other groups of rats (SHR, n=8; WKY rats, n=5) were allowed 2–3 days to recover before experimentation. These rats then underwent the protocol outlined above.

**Propranolol treatment.** A group of young SHR (n=8) were prepared as outlined above except that a catheter was also inserted into a femoral vein. Two days after surgery, the rats were brought into the laboratory and placed in restraining cages. As before, a blood sample was collected after a 1-hour stabilization period; then renal perfusion pressure was reduced to 90 and 50 mm Hg for 5-minute periods, and blood was withdrawn at the end of each period. The aortic constriction was released, and the rats were allowed to recover for 1 hour. A bolus injection of propranolol (Sigma Chemical Co., St. Louis, Missouri) was then given into the femoral vein (6.5 mg/kg). In five of the rats, the air was turned on and the constriction protocol was repeated in the presence of the stress. In the other three rats, the constriction protocol was repeated but the air was not turned on. At the end of the experiment, a bolus injection of isoproterenol (0.1 mg/kg) was given intravenously. The absence of an increase in heart rate was considered evidence of adequate β-adrenergic blockade.

**Young animals, unrestrained.** The potential for restraint stress to affect the sensitivity of the renin response to reduced renal perfusion pressure was investigated by using some rats in the freely moving state. These rats (SHR, n=11; WKY rats, n=7) were tested 2–3 days postoperatively. In these experiments, only the baseline renin response to reducing
renal perfusion pressure to 90 and 50 mm Hg was assessed. The occlusion in the presence of the air was not carried out. The pressure transducer and syringe for inflating the occluding cuff were connected to the rats with long extender tubes that allowed free movement anywhere in the cage.

**Adult animals.** All experiments in the adult SHR (n = 7) and WKY rats (n = 5) were performed 2 or more days postoperatively with the rats restrained. The protocol was similar to that of the young, restrained rats except the renal perfusion pressure was reduced to 100, 75, and 50 mm Hg for 5-minute periods before and in the presence of the air stress. In these rats, eight blood samples (totaling 2 ml) were withdrawn. Renal blood flow was also monitored throughout the experiment.

**Renal denervation.** An additional four adult SHR underwent bilateral renal denervation at the time of the initial surgery. The kidneys were exposed through flank incisions, and the renal arteries and veins were stripped of all connective tissue and then were painted with 10% phenol in absolute ethanol. In these rats, the occluding cuff was placed around the aorta through the right flank incision so that a ventral incision was not required. Doppler flow probes were not used in this group. Two to three days after surgery, the rats were brought to the laboratory and were restrained as above. The aortic constriction protocol was then carried out before and in the presence of the air stress.

**Plasma Renin Activity Assay**

Blood samples were collected in chilled microcentrifuge tubes containing EDTA (final concentration, 1 mg/ml). The samples were immediately centrifuged at 4°C, and the plasma was withdrawn and frozen for subsequent assay. PRA was measured by using a modification of a commercially available (New England Nuclear, Boston, Massachusetts) radioimmunoassay kit. Plasma (100 μl) was incubated for 1 hour at 37°C in the presence of dimercaprol and 8-hydroxyquinoline (2 μl each) and a maleate buffer (200 μl, pH 6.0). The angiotensin I generated during this incubation was then determined by radioimmunoassay. The intra-assay variability determined in this laboratory is 8.5%, and the interassay variability is 11%. The assay sensitivity is reported by the manufacturer to be 40 pg/ml angiotensin I per tube.

**Data Analysis**

**Young rats.** For each rat the relation between renal perfusion pressure and PRA was divided into two components, a steep phase and a plateau phase. For the steep phase, the equation of the line joining the PRA values at the two lowest levels of perfusion pressure (90 and 50 mm Hg) was determined. For the plateau phase, a horizontal line was constructed at the PRA value corresponding to spontaneous arterial pressure. The intersection between these two lines was estimated to be threshold pressure. The effect of surgical stress was determined with two-way analysis of variance to compare the values for the slope of the steep phase or the threshold pressure obtained from SHR and WKY rats used 1 or 2–3 days postoperatively. The effect of restraint stress was determined by comparison of the slopes and threshold pressures in SHR and WKY rats that were restrained or freely moving. The effect of the air stress was determined in a similar manner except that analysis of variance for repeated measures was used. The effect of propranolol on the slope and threshold pressure in the SHR was determined with the paired t test (two-tailed).

**Adult rats.** For each rat, linear regression was used to determine the equation of the line relating PRA and the three lower levels of renal perfusion pressure (100, 75, and 50 mm Hg). As before, the plateau phase was defined by a horizontal line constructed at the level of spontaneous PRA. The threshold pressure was defined as the intersection of these two lines. Comparisons were made as outlined above. The effect of renal denervation in the SHR was determined using the paired t test (two-tailed).

Any post hoc pairwise comparisons were made with Fisher’s New Multiple Range Test. For all analyses, p<0.05 was considered to be significant.

**Results**

**Effect of Surgical Stress in Young Rats**

Figure 1 compares the renin response to reduced renal perfusion pressure in young SHR and WKY rats used 1 day after surgery with rats used 2–3 days postoperatively. Both groups of rats exhibited an increased sensitivity (slope of the steep phase) of the response when the experiments were performed 1 day postoperatively. However, the threshold pressure was not significantly affected by the surgery. Analysis of variance showed that the slope of the steep phase
was significantly greater in the SHR compared with the WKY control rats both at 1 day and at 2–3 days postoperatively. In the rats used 1 day postoperatively, application of the air had no significant effect on the renin response to reduced renal perfusion pressure for SHR or WKY rats (data not shown). The effect of the air stress in rats given 2–3 days to recover from surgery is presented below.

**Effect of Air Stress**

The effect of application of the air stress on the renin response to aortic constriction in young, restrained SHR given 2–3 days to recover from surgery is shown in Figure 2A. The control response (absence of air) in these rats is the same as that depicted in Figure 1A (filled circles). The air-stress protocol requires that the rats be restrained. Application of the air produced a significant rightward shift in the threshold pressure. However, the air stress did not produce a significant increase in sensitivity (slope) of the response. In the WKY rats, the threshold pressure and slope were 103±9 and -0.32±0.07, respectively, before the air (see Figure 1B, filled circles) and 97±3 and -0.39±0.05 during the air. Neither variable was significantly affected by the stress.

**Effect of Propranolol**

Figure 2B shows the effect of application of the air stress on the renin response in a different group of rats that were pretreated with propranolol. In these rats, the control response to reduced renal perfusion pressure was obtained and then the propranolol was given. In the presence of β-adrenergic blockade, the slope of the steep phase of the response was significantly decreased and the threshold pressure was unchanged during the air stress. In the three rats given propranolol but not air, the slope of the response also decreased (Δ = -0.33±0.1).

**Effect of Restraint Stress**

Comparison of the response in restrained versus freely moving SHR and WKY rats showed that the slope of the steep phase was significantly greater in the restrained SHR (−0.62±0.05 vs. −0.39±0.05). Threshold pressure was not different in the restrained SHR. In the WKY rats, restraint had no effect on the slope or the threshold pressure. In Figure 3 the relation between renal perfusion pressure and PRA in young SHR allowed to move freely in their cage is compared with age-matched WKY rats. For the WKY rats, data from restrained (n=5) and unrestrained (n=7) animals were pooled as there was no significant difference between these groups. Although the SHR started at a higher spontaneous arterial pressure, both groups exhibited similar threshold pressures and slopes.

**Adult Rats**

Figure 4 shows the relation between renal perfusion pressure and PRA under control conditions (absence of air) in adult SHR and WKY rats. Spontaneous arterial pressure was significantly greater in the SHR as expected. However, the threshold pressure and slope of the steep phase were not statisti-
压力依赖性血管紧张素原释放在成年自发性高血压大鼠中的作用

目的

在本研究中，我们旨在确定空气刺激是否可以作为刺激血管紧张素原释放的刺激。

方法

在空气刺激下，肾脏对压力的反应有所不同。空气在WKY大鼠中没有对肾脏血流产生任何影响。在空气刺激下，肾血流在SHR大鼠中显著增加。

结果

表1总结了空气刺激对WKY和SHR大鼠肾血流的影响。在空气刺激下，肾血流在SHR大鼠中显著增加。

讨论

空气刺激和血管紧张素原的释放关系不线性。研究人员通常将血管紧张素原的释放分为“平台”期和“陡峭”期。在空气刺激下，肾脏血流在SHR大鼠中显著增加。
thetic hyperactivity that is present in the SHR was sufficient to increase the sensitivity of the renin response to reduced renal perfusion pressure (a nonneural stimulus). Animals were studied both in the developmental stage and the established stage of hypertension. In addition, the ability of a stressful stimulus (air to the face) to further augment the responsiveness of renin release mechanisms was also investigated.

In general, PRA is not thought to be elevated in SHR during the development of hypertension. However, there are several lines of evidence that are consistent with a contributory role of an interaction between the sympathetic nervous system and the renin-angiotensin system in the development of this form of genetic hypertension. First, long-term treatment of young SHR with angiotensin converting enzyme inhibitors is known to prevent the development of hypertension. Second, long-term treatment with \( \beta \)-adrenergic antagonists reduces renin secretion and attenuates the development of the hypertension in SHR. Third, renal denervation also delays the development of hypertension in these animals. These data are consistent with the potential for increased sympathetic outflow to the kidney, whether constant or intermittent, to enhance renin release mechanisms that have the potential to produce a long-term increase in arterial pressure in SHR.

However, data from the present investigation suggest that under normal, low-stressed conditions, SHR do not exhibit an increased renin response to reduced renal perfusion pressure compared with WKY control rats. This lack of difference was present both in young SHR in the developing stages of hypertension and older rats with established hypertension. Apparently, the pressure-dependent control of renin secretion is one regulatory mechanism that is not "reset" in the SHR to a higher sensitivity or threshold pressure. Because of this lack of resetting, adjustments in renin secretion rate in response to intermittent decreases in arterial pressure that may occur throughout the day in unstressed SHR probably are not sufficient to contribute to the hypertension.

This result was unexpected as SHR are known to have increased renal nerve activity compared with WKY control rats. Various investigators have shown in dogs and rats that only very small increases in renal nerve activity are needed to enhance the sensitivity of pressure-dependent renin secretion. Larger increases in renal nerve activity can cause significant antinatriuresis. It is known that tonic sympathetic activity to the kidney is sufficient in the SHR to produce an antinatriuresis, and so one would predict that renin release mechanisms would be enhanced also. Several possibilities for the lack of effect could be proposed. The elevated arterial pressure that was present in both the young and the older SHR could be exerting an inhibitory effect that is sufficient to overcome the neural stimulus for increased sensitivity. Alternatively, the manner in which renal sympathetic activity influences renal functions may be different in the SHR than in dogs or Sprague-Dawley rats. Perhaps the juxtaglomerular cells are less responsive to neural activation than are renal tubule cells in the SHR. Also, a chronically increased renal nerve activity might not affect renal functions in the same manner that an acute increase would. Finally, differences in sodium balance could play a role. Fahri et al have shown that sodium restriction can increase the slope of the steep part of the renal perfusion pressure–PRA relation. Sodium balance was not monitored in the present study. The rats all ate and drank ad libitum before and after surgery. If the WKY rats were in a relatively sodium-depleted state compared with the SHR, then they might exhibit an exaggerated responsiveness that could mask a difference between the two groups.

Although no differences were found under baseline conditions, renin release mechanisms in the SHR appear significantly influenced by stressful conditions. Young SHR showed an increase in the sensitivity of pressure-dependent renin release in response to surgery. This stress was apparently severe enough that WKY rats also exhibited an enhanced sensitivity. These data suggest that a minimum of 2 days be allowed after surgery whenever renin release mechanisms are investigated in these rats. Restraint stress also increased the sensitivity of the relation between renal perfusion pressure and plasma renin activity in the young SHR but not the young WKY rats. In the young SHR, but not the WKY rats, the application of an acute air stress also shifted the response curve to the right but did not increase the slope. However, these rats seem to be extremely sensitive to stressful stimuli and the air-stress design requires that the animals be restrained. Thus, the control renin-response curve (in the absence of air) in the young rats tested 2–3 days postoperatively, but restrained, already exhibited an increase in slope compared with freely moving animals. This increase in sensitivity would tend to minimize any further effect produced by the air stress. The adult SHR responded to the air stress with an increase in slope but not threshold pressure. This suggests that acute stress may affect pressure-dependent renin release differently in animals with established hypertension.

These data raise the possibility that, under certain conditions, the SHR may exhibit increased sensitivity of renin control mechanisms. During times of stress, the juxtaglomerular cells may respond to normal daily nonneural stimuli with exaggerated increases in renin secretion if the threshold pressure or sensitivity are increased sufficiently. Intermittent increases in PRA and thus plasma angiotensin II, if persistent, could then contribute to the increased arterial pressure. Ehmke et al argue that the pressure-dependent control of renin secretion is a primary determinant of arterial pressure. It is presently not possible to quantify the magnitude of this effect, but the ability of converting enzyme inhibitors to prevent the development of hypertension in the SHR argue...
that it may be significant. It is also recognized that an increased end-organ responsiveness to angiotensin II could explain part or all of the contribution of the renin-angiotensin system in the SHR.24

The propranolol study in the young SHR suggests that the increase in threshold pressure produced by the air stress was mediated by β-adrenergic receptors. The propranolol presumably acted at the juxtaglomerular cells to block the effects of neurally released norepinephrine or increased adrenal catecholamines. It is interesting that propranolol alone produced a significant decrease in the slope of the response. These data agree with the effect of propranolol in conscious Sprague-Dawley rats.6 It should be noted that the slope produced by propranolol treatment in these restrained rats (see Figure 2B) was nearly identical to the slope in the freely moving SHR (see Figure 3). This suggests that the small increase in sensitivity imparted by restraint stress in the young SHR is also mediated by β-adrenergic receptors.

In the adult SHR, most of the increase in sensitivity produced by the air stress can be attributed to increased renal nerve activity, since in the renal-denervated rats the air had no significant effect. However, the response in the denervated rats was not identical to the innervated rats, which may suggest that circulating catecholamines can also contribute to the enhancement. A greater decrease in renal blood flow in the presence of the air could not explain the augmented response in the adult SHR as it was reduced similarly at each level of renal perfusion pressure.

Both age groups of WKY rats were unresponsive to the air stress. These data are consistent with a report that air stress does not increase renal nerve activity in the WKY rat.11 The observation that another normotensive strain (Sprague-Dawley) of rats did respond to the air stress with increased responsiveness6 raises the possibility that WKY rats may be genetically hyporesponsive to stressful stimuli. If so, the differences reported in the present investigation between SHR and WKY rats may be due more to the WKY rat than the SHR. It has been reported that Wistar rats (the parent strain for SHR and WKY rats) have a hyporesponsive renin system.25

In summary, the data from the present investigation show that under relatively unstressed conditions, SHR and WKY rats respond to decreases in renal perfusion pressure with similar increases in PRA. This similarity was present in young and adult rats. The young SHR were extremely sensitive to stressful conditions. Chronic stress (surgical), restraint stress, and air stress all produced an enhanced sensitivity or increase in threshold pressure of the renin response to reduced arterial pressure. Adult SHR also responded to the acute stress (air) with an enhanced sensitivity. Except for surgical stress in the young WKY rats, the renin response in both age groups of WKY rats was not affected by the stressful stimuli. These data raise the possibility that under certain stressful conditions the renin response to ordinary nonneural stimuli may be exaggerated in SHR during the development or maintenance of hypertension. An intermittent elevation in PRA and thus angiotensin II could then contribute to the elevated arterial pressure. This hypothesis is consistent with reports that converting enzyme inhibitors can prevent the development of hypertension in the SHR.

References


KEY WORDS • renin • spontaneously hypertensive rat • stress • essential hypertension
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