Baroreceptor–Heart Rate Reflex Function Before and After Surgical Reversal of Two-Kidney, One-Clip Hypertension in the Rat

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Baroreflex function was studied in conscious early phase (<6 weeks) two-kidney, one-clip hypertensive rats before and 24 hours after surgical reversal of hypertension by removal of the constricting renal artery clip or after pharmacological reduction of blood pressure by an infusion of hydralazine or captopril. A normotensive sham-clipped group was included. Another group of two-kidney, one-clip rats was studied 3 weeks after unclipping. Baroreflex sensitivity, as assessed by the steady-state method using a graded phenylephrine infusion, mean arterial pressure, and heart rate were measured preoperatively and at 24 hours postoperatively. Two-kidney, one-clip rats were significantly hypertensive preoperatively compared with control (mean arterial pressure, 183±4 vs. 106±2 mm Hg, p < 0.001), heart rate was similar (420±9 vs. 401±9 beats/min, p > 0.05), and baroreflex sensitivity was significantly reduced (0.76±0.07 vs. 1.50±0.20 msec/mm Hg; p < 0.001). There was a minimal change in heart rate despite the fall in mean arterial pressure in all hypertensive groups, indicating resetting of the baroreflexes. At 24 hours after the operation, baroreflex sensitivity was unchanged in all groups compared with the preoperative value. By 3 weeks, baroreflex sensitivity was significantly greater than in the hypertensive two-kidney, one-clip rats before the operation and 24 hours after they were unclipped, but not compared with normotensive sham-clipped rats. Thus, although resetting occurs within 24 hours, whatever the method of blood pressure reduction, baroreflex sensitivity remains impaired at this time. However, baroreflex sensitivity had returned to normal at 3 weeks after the rats were unclipped, suggesting the importance of structural change. These baroreflex alterations appear to be independent of the renin-angiotensin system but consequential to the fall in blood pressure. (Circulation Research 1990;66:1673–1680)

Surgical reversal of early phase two-kidney, one-clip (2K-1C) renovascular hypertension by removal of the constricting clip results in a rapid, profound fall in blood pressure, associated with a marked reduction in peripheral vascular resistance.1 However, in hemodynamic studies in anesthetized rats, there was no significant increase in heart rate 2 hours after unclipping,2 and later studies in conscious rats also show no tachycardia 24 hours after the clips were removed.1 Arterial baroreflexes provide a powerful cardiovascular reflex to counteract sudden changes in blood pressure. A reduction in arterial pressure would be expected to activate baroreceptors, resulting in reduction of cardiac vagal efferent activity and increased sympathetic activity, with an increase in heart rate. Therefore, the absence of a tachycardia with unclipping suggests either an alteration in baroreflex function or a direct inhibition of autonomic nervous system activity.

Sustained alterations in arterial pressure for even short periods (15 minutes) result in acute resetting of the baroreceptor threshold and operating range in the direction of the change in blood pressure. This is accompanied by an even greater degree of resetting of the baroreceptor–heart rate reflex. However, acute resetting is not usually accompanied by a change in the sensitivity of the reflex.3

In a number of models of hypertension, baroreflexes are reset to operate at higher arterial pressures and have a reduced sensitivity to pressure changes.4 Reversal of renovascular hypertension results in progressive resetting of the baroreceptor pressure range, with almost complete resetting at 6 hours after the clips were removed.5 Assessment of the vagal com-
ponent of the baroreceptor–heart rate reflex at 24 hours after the operation by using the ramp method also showed a significant increase in sensitivity, occurring before regression of structural changes could have occurred. Angiotensin levels are known to be increased in early phase 2K-1C hypertension, falling rapidly after unclipping. The changes in the sensitivity of the baroreflex may therefore be partly due to angiotensin II. It has also been suggested that release of renomedullary lipid after unclipping may inhibit sympathetic nervous system activity.

Therefore, the aim of these experiments was to study in more detail the time course of alterations in the baroreceptor–heart rate reflex after unclipping in the 2K-1C hypertensive rat. In addition, baroreflexes were assessed after pharmacological reversal of hypertension with the vasodilator hydralazine or the angiotensin converting enzyme inhibitor captopril to further elucidate the influence of angiotensin II as opposed to that of blood pressure reduction on the baroreflex in this model. Baroreflex sensitivity was measured by the steady state infusion method with a graded phenylephrine infusion to assess both the vagal and sympathetic components of the reflex.

Materials and Methods

Female Wistar rats weighing 170–190 g were used. All operative procedures were performed during ether anesthesia. 2K-1C hypertension was produced by placing a silver clip (0.2 mm i.d.) on the left renal artery through a loin incision. In the control sham-clipped group, a clip was placed near, but not constricting, the left renal artery. The right kidney was not disturbed. Indirect blood pressures were measured by a photoelectric method under light ether anesthesia, and animals with 2K-1C hypertension and systolic pressures greater than 150 mm Hg at 3–6 weeks after clipping were selected for further study. Blood samples for measurement of plasma renin concentration were taken under light ether anesthesia by amputation of the tail and according to a previously established protocol. The blood sample was obtained at least 1 week before cannulation to allow recovery of the blood volume before further studies. The volume of the blood sample taken was 0.8 ml, that is, between 5% and 10% of the blood volume of the animal. Other studies in rats have shown that acute bleeding of this degree is followed by full recovery of the hematocrit within 8 days.

Operative Procedures

During ether anesthesia, a polyethylene catheter was inserted into the right femoral artery (P-25, 0.4-mm i.d., 0.8-mm o.d.) and two polyethylene catheters were inserted into the right femoral vein and advanced to the inferior vena cava (P-10, 0.28-mm i.d., 0.61-mm o.d.). All catheters were exteriorized between the scapulae and protected by a light flexible metal coil attached to the animals by a linen jacket. The coil was attached to an overhead, lightly counterbalanced arm to ensure minimal tension as previously described. The femoral arterial catheter was connected to a Statham P23 ID transducer (Gould, Cleveland, Ohio), and arterial pressure and heart rate were recorded on a multichannel recorder (Grass Instruments, Quincy, Massachusetts). The arterial catheter was flushed with 0.2 ml heparinized dextrose (50 g dextrose/l, 10 units heparin sodium/ml). Heparinized dextrose was then constantly infused at a rate of 0.25 ml/hr through both P-10 venous catheters (total of 0.5 ml/hr) overnight.

Baroreceptor–Heart Rate Reflex

Baroreflex sensitivity was measured using the steady-state infusion technique. Blood pressure was elevated by a continuous infusion of phenylephrine hydrochloride (Sigma Chemical, St. Louis, Missouri) at progressively increasing rates (0–0.06 ml/min) to give stepwise increases in phenylephrine doses of 0, 4, 10, 20, and 80 μg/kg/min. Each dose of phenylephrine was given for 1.5 minutes, and heart rate and mean arterial pressure were recorded at each plateau before increasing the dose. Heart period, the beat-to-beat interval, was then calculated from heart rate. Heart period was then plotted against mean arterial pressure for each dose of phenylephrine by linear regression, and the slope of the line was taken as an index of baroreflex sensitivity, expressed as milliseconds per millimeter of mercury. It was apparent that although the values obtained for heart period and mean arterial pressure for the doses 0–20 μg/kg/min resulted in a linear plot (mean correlation coefficient, 0.93), there was considerable variation in the values obtained at the higher dose of 80 μg/kg/min, this dose sometimes resulting in cardiac arrhythmias. Therefore, this higher dose was not included in the calculation of baroreflex sensitivity.

Study 1: Groups

The next day, 15 hours after cannulation, two baseline recordings of mean arterial pressure (in millimeters of mercury), heart rate (in beats per minute), and baroreceptor–heart rate reflex sensitivity (in milliseconds per millimeter of mercury) were made with a 30-minute interval between recordings. The 2K-1C rats were then randomly divided into groups and subjected to a brief ether anesthetic (<10 minutes) during which either the constricting renal artery clip was removed (uncipping; n = 10) or a sham operation was performed. In this procedure the renal artery clip was exposed and cleaned but not removed (sham unclipping, n = 8). The sham-clipped control group was also subjected to removal of the nonconstricting clip (n = 10). All rats were subsequently studied conscious and unrestrained. In two groups of sham-unclipped 2K-1C hypertensive rats, an infusion of heparinized dextrose with either hydralazine (0.5 mg/kg/hr, n = 7; CIBA Laboratories, Horsham, West Sussex, UK) or captopril (a bolus of 500 μg followed by 8.3 μg/kg/min, n = 7; E.R. Squibb & Sons, Inc, Princeton, New Jersey) was begun at the time of this operation at the rate of 0.56 ml/hr via one
of the P-10 catheters. In the captopril-infused group, adequate blockade of angiotensin converting enzyme was demonstrated by the absence of a significant pressor response to a 50-ng bolus dose of angiotensin I (Beckman Bioproducts, Geneva, Switzerland) given after 24 hours of captopril infusion. The other groups received an infusion of 0.56 ml/hr of heparinized dextrose alone. Mean arterial pressure, heart rate, and baroreflex sensitivity were measured at hourly intervals postoperatively for 9 hours and again after 24 hours.

Hence, the groups studied were 1) sham clipped, infused with dextrose; 2) 2K-1C, sham unclipped, infused with dextrose; 3) 2K-1C, unclipped, infused with dextrose; 4) 2K-1C, sham unclipped, infused with hydralazine; and 5) 2K-1C, sham unclipped, infused with captopril.

**Study 2**

Another group of rats was studied to further elucidate the time course of changes in the baroreceptor–heart rate reflex. 2K-1C hypertension was produced as above. Three to six weeks later, after indirect blood pressure recording, these rats were subjected to a brief ether anesthetic and the constricting clip was removed. Three weeks after unclipping, the rats were cannulated as in study 1 and allowed to recover overnight (n=11). Fifteen hours postcannulation, two sets of measurements of mean arterial pressure, heart rate, and baroreflex sensitivity were made by using the same procedures as in study 1.

**Plasma Renin Concentration**

Blood was collected in a precooled tube containing 0.1 ml concentrated dipotassium EDTA. After centrifugation at 4°C, the plasma was stored at −20°C. Plasma renin concentration is a measure of the concentration of plasma renin in terms of its activity. This is accomplished by inactivation of the endogenous substrate and then the addition of an excess of exogenous substrate in the form of plasma from binephrectomized rats. The generation of the product, angiotensin I, is then measured by radioimmunoassay, and the results are expressed as the quantity of angiotensin I generated under standardized conditions over a fixed time (picomoles of angiotensin I per milliliter per hour).11,14

**Statistics**

Results are expressed as mean±SEM. Groups were compared by using analysis of variance. If this demonstrated a statistical difference or if further comparisons were of particular importance, then differences between individual groups were analyzed further with Student’s unpaired t test with Dunnett’s correction for multiple comparisons when appropriate.13 Plasma renin concentration was not normally distributed and therefore was logarithmically transformed before statistical analysis. In addition, Student’s paired t test was used to compare mean arterial pressure, heart rate, and baroreflex sensitivity before and 24 hours after operation in each group. To examine whether there was any progressive change in sensitivity (gradient of slope) or set point (intercept) of the baroreflex with time through the experiment, the heart period at 140 mm Hg and the gradients were compared between rats and between times by using linear modeling on GLIM.16 The validity was tested and was satisfactory. The method assumes that the effect of the previous dose of phenylephrine does not influence the sensitivity at the beginning of the next estimate of sensitivity.

**Results**

**Study 1**

The weight [overall mean, 233±4 g, F(4,37)=0.51, p>0.05] and number of days postoperation [33±1 days, F(4,37)=2.51, p>0.05] were similar in all groups. Plasma renin concentration was significantly elevated in the 2K-1C groups compared with the sham-clipped group (386±41 vs. 58±9 pmol angiotensin I/ml/hr, p<0.001). The initial observations demonstrated that the mean arterial pressure was significantly elevated in the 2K-1C groups compared with the sham-clipped controls (183±4 vs. 106±2 mm Hg, p<0.001). The heart rate was not significantly different (420±9 vs. 401±9 beats/min, p>0.05). Baroreflex sensitivity was significantly reduced in the 2K-1C group compared with the sham-clipped controls (0.76±0.07 vs. 1.50±0.2 msec/mm Hg, p<0.001).

There was a significant and marked reduction in blood pressure in the unclipped group at 24 hours after operation compared with the initial preoperative value. There was a smaller but significant fall in blood pressure in the groups infused with hydralazine or captopril, and a very small reduction in mean arterial pressure with sham unclipping in the 2K-1C model (see Figure 1). Blood pressure was not significantly different before and 24 hours after operation in the sham-clipped controls. There was a small but significant tachycardia at 24 hours compared with the initial heart rate in the unclipped group (439±11 vs. 406±10 beats/min, p<0.05). There was no significant change in the heart rate before and 24 hours after treatment in the other groups. Baroreflex sensitivity was not significantly altered in any group 24 hours after the operation.

There was a significant negative correlation between the initial mean arterial pressure and baroreflex sensitivity in the hypertensive groups [−0.709, F(1,30)=30.27, p<0.001; see Figure 2], that is, the more hypertensive rats had the least sensitive baroreflex. In the hypertensive groups, there was no significant correlation between the plasma renin concentration 1 week before the experiment and the initial baroreflex sensitivity [0.165, F(1,30)=0.84, p>0.05] or between the plasma renin concentration and the mean arterial pressure [0.135, F(1,30)=0.56, p>0.05]. When measured at 24 hours after the sham operation, unclipping, or reduction of the blood pressure
by pharmacological means, there was no significant correlation between mean arterial pressure and baroreflex sensitivity \([-0.140, F(1,30)=0.60, p>0.05]\).

To examine whether there was any progressive resetting (shift of the curve) or change in sensitivity (gradient of slope) of the baroreflex with time through the experiment, the heart period at 140 mm Hg and the gradients were compared between rats and between times by using linear modeling. There was no significant resetting of the baroreflex with time in the sham-clipped control \([F(10,90)=0.73, p>0.05]\) or the 2K-1C sham-unclipped group \([F(10,70)=1.47, p>0.05]\). The other three groups showed a significant shift of the baroreflex curve with time after unclipping \([F(10,90)=3.224, p<0.001]\), sham unclipping with hydralazine infusion \([F(10,60)=14.1, p<0.0001]\), and sham unclipping with captopril infusion \([F(10,60)=2.167, p<0.05]\). In the sham-clipped control group there were small changes in sensitivity (slope) with time, showing a slight increase in gradient in the first few hours after operation, but then returning to original values \([F(10,90)=2.09, p<0.05]\). There was no significant change in sensitivity with time throughout the 24 hours after sham unclipping \([F(10,70)=0.7, p>0.05]\), unclipping \([F(10,90)=0.75, p>0.05]\), hydralazine infusion \([F(10,60)=1.16, p>0.05]\), or captopril infusion \([F(10,60)=0.72, p>0.05]\).

The degree of shift to the left in the baroreflex curves was related to the amount of blood pressure reduction achieved (see Table 1 and Figure 3). This parallel shift indicates resetting of the baroreceptor–heart rate reflex with the fall in blood pressure, irrespective of whether the reversal was by surgical or pharmacological means.

**Discussion**

This study confirms that the baroreceptor–heart rate reflex is reset to operate at higher mean arterial pressures in 2K-1C hypertension, with reduced sensitivity. With reduction of the blood pressure, the reflex is reset within 24 hours, irrespective of the method of blood pressure reduction, the degree of resetting being dependent on the level of blood pressure achieved. This resetting occurs without a change in the baroreflex sensitivity. However, at 3 weeks after unclipping, the baroreflex sensitivity had returned to normal.
The present study of female rats showed no change in baroreflex sensitivity during the first 24 hours after unclipping. This is at variance with the study performed by Floras and coworkers, who demonstrated a significant increase in the baroreflex sensitivity by 24 hours after unclipping male 2K-1C rats. This apparent contradiction may be accounted for by methodological differences in the two studies. In the earlier study by Floras, the ramp technique was used. With this method, beat-to-beat heart period is correlated with the rising blood pressure in response to a bolus injection of a vasoactive drug. There are differences between the time constants of the vagal and sympathetic cardiac efferent responses, the vagal responses occurring within 1–2 seconds of the blood pressure changes but the sympathetic responses taking several seconds. Therefore, the ramp method essentially measures only the vagal component of the reflex, whereas the steady state method used in the current study gives adequate time for both the vagal and sympathetic components to contribute. In addition, there may be other subtle differences in the responses elicited by the different methods, such as the relative contribution of the cardiopulmonary baroreflexes.

Phenylephrine hydrochloride was used in this study. At the doses of 0–20 \( \mu \text{g/kg/min} \), the rise in blood pressure and bradycardia were reproducible. At the higher dose of 80 \( \mu \text{g/kg/min} \), there was a variable heart rate response, suggesting possible direct chronotropic effects of phenylephrine, and this dose was therefore not included in the analysis of results. At the lower doses, although some chronotropic effects have been noted, these have been small compared with the baroreflex-mediated changes and should therefore have little influence on the results obtained.

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Heart period–mean arterial pressure baroreflex curves in rats before (top panel) and 24 hours after (bottom panel) operation. ▲, Sham unclipped; ◊, sham unclipped with captopril; ◊, sham unclipped with hydralazine; ■, unclipped; □, sham clipped.

![Figure 4](http://circres.ahajournals.org/)

**Figure 4.** Heart period–mean arterial pressure baroreflex curves in two-kidney, one-clip rats before operation (○), 24 hours after unclipping (■), and 3 weeks after unclipping (△), and in sham-clipped rats (●).
In the hypertensive 2K-1C rats, the baroreflexes are reset to operate at higher arterial pressure levels with a shift in the pressure response curve to the right. This confirms the early observations by McCubbin et al., who showed a shift in the threshold response of the baroreceptors with established hypertension. There was also a significant reduction in baroreflex sensitivity, with an inverse correlation between initial mean arterial pressure and sensitivity.

Reduction in blood pressure is associated with rapid resetting of the reflex. The mean arterial pressure–heart period curve when plotted over the entire response range is sigmoid, with an upper and lower plateau. In hypertension, the resting point of mean arterial pressure–heart period may be eccentrically placed, lying near the upper plateau. Therefore, it is possible that this is reflected in the reduced sensitivity of the reflex to further blood pressure elevation. If reduction of the blood pressure simply returned the resting point to nearer the midpoint of the curve, an increase in sensitivity to blood pressure elevation could be observed without any change in the entire baroreflex response curve. Although in this study the entire baroreflex curve was not obtained, the observation of a parallel shift in the curve must indicate actual resetting of the reflex, not just change in the position of the resting point on the curve.

The degree of resetting of the reflex is dependent on the level of mean arterial pressure attained at 24 hours after the operation. This is independent of the means of blood pressure reduction—pharmacological, with and without blockade of the renin-angiotensin system, or unclipping. The mechanisms responsible for this acute resetting are still not fully understood. Even short sustained changes in arterial pressure result in resetting of the baroreceptor response curve in the direction of the blood pressure change. It has been suggested that this may be due to changes in the local mechanical characteristics of the vessels in the region of the receptors, by viscoelastic creep, or alternatively, ionic changes secondary to the activity of the Na⁺,K⁺-ATPase pumps of the receptors may contribute.

The rate of resetting was more rapid than the changes in sensitivity of the reflex. Regulation of sensitivity is complex and, in addition to dependence on baroreceptor input, may also be determined by central interactions with other afferent input or neurohumoral influences. It has been suggested that high levels of angiotensin II may excite sympathetic and suppress cardiac vagal neurons, thus reducing the sensitivity of the reflex. In support of this, blockade of the renin-angiotensin system in the sodium-depleted rabbit (a state associated with high endogenous levels of angiotensin II) increased baroreflex sensitivity, as assessed by the ramp method. However, there may be species differences because studies in the conscious rat showed no alteration in sensitivity with sodium deprivation, saralasin, or captopril. One possible explanation is that the area postrema does not mediate a central action of angiotensin II in the rat. In the present study, there was reduction in the sensitivity of the baroreceptor–heart period reflex in the hypertensive 2K-1C rats, which as a group had elevated plasma renin concentrations. However, there was no significant correlation between plasma renin concentration and baroreflex sensitivity. In this model, unclipping the renal artery results in a rapid reduction of angiotensin II levels within 3 hours with the vascular renin levels taking a little longer to fall (complete in 24 hours). In this study, there was no change in baroreflex sensitivity in the first 24 hours after unclipping or with blockade of the renin-angiotensin system with captopril. This indicates that the reduced sensitivity in 2K-1C hypertension in the rat is not dependent on plasma or tissue angiotensin II levels.

Other neurohumoral systems have also been posulated as modulators of sympathetic activity, possibly through the arterial baroreflexes. Renal venous effluent from an unclipped kidney lowers the blood pressure of a normal rat, associated with an acute fall in sympathetic nerve activity, whereas similar reduction of blood pressure achieved by either graded blood loss or infusion of sodium nitroprusside results in increased sympathetic nervous activity. Therefore, it is possible that renomedullary lipid released from the unclipped kidney may result in the central suppression of sympathetic discharge, contributing to the reduction of sympathetic nerve activity produced by surgical reversal of 2K-1C hypertension, as assessed by direct recording of electrical impulses in efferent renal sympathetic nerves. In the present study, there was no evidence that the baroreceptor–heart period reflex was specifically affected by unclipping, at least in the immediate postoperative period, since resetting was independent of the method of blood pressure reduction. However, it is possible that there are differential effects on baroreflexes such that control of renal sympathetic nerve activity may not always be affected in the same way as the control of heart rate.

An important determinant of sensitivity of the reflex may be the inhibitory effect of cardiopulmonary baroreceptor input. There is some evidence that this is enhanced in hypertensives, possibly associated with changes in the venous circulation and centralization of blood volume. There is evidence in 2K-1C hypertension of reduced vascular capacity and increased mean circulatory filling pressure. Therefore, it is possible that changes in cardiopulmonary receptor discharge were still increased within 24 hours of unclipping or reduction of blood pressure by pharmacological means, but had returned to normal by 3 weeks.

Mean circulatory filling pressure falls within 6 hours of unclipping, and therefore, one might expect a reduction in cardiac afferent discharge. However, mean circulatory filling pressure reflects changes in vascular capacitance as a whole and does not provide direct measurements of pressure or distension of the pulmonary circulation or left atrium. It would be of
interest to measure cardiac afferent activity directly in this model.

Although there was no change in baroreflex sensitivity during the first 24 hours after unclipping, by 3 weeks the sensitivity of the reflex had significantly increased, being no different from the level in normotensive controls. This slow recovery suggests the importance of structural change. Histological studies in rabbits with chronic renal wrap hypertension demonstrated changes in the arterial walls in the region of the baroreceptors, suggesting that these may, at least in part, account for the reduced sensitivity of the baroreflex response.30 However, if this is the case, the changes must be reversible. Baroreflex sensitivity was completely restored by 6 weeks after reversal of renal wrap hypertension in rabbits31 and by 3 weeks after unclipping in the present study. Long-term reduction of blood pressure in hypertensive subjects by pharmacological means has also been associated with some recovery of baroreceptor–heart rate reflex sensitivity, despite different modes of action of the agents used, for example, with calcium channel antagonists32 and β-blockers.33 Regression of structural change in vascular beds has been demonstrated after unclipping.34 It is possible that changes in the venous circulation or cardiac hypertrophy may contribute to central modulation of the reflex by alteration of cardiac afferent fiber discharge. Unpublished observations (Thurston and coworkers) have shown that the ratio of heart weight to body weight is increased in the 2K-1C hypertensive rat compared with sham-clipped controls (0.42% vs. 0.35%). At 24 hours after the arteries were unclipped, the ratio remained increased (0.40%), but 3 weeks later the ratio had returned to 0.35%, illustrating regression of cardiac hypertrophy over this period.

In summary, resetting of the baroreflex occurs rapidly with reduction of arterial pressure, irrespective of the method by which the hypertensive effect is achieved. This resetting alone is sufficient to account for the minimal resting tachycardia after unclipping. However, baroreflex sensitivity takes longer to normalize. This appears to be independent of the influence of angiotensin II, and in view of the time course, may be secondary to regression of structural change.

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