Tachycardic Responses During the Development of Renal Hypertension

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SUMMARY Basal heart rate (HR) of conscious rats under resting conditions was measured daily by electrocardiogram (ECG) during the development of one-kidney, one clip (1K1C) hypertension. A progressive increase in HR (mean ± SEM) was observed from Day 1 to 7: 318 ± 11; 330 ± 18, 338 ± 18; 344 ± 19; 372 ± 14; 374 ± 11, and 388 ± 12 bpm, respectively. During the same period, mean arterial pressure (MAP) also increase progressively: 126 ± 2; 129 ± 2; 134 ± 4; 135 ± 7; 144 ± 2 and 157 ± 4 mm Hg, respectively. From Day 7 onward, the HR declined, reaching values of 336 ± 13 bpm on Day 9, with no further alterations for the next 21 days. The MAP continued to rise, however, being 158 ± 4 on Day 8 and 175 ± 7 mm Hg on Day 30. Sham-operated rats showed no changes in HR or MAP. During the development of hypertension, blockade of converting enzyme with captopril (10 mg/kg, i.v.) caused a significant blood pressure fall on Day 1 (-27 ± 1 mm Hg) and Day 3 (-18 ± 2 mm Hg), whereas on Day 6 (-9 ± 3 mm Hg) and Day 14 (-8 ± 3 mm Hg) the fall was not different from that of the normotensive control rats (NCR) (-6 ± 1 mm Hg). Reflex bradycardia, produced by increasing doses of phenylephrine which elevated the MAP by 10 to 40 mm Hg, was studied in conscious NCR and renal hypertensive rats (RHR) 3 and 7 days after surgery during the development of renal hypertension. Impaired baroreflex function was demonstrable on Day 3 for RHR, with a sensitivity of 0.425 ± 0.05 vs 0.954 ± 0.11 msec/mm Hg for NCR, but baroreflex function was normal in RHR on the Day 7. These data show that, during the early development phase of hypertension, rats exhibited a transitory tachycardia, which indicated an impaired baroreflex control of the HR.

Key Words • heart rate • baroreceptor resetting • baroreflex sensitivity • vagus nerve • renin-angiotensin system

Bradycardia is one of the most conspicuous consequences of increased excitation of the baroreceptors during acute hypertension. However, when the rise in pressure is permanently maintained, the reflex bradycardia progressively declines, because resetting of the baroreceptors returns the increased discharge rate progressively to normal levels. Therefore, the evolution of reflex bradycardia in acute hypertension can provide a valuable index for monitoring the sequence of baroreceptor resetting. Soato and Krieger measured the heart rate (HR) of conscious rats, by means of electrocardiographic recordings in the same model. In their study of the heart rate (HR) of conscious rats, Soato and Krieger measured the heart rate (HR) of conscious rats, by means of electrocardiographic recordings in the same model. In their study of the heart rate (HR) of conscious rats, Soato and Krieger measured the heart rate (HR) of conscious rats, by means of electrocardiographic recordings in the same model. In their study of the heart rate (HR) of conscious rats, Soato and Krieger measured the heart rate (HR) of conscious rats, by means of electrocardiographic recordings in the same model. In their study of the heart rate (HR) of conscious rats, Soato and Krieger measured the heart rate (HR) of conscious rats, by means of electrocardiographic recordings in the same model. In their study of the heart rate (HR) of conscious rats, Soato and Krieger measured the heart rate (HR) of conscious rats, by means of electrocardiographic recordings in the same model. In their study of the heart rate (HR) of conscious rats, Soato and Krieger measured the heart rate (HR) of conscious rats, by means of electrocardiographic recordings in the same model. In their study of the heart rate (HR) of conscious rats, Soato and Krieger measured the heart rate (HR) of conscious rats, by means of electrocardiographic recordings in the same model.
Methods

When the HR of conscious rats is measured over a short period of time by means of the arterial pressure pulse, there is usually an overestimation of the true basal resting HR because of excitement of the rat. Excitement can be avoided by using ECG measurements with implanted electrodes.2,4 We introduced minor modifications in the design of the electrodes to permit measurements for long periods of time with negligible interference from the spontaneous movements of the rat. Three electrodes (fine stainless steel wire, 5 cm in length and 0.50 mm in diameter) were implanted subcutaneously in the back of the rat and connected to a rotating socket and then to a multichannel recorder (Physiograph Four-A, E & M Instruments Company, New York, New York). Since the HR of the rat is affected by behavior,4 basal HR was measured under undisturbed resting conditions during a 10 to 15-minute period. The daily values reported are the average of at least three measurements performed at 8 a.m., 2 p.m., and 5 p.m.

Direct arterial pressure was measured in unanesthetized rats by means of a plastic cannula (PE 10 connected to PE 50) inserted into the abdominal aorta through the femoral artery under the ether anesthesia, 24 hours before the recording session. The cannula emerged through the back of the rat and was connected to a Statham P23-Dp pressure transducer and a Hewlett-Packard (Model 7848 A) multichannel recorder for blood pressure measurements. 1K1C hypertension was produced by applying a silver clip (6 × 2 mm) to the main left renal artery under light ether anesthesia by the technique described by Schaffenburg3 with simultaneous right nephrectomy. The degree of constriction was related to the weight of the rat, i.e., 0.30 mm for the 180–200 g rats used in the present study.

The sensitivity of reflex bradycardia was determined in conscious rats using a technique similar to that described by Jones and Floras6 and Gordon et al.7 Increasing doses of phenylephrine (0.5–4.0 μg/kg) were injected to produce at least four pressure responses ranging from 10 to 40 mm Hg. Sufficient time was allowed between injections for HR and MAP to return to control levels. Maximum bradycardia (Δ pulse interval in msec) was determined at the peak of the pressure response. The slope of the baroreceptor bradycardia to increasing amounts of phenylephrine was determined by linear regression analysis of Δ pulse interval and Δ mean arterial pressure data for each group of rats.

The decrease in the HR produced by electrical stimulation (5 V, 2 msec, 1–128 Hz) of the right vagus nerve was studied in the rats anesthetized with chloralose (60 mg/kg i.v.). The interval between stimulation was determined by the time required for the HR to return to the prestimulation level. Body temperature was maintained at 37°C by external heating. The response of MAP to captopril (10 mg/kg i.v.) in conscious rats was used to assess the activity of the RAS. The results are expressed as means ± SEM. Student's paired and unpaired t tests were used when appropriate, and the differences were considered significant at p < 0.05.

Results

Time Course of Hypertension and Tachycardia after Clipping

Figure 1 shows that at 1 day after clipping the renal artery, the average MAP was elevated (126 ± 2 vs 117

\[ \text{MAP} = 126 \pm 2 \text{ mm Hg} \]

\[ \text{Basal HR} = 380 \pm 10 \text{ beats/min} \]

\[ \text{MAP} = 167 \pm 6 \text{ mm Hg} \]

\[ \text{Basal HR} = 340 \pm 8 \text{ beats/min} \]

\[ \text{MAP} = 118 \pm 2 \text{ mm Hg} \]

\[ \text{Basal HR} = 320 \pm 6 \text{ beats/min} \]

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± 2 mm Hg in the control group). Daily measurements showed that hypertension was progressive, and the MAP reached 157 ± 4 mm Hg on the 6th day. No change in pressure was observed in the sham-operated animals during the same period of observation (MAP range = 117 ± 2 to 121 ± 3 mm Hg).

Resting HR measured daily in another group of conscious rats showed that the HR of clipped rats increased progressively, reaching 388 ± 12 bpm on the 7th day. When compared to the HR of the sham-operated group, the differences in the daily values of HR in the renal clipped rats began to be significant on the 5th day. No changes were observed in the sham-operated rats, whose resting HR values were within the range of 325 ± 4 to 330 ± 6 bpm. At the end of Day 7, the direct MAP of conscious clipped rats was 167 ± 6, and of sham-operated rats, 118 ± 2 mm Hg. Measurement of resting HR was started 8 days after clip implantation in another group of rats to study the evolution of tachycardia. From the value of 370 ± 13 bpm on the Day 8, the resting HR dropped to 336 ± 13 and 329 ± 9 bpm on the Days 9 and 10, respectively, and remained within the normal range (324 ± 9 to 326 ± 6 bpm) during the subsequent days. The direct MAP of this group measured on Day 15 was 164 ± 7 mm Hg. No changes were observed in either HR or MAP of the sham-operated group during the same period.

The fact that tachycardia after the clipping was transitory while hypertension persisted is illustrated in figure 2, which shows the results obtained in different groups of rats studied for 6-8 and 14-16 days after clipping (158 ± 3 and 158 ± 4 mm Hg respectively) and was higher (175 ± 7 mm Hg) in the rats studied for 29-31 days. Tachycardia (371 ± 10 bpm) was only observed at the end of the first week, the HR being normal in the groups studied 2 and 4 weeks after clip implantation. Resting HR was even decreased when compared to the sham-operated animals (303 ± 5 vs 323 ± 5 bpm) in the group studied at the end of the second week.

**MAP Responses to Converting-Enzyme Inhibition**

To assess the activity of the RAS, the response of MAP to a single injection of captopril (10 mg/kg i.v.) were studied in conscious rats at different times after clipping (table 1). The average drop in MAP of clipped rats was greater than controls only 1 and 3 days after clipping when all the individual responses were greater than 10 mm Hg. Six and 14 days after clipping only three of eight and two of six rats, respectively, had responses greater than 10 mm Hg. The average responses were — 9 ± 3 and — 8 ± 3 mm Hg, respectively. The average response of the sham-operated animals was — 6 ± 1 mm Hg and only four of 24 rats had decreases larger than 10 mm Hg.

**Baroreflex Bradycardia**

The sensitivity of baroreceptor bradycardia was assessed in conscious normotensive rats (117 ± 2 mm Hg) and in rats 3 (146 ± 3 mm Hg) and 7 days (149 ± 3 mm Hg) after clip implantation by studying the decrease in HR produced by increasing doses of phenylephrine (Fig. 3). The sensitivity of the reflex was normal in the group studied 7 days after clipping (0.932 ± 0.012 vs 0.954 ± 0.111 msec/mm Hg in the control animals) and was significantly decreased in the group studied after 3 days (0.425 ± 0.05 msec/mm Hg).

**Bradycardia in Response to Vagus Stimulation**

The decreases in HR produced by electrical stimulation of the vagus (5 V, 2 msec, and 1-128 Hz) were studied in chloralose-anesthetized rats 3 and 7 days.
after clipping. Maximum bradycardia was observed in all groups with electrical stimuli of 32–64 Hz. While 3 days after clipping the rats had the maximum bradycardia, which was similar to that observed in the controls (60 ± 22 and 77 ± 20 bpm, respectively), the HR of rats 7 days after clipping dropped only to 262 ± 41 bpm. Thus, the HR levels during maximal response to vagal stimulation were 18% of the control values in the controls and 19% and 61% 3 and 7 days after clipping respectively (fig. 4).

Discussion

Our data show for the first time that during the onset of IKIC hypertension the conscious rat exhibits a marked and progressive increase in the resting HR up to the 7th day, with the HR returning to normal levels 9 to 10 days after clipping. This tachycardia was unexpected, in view of the physiological role of the barore-
ceptors in counteacting any elevation in pressure with bradycardia and decreased total peripheral resistance. Indeed, marked bradycardia was observed in the rat when an immediate arterial hypertension was produced by subdiaphragmatic aortic constrictor, maximum bradycardia was reached 3.5 hours after constriction, and subsequently the HR returned to normal levels (48 hours). Therefore, during the time required for the baroreceptors to complete the process of resetting, the rats showed some degree of bradycardia. Since the resetting of the baroreceptors lags behind the pressure rise, the process should be slower when the hypertensive stimuli are applied progressively, and should be complete only when the hypertension attains a stable level. Since arterial pressure increases progressively during the first week after clipping, complete resetting of the baroreceptors should be expected to occur only during the 1st to 2nd week when the hypertensive level is stable. Jones, analyzing the development of renovascular hypertension in the rat, reported that the baroreceptor threshold in the carotid sinus was completely reset 2 weeks but not 1 week after implantation of the clip around the renal artery. Since hypertension in 1K1C rats is progressive and since the resetting process is occurring simultaneously, the net increase of baroreceptor discharge may not be of sufficient intensity to produce a conspicuous decrease in the resting HR. However, there is no reason to expect tachycardia unless some other factors are altering the baroreflex control of the HR. Actually, a transient decrease in the sensitivity of the reflex bradycardia was observed 3 days but not 7 days after clipping. In contrast, the bradycardia produced by electrical stimulation of the vagus was decreased 7 days but not 3 days after clipping. These data indicate an abnormality in the baroreflex control of the HR during the first week of 1K1C hypertension in the rat even though these data do not allow a complete characterization of the time course of the phenomenon.

There is evidence that angiotensin II (All), leucine-enkephalin, and substance P suppress the baroreflex. All acts on the central nervous system (CNS) to inhibit the baroreceptor-mediated vasodilation9 and decrease the bradycardiac response produced by baroreceptor stimulation. Junqueira and Krieger10 showed that rats with chronic severe 1K1C hypertension had altered baroreceptor function during natural sleep, probably due to modification of the central integration of the baroreceptor reflex. Contrary to reports that chronic 1K1C hypertension rats do not respond to acute administration of converting enzyme inhibitors, rats with severe renal hypertension actually do (Krieger and Moreira, personal communication). There is general agreement that during the early phase of 1K1C hypertension, the RAS in the rat is overactive. Our data confirm this view by showing that, on the basis of the response to captopril, the RAS was hyperactive only 1 and 3 days after clipping, but was normal on the 6th and 14th day.

Since the early observation of Bickerton and Buckley16 that blood-borne All can elevate arterial pressure by a direct action on the brain, several lines of evidence have indicated the important role of All as a neuropeptide present in the brain capable of modulating the autonomic nervous system. Recently, Suzuki et al., studying the early phase of the development of two-kidney, one clip hypertension in conscious dogs, observed that the activation of the renal pressor system was associated with time-related changes in the concentrations of norepinephrine and All in both plasma and cerebrospinal fluid, which indicates an early involvement of both the sympathetic nervous system and RAS in the pathogenesis of renovascular hypertension. They also observed that the early phase of hypertension was accompanied by tachycardia. Faber and Brody9 showed that hypertension induced by acute stenosis of the renal artery in conscious rats was not only renin-dependent but also associated with high neurogenic vasoconstrictor tone, presumably activated by the indirect neural action of All.

The data reported here are consistent with, but do not prove, the view that the progressive tachycardia observed during the onset of one-kidney, one clip hypertension in rats could be due to a decreased sensitivity of the baroreceptor reflex at the beginning (3rd day) of the tachycardia, followed by a decreased efferent vagal activity concomitant with the peak of tachycardia (7th day). Our observations point to a complex alteration of the HR regulation during the onset of one-kidney, one clip hypertension. However, it must be pointed out that this phenomenon could be also associated with a more general activation of the sympathetic system which could override the baroreceptor reflex, and this possibility has not been examined in the present research. The role of the baroreceptor in the pathogenesis of hypertension needs to be assessed both in terms of changes in HR and vascular resistance control. Thus, other studies are necessary to evaluate whether the control of peripheral resistance by the baroreceptors is also affected during the onset of renal hypertension, as observed for the control of the HR.

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Hypertension. 1983;5:V122
doi: 10.1161/01.HYP.5.6_Pt_3.V122

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