Loss of Baroreflex Bradycardia in Renal Hypertensive Rabbits

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ABSTRACT
The degree of reflex bradycardia elicited by a sudden, brief rise in arterial pressure was used as an index of sino-aortic reflex activity in conscious rabbits with early renal hypertension. Change in heart rate was measured every 6 seconds after injected angiotensin which caused a rise in blood pressure of 15 to 60 mm Hg. Ninety-one tests for reflex bradycardia were made in 30 normal rabbits; 14 of them were tested intermittently during the 1 to 40 day period following unilateral nephrectomy and latex encapsulation of the opposite kidney. A total of 62 tests were made after arterial pressure had risen 10 to 80 mm Hg, average 35, above control values. Average decrease in heart rate was significantly less and frequency of negligible reflex bradycardia was much higher in hypertensives than controls. Individual rabbits showed no tendency for reflex bradycardia to return toward normal magnitude. However, the vasomotor component of the sino-aortic reflex mechanism in renal hypertensive rabbits buffered the pressor response to angiotensin in a normal manner. Resetting of sino-aortic reflex activity under hypertensive conditions is discussed.

ADDITIONAL KEY WORDS
heart rate reflex resetting of sino-aortic reflexes baroreceptors and heart rate hypertension carotid sinus and aortic nerves conscious rabbits

Information concerning function of sino-aortic reflexes in man or in conscious laboratory animals with arterial hypertension is scanty, although it is important in the broader question of baroreflex resetting in hypertension. The term "reset" as applied to the sino-aortic baroreflex system has not been defined precisely in the literature, but is used here to mean that the effector response has returned to normal magnitude or rate under conditions that initially altered it. In this definition sino-aortic receptor activity, afferent or efferent nerve discharge can be substituted for effector response, the effector organs being, of course, the heart and blood vessels.

When considering resetting it is important to distinguish between steady state conditions and acutely induced changes at the sino-aortic receptor sites. The extent of resetting of effector responses (or other parts of the reflex pathways) to a continuously high arterial pressure or to an occasional brief stretch of the receptor areas can be very different.

In normal and hypertensive human beings, Pickering et al. found no clear differences in the magnitudes of cardiac and blood pressure responses to digital compression of the carotid sinus regions. Pickering indicated later, however, that this experiment was too crude to test the really important point of whether the reflex responses were excited to the same, or to a lesser or greater, extent in hypertensive than normotensive subjects. That would require knowledge concerning the activity of carotid sinus nerve potentials but if the applied stimulus is measurable, which was not the case in the study by Pickering et al., one has a basis for comparison of responses. Itahara et al. subjected hypertensive people...
with normal resting heart rates to a standardized passive tilt in order to activate reflex cardiac rate responses and found they were reduced in magnitude. Regnier's had noted earlier that release of carotid occlusion did not cause bradycardia in patients with high blood pressure as it did in normal subjects.

The latter two studies indicated that effector responses to acutely induced activation of sino-aortic receptors had not reset under hypertensive conditions. Conway, on the other hand, studied cardiovascular reflexes in conscious hypertensive and normotensive rabbits and found no differences in the magnitudes of pressor or reflex bradycardia responses to injected noradrenaline. When, however, the sympathetic outflow was blocked by hexamethonium, the arterial pressor response was greatly enhanced in hypertensive rabbits. This indicated that prior to block their vasomotor regulatory reflexes were extremely active. Other studies that did not involve superimposed activation of baroreceptors but simply examination of one parameter under the steady state condition of hypertension, include those of McCubbin and Wennemark. The former found that carotid sinus nerve activity in dogs did not reset completely for at least one or two weeks after development of renal hypertension. Kezdi and Wennemark came to the same conclusion about the tone of the sympathetic nervous system in their study of anesthetized dogs with early renal hypertension, but found resetting had occurred in dogs with chronic hypertension. The latter was also true of carotid sinus nerve activity.

In the present study we tested conscious rabbits for the reflex bradycardia that occurs acutely in response to a sudden brief rise of arterial pressure, caused by a single injection of angiotensin. The results will show that reflex bradycardia was absent or diminished in magnitude during the first three to five weeks of renal hypertension. Nor was there any tendency for the response to reset during this time. In contrast to this, the capacity of the sino-aortic reflex system to buffer or reduce the arterial pressor response to angiotensin remained apparently normal.

**Methods**

New Zealand, female rabbits weighing 2 to 3 kg were used for this study. After the local injection of 2% procaine, a femoral or central ear artery was cannulated with plastic tubing and heparin solution (10 mg/ml) was infused to prevent blood clotting. The conscious, unrestrained rabbit was then placed in a small cage inside a sound-attenuated cubicle. The plastic cannula was connected to a pressure transducer (Sanborn 267B) and systolic and diastolic pressures, along with heart rate, were recorded by an electrical recorder. The heart rate record was obtained from a cardiotachometer triggered by the electrical output of the transducer.

**RENAL HYPERTENSION**

Rabbits were anesthetized with ether and both kidneys were exteriorized through dorsal incisions. The right kidney was removed and the left was enclosed in a molded latex capsule. Arterial pressure began to rise within 24 hours and often reached a maximum value at three to five days, or continued to rise slowly for several weeks.

**TEST FOR REFLEX BRADYCARDIA**

We elected to test for the reflex bradycardia that results from a sudden, brief arterial pressure rise because carotid sinus and aortic arch regions are subjected often to this type of pressure change as the result of postural shifts or brief increases in cardiac output. Other workers have also used this type of transient stimulus to study reflex control of heart rate.

Angiotensin was the pressor agent of choice because it has no direct chronotropic cardiac effect nor does it depress blood pressure when applied locally to the carotid sinus. After the rabbit had been sitting quietly in the cage for about 30 minutes, the paper speed of the recorder was set to 0.5 cm/sec and angiotensin, immediately followed by saline, was injected into the marginal ear vein. The total volume of injected fluid was always 1.0 ml. The injection took five to six seconds of the total one-minute test period and usually two tests were run five minutes apart; the doses of angiotensin were 0.2 and 0.4 µg/kg. It was not necessary to touch the animal during the injection because the two syringes, one with angiotensin and the other with saline, were mounted on a board and connected by a T-arrangement to a plastic tube leading to a needle in the ear vein. In some animals, the injection was accompanied by a change of heart rate but this never interfered with the reflex cardiac response to rising arterial pressure.
pressure which began after the first six seconds. Control saline injections did not change arterial pressure or heart rate.

Test results were discarded if the animal moved during the sixty-second test period because blood pressure and heart rate records of conscious rabbits showed characteristically that body movements often caused a two to five second, 5 to 15 mm Hg change in arterial pressure and a 20 to 30 beats/min change in heart rate. In sino-aortic denervated rabbits (see below) these changes were larger and lasted longer.\textsuperscript{17} When the conscious rabbit sits quietly, however, heart rate varies only ± 10 beats/min.

Pressure and heart rate values were averaged for consecutive six-second intervals during the minute after the beginning of injection and reflex bradycardia was evaluated at times specified in Results. "Initial heart rate" (HR\textsubscript{i}) and "initial diastolic pressure" (DP\textsubscript{i}) were the average values recorded in the six seconds preceding the injection. For simplification of presentation only diastolic pressure values were used. The change in systolic pressure was usually less than 5 mm Hg, and at most 10 mm Hg, different from the change in diastolic pressure. Pulse pressure changes of this order did not affect the reflex cardiac response. Previous work\textsuperscript{18} also showed that reflex bradycardia, produced by a ten-minute pressure rise, was unaffected by pulse pressure changes.

**Removal of Moderator Nerves**

Rabbits were anesthetized with sodium thio-pental, iv 30 mg/kg, and a midline incision was made in the neck from the lower jaw to the sternum. Under low power magnification, the aortic nerve, which is in the carotid sheath but separate from the vagus in rabbits, was resected bilaterally from its origin to just above the sternum. To remove the carotid sinus nerves, the carotid artery bifurcation was exposed and a ligature was placed around all the tissue between the internal and external carotid arteries and tied as closely as possible to the bifurcation. All tissue was removed between this tie and a second one at the origin of the sinus nerve. The bifurcation was painted with 5\% phenol.

**Results**

After initiation of renal hypertension, 14 rabbits were tested for reflex bradycardia one to eight times (average four) between day 1 and 21, and 2 of them were tested again several times between days 27 and 40 for a total of 62 tests in hypertensive rabbits. Between day 1 and day 40, diastolic pressure in these animals had risen a minimum of 10 and a maximum of 80 mm Hg (average 35 mm Hg). The same rabbits were also tested one to four times, average twice, during the two days preceding the operation. The latter tests along with tests in 10 other normal rabbits gave a total of 91 control tests for reflex bradycardia in 30 normal rabbits.

Results were based on the decrease in heart rate 30 seconds after the injection of angiotensin because that was the time, ±6 seconds, of the maximum diastolic pressor response, which ranged from 15 to 60 mm Hg, average 30 mm Hg. By selecting heart rate values at a specific time they could be compared at equivalent rates of arterial pressure rise. Similar results were obtained by using values of the maximum decrease in heart rate or the decrease at the end of the initial pressure rise; examples of each of the latter are also included below.

In control and hypertensive rabbits HR\textsubscript{i} values ranged from 160 to 300 beats/min. As testing of control animals proceeded it became apparent that the magnitude of reflex bradycardia, which varied widely, was closely related to HR\textsubscript{i}. Rabbits with the highest HR\textsubscript{i} values showed more reflex cardiac slowing than others. Test results from hypertensive and control rabbits were compared there-
before after the data were divided into high (230 to 300 beats/min) and low (160 to 229 beats/min) HRi ranges. Both groups had essentially the same mean values for the two HRi ranges: 259 and 251 beats/min and 202 and 203 beats/min for hypertensives and controls, respectively. Figure 1 shows the average reduction of heart rate at 30 seconds for all tests of controls, DPi, 50 to 89 mm Hg, and hypertensives, DPi, 70 to 145 mm Hg. Control rabbits had more reflex cardiac slowing than hypertensives within both HRi ranges, and both groups generally showed more reflex bradycardia at high than low HRi levels. The data in figure 1 were pooled and a statistical analysis is shown in table 1. The hypertensive rabbits showed significantly less reflex bradycardia than controls with similar HRi values (P < 0.001). Moreover, even hypertensives with high HRi showed significantly less slowing than controls with low HRi (P < 0.001, not shown in table 1). Among controls, HRi made a significant difference in the absolute heart rate change (P < 0.001, table 1) and in the relative change also. For example, the mean decrease of 51 beats was an average change of 20% as compared to only 13% represented by the mean decrease of 26 beats. Among hypertensives, the magnitude of reflex bradycardia was uniformly so low that HRi made very little difference to the magnitude of response (P = 0.1, table 1).

Figure 2 shows that the reduced magnitude of reflex bradycardia among hypertensives was independent of the magnitude of the 30-second pressor response (range 15 to 60 mm Hg). In the high HRi range, a 15 to 29 mm Hg increment caused a 40 to 50 beat decrease of rate among controls, whereas, among hypertensives, even 45 to 60 mm Hg did not elicit more than a 20 beat drop. Sometimes, regardless of the magnitude of pressor response, control animals with low HRi, but never those with high HRi, showed negligible bradycardia (10 beats or less) or even an acceleration of rate. This occurred with much greater frequency, however, among hypertensives with low HRi, as shown in table 2, and, moreover, it occurred also with similar frequency in hypertensives with high HRi.

Figure 3 shows data from four individual rabbits before and after renal encapsulation. The maximum heart rate decrease during the one-minute tests was plotted along with the decrease at 30 seconds for each animal. The absolute and relative changes of the former values were essentially the same as those for

### Table 1

| HRi Range | Controls (beats/min) | Hypertensives (beats/min) | P
|-----------|----------------------|---------------------------|---------
| High      | 51 ± 23              | 9 ± 12                    | < .001  |
| Low       | 28 ± 24              | 3 ± 16                    | < .001  |
| P         | < .001               |                           | = .100  |

*Pooled data from figure 1.
†HRi low: 160 to 229 beats/min; HRi high: 230 to 300 beats/min.

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the latter. Note that there was no tendency for the magnitude of reflex bradycardia to return toward the preoperative magnitude during the two- or three-week period of hypertension studied. The same was true for the two rabbits studied beyond 21 days.

It was possible that the amount of reflex bradycardia shown by hypertensives would compare more closely to that of controls during the time of most rapid pressure and heart rate change, which was the first 12 seconds of the pressor response to angiotensin. Records were selected that showed fast and constant

| TABLE 2 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Number and Per Cent of Tests Showing Negligible Reflex Bradycardia or Acceleration at Thirty Seconds** |
| **Controls** & **Hypertensives** | **Lowt HRI** | **High HRI** | **Lowt HRI** | **High HRI** |
| Total no. of tests | 64 & 27 | 34 & 28 |
| Number | 19 & 0 | 25 & 21 |
| Per cent | 30 & 0 | 74 & 75 |

*Negligible bradycardia: 10 beats or less. Acceleration: maximum 20.
†See footnote, table 1.

Comparison of reflex bradycardia at the end of the initial 12-second pressure rise (range 2.3 to 3.7 mm Hg/sec) in controls and hypertensive rabbits with similar HRi values. 18 control tests (o); 15 tests of hypertensive rabbits (•).

In contrast to the cardiac slowing component of the baroreflex mechanism, the vasomotor component functioned adequately in hypertensives, as indicated by the magnitude of pressor responses. One might expect the pressor response to angiotensin to be augmented in hypertensives by the reduction or absence of the reflex bradycardia. As a group, however, hypertensives had the same range of pressor responses to angiotensin as controls. Individually, only four hypertensives had a 10 to 20 mm Hg greater response than before operation to the same dose of angiotensin. Moreover, complete sino-aortic denervation of a renal hypertensive rabbit enhanced the pressor response to angiotensin as shown on the right hand side of figure 5. In eight normotensive and two renal hypertensive rabbits from which both sets of moderator nerves were removed, the 30-second pressor responses increased from a range of 20 to 40 mm Hg to one of 40 to 80 mm Hg.

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Pressor and reflex cardiac slowing responses to the injection of angiotensin before and after complete sino-aortic denervation of one normotensive and one renal hypertensive rabbit. Note increase in pressor response and absence of bradycardia after denervation.

When four of the eight sino-aortic denervated rabbits were subsequently made hypertensive by renal encapsulation their pressor responses still exceeded those of hypertensives with both sets of moderator nerves intact. In addition, after the injection of angiotensin into sino-aortic denervated, renal hypertensive rabbits, arterial pressure remained high for five to ten minutes instead of returning to pre-injection pressures.

Figure 5 demonstrates that after sino-aortic denervation, reflex bradycardia did not accompany the pressor response to angiotensin; a consistent finding in all 10 rabbits mentioned above. However, about one month after denervation the cardiac slowing response returned in four of the ten; they were re-operated and tissue that had grown around the carotid bifurcation and between the cut ends of the aortic nerves was removed. In all four, this again eliminated reflex bradycardia. We concluded that carotid sinus and aortic nerves were the only ones involved in the reflex bradycardia that is evoked by the injection of angiotensin.

Discussion

The present study was done to determine whether conscious rabbits with early renal hypertension had normal sino-aortic reflex activity. We found the reflex bradycardia that is the normal response to an acutely induced pressure rise was absent or diminished, i.e., this particular reflex effector response did not reset. On the other hand, and as Conway also noted in his study of hypertensive rabbits, vasomotor reflex activity was normal. Since receptors for the vasomotor and cardiac rate components of the sino-aortic reflexes are all located in the same general areas, absence of response from one component only cannot be attributed to inadequate stretch of the vessel wall in which the receptors lie. Furthermore, the pressor response to angiotensin was enhanced after sino-aortic nerves were sectioned.

As indicated in the introductory sentences of this paper, one must distinguish between effector responses to the steady condition of hypertension and to acutely induced changes at the receptor level. Our rabbits with early renal hypertension had resting heart rates, HRi levels, within normal limits and not below normal. It is well accepted that hypertensive animals and man have normal resting heart rates. Thus this particular effector response, resting heart rate, resets in the presence of a steadily elevated arterial pressure. We showed in a previous study of conscious rabbits that in the presence of continuously elevated arterial pressure, maintained by the infusion of angiotensin, the slowed heart returned to normal within an average time of one hour, maximum time, seven hours. The degree of post-infusion tachycardia, when
pressure was normal, varied directly with the amount of restoration in heart rate. This suggested that resetting resulted from the development of a new level of neural activity within the central nervous system during the time pressure was high.

No attempt was made in the present study to determine why the acutely induced reflex heart rate response was absent during the first three to five weeks of hypertension. If, however, steadily elevated arterial pressure caused sino-aortic nerves to discharge continuously at a high rate, as was reported for dogs, then perhaps only certain receptors were further activated by the acutely superimposed pressor effect of angiotensin. There is evidence, from Landgren's study of individual baroreceptors in the carotid sinus area of cats, for a limited range of intrasinus pressure within which a pressure rise can elicit increased impulse discharge into afferent nerve fibers. Also variation in threshold sensitivity of aortic nerve fibers has been observed. Although there is no direct evidence for specialized function of specific receptors, a pressure rise above an already elevated level of arterial pressure might not discharge receptors specifically connected to reflex pathways affecting heart rate, but could increase further the discharge of other receptors connected to vasomotor pathways.

Of the 64 tests done when the control rabbits had low HRi values (high vagal tone), 30% showed negligible slowing or, occasionally, acceleration. This never occurred when control rabbits had high HRi values. Why a high vagal tone interferes with slowing is obscure but other workers have noted that HRi influences the heart rate response in dogs. The same rabbits with low HRi that did not develop reflex cardiac slowing in response to a brief rise in arterial pressure did so in response to other kinds of sensory stimuli, such as body movement, noise or odors. This was also true of hypertensive rabbits in which the incidence of negligible slowing was 75%, regardless of HRi. Absence of reflex slowing, therefore, was not due to a change in the efferent nerve pathways to the heart or in the heart itself but rather to a lack of effective activation of the afferent side or central connections of the sino-aortic reflex pathways.

References
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