Impaired Responses of Sympathetic Nerves to Cardiac Receptor Stimulation in Hypertension

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SUMMARY We recently reported that the vagal cardiopulmonary baroreceptor reflex inhibition of renal nerve traffic is impaired in rabbits with renal hypertension. The purpose of this study was to determine if the locus of the abnormality is mainly in the brain or in the afferent limb of the reflex. Experiments were done in α-chloralose-anesthetized rabbits with (n = 10) or without (n = 10) hypertension induced 6 to 8 weeks before study by wrapping the left kidney in cellophane followed by removal of the right kidney. The left side of the chest was opened, and a pericardial cradle was made. Nicotine was applied to the epicardial surface of the heart in concentrations of 10 to 500 μg/ml, and changes in arterial pressure and renal nerve traffic were measured. Dose-dependent decreases in traffic and arterial pressure resulted that were significantly smaller in hypertensive than in normotensive rabbits. After sinoaortic baroreceptor denervation, a similar impairment in the responses of hypertensive rabbits was observed. Vagotomy nearly abolished the responses of the renal nerves to epicardial nicotine. The responses of the lumbar sympathetic nerves to epicardial nicotine also were impaired in renal hypertensive (n = 8) compared with normotensive rabbits (n = 8). If the behavior and number of chemically sensitive endings are assumed to be unaltered in hypertension, then these findings are explained best by an abnormality in the central nervous system. These results support the view that the previously reported impairment in the vagal cardiopulmonary baroreceptor reflex control of renal nerve traffic is due mainly to a central abnormality, although they do not exclude an abnormality in the afferent limb of the reflex. (Hypertension 9: 478-484, 1987)

KEY WORDS * chemosensitive endings * sinoaortic denervation * vagal afferents * nicotine

My colleagues and I reported recently1 that the vagal cardiopulmonary baroreceptor reflex control of renal sympathetic nerve traffic during volume expansion is impaired in rabbits with renal hypertension. In those experiments, we did not determine the locus of the abnormality in reflex control (i.e., sensory receptors vs central nervous system). However, we did find that the degree of left ventricular hypertrophy was modest, and we suggested that the cause of the abnormality might well be in the central nervous system rather than in receptor behavior that could result from cardiac hypertrophy. Studies in spontaneously hypertensive rats2 and in prehypertensive Dahl salt-sensitive hypertensive rabbits3 indicate that abnormalities exist in the cardiopulmonary baroreceptor reflex control of renal nerve traffic that are due primarily to alterations in the behavior of the receptors themselves.

The purpose of the present study was to determine the locus of the abnormality in the cardiopulmonary baroreceptor reflex control of the renal nerves in renal hypertensive rabbits. The strategy of these experiments can be summarized as follows. A stimulus was selected that activated cardiac receptors by chemical rather than mechanical stimulation. The study was based on three assumptions: 1) chemical stimulation of chemosensitive (in contrast to cardiac mechanoreceptors) epicardial receptors would not be affected by cardiac hypertrophy; 2) the number of chemosensitive endings in the heart is not altered by hypertension or by the resulting mild hypertrophy found early in renal hypertension; 3) application of a chemical excitatory agent to the epicardial surface of the heart would result in similar degrees of cardiac receptor activation in hypertensive and normotensive rabbits. The results of these experiments are consistent with the view that there is a striking central neural abnormality in the vagal cardiopulmonary baroreceptor reflex control of...
renal nerve traffic. This finding is consistent with our prior observation of a similar central abnormality in the arterial baroreceptor reflex control of the renal nerves in renal hypertension. A similar impairment in responses to epicardial nicotine was observed in the lumbar nerves. This finding suggests that a general impairment in responses to cardiac receptor stimulation may be present in renal hypertension.

Materials and Methods

Production of Renal Hypertension

Eighteen New Zealand white male rabbits (2.4–3.5 kg; Blue and Gray Rabbity, Aylett, VA, USA) were rendered hypertensive by unilateral renal wrapping (cellophane) with contralateral nephrectomy under general anesthesia. Anesthesia was induced with sodium thiopental (25 mg/kg i.v.) followed by nitrous oxide administered with oxygen. Nephrectomy was done 3 to 7 days after renal wrapping.

Preparation for Short-term Experiments

The short-term experiments were performed 6 to 8 weeks after renal wrapping. The rabbits were anesthetized with intravenously administered (ear vein) sodium thiopental (25 mg/kg) followed by α-chloralose (50 mg/kg i.v.). Supplemental doses of chloralose (10 mg/kg) were administered hourly. After tracheal intubation the animals were ventilated artificially with a mixture of oxygen and room air. Decamethonium bromide (2 mg/kg) or pancuronium bromide (0.5 mg) was administered as needed to block muscle activity during periods of nerve recording. To ensure adequate general anesthesia, the effect of each dose of neuromuscular blocker was permitted to dissipate before the administration of a subsequent dose. A catheter was positioned in the aorta through the left femoral artery for measurement of arterial pressure. Mean arterial pressure was normalized with intravenously administered (ear vein) sodium thiopental (25 mg/kg i.v.) followed by nitrous oxide administered with oxygen. Arterial blood gases and pH were measured periodically and corrected when necessary by adjusting the respiratory frequency. Arterial oxygen tension was always above 200 mm Hg, and carbon dioxide tension and pH were maintained between 25 and 35 mm Hg and between 7.35 and 7.5, respectively. Body temperature was maintained between 37 and 39°C by external warming.

Denervation of Reflex Pathways

The aortic nerves were identified as they course near the vagus and join the superior laryngeal nerve. Fine silk ties were carefully looped around these nerves for subsequent section. The carotid sinus nerves were prepared for interruption by carefully passing silk ties around all the structures that course between the internal and external carotid arteries. Carotid baroreceptors were denervated by ligating and sectioning these structures. Interruption of aortic and carotid sinus nerves resulted in sinoaortic baroreceptor denervation. The completeness of arterial baroreceptor denervation was established in the studies in which lumbar nerve traffic was recorded by the demonstration that large phenylephrine-induced increases in arterial pressure caused no change in lumbar nerve traffic or heart rate. The completeness of baroreceptor denervation was not determined in the studies on the renal nerves. The vagi were prepared for subsequent denervation by carefully positioning a silk tie around each cervical vagus.

Recording and Quantitation of Nerve Traffic

The left renal sympathetic nerves were exposed using a flank incision and retroperitoneal approach. A branch of the nerves was sectioned and, after removal of the sheath from the cut central end, immersed in mineral oil and placed on bipolar Ag/AgCl electrodes for recording of action potentials. The technique for quantitation of nerve traffic has been described in detail previously. In brief, the recorded spikes were amplified using a Grass P511 preamplifier (Quincy, MA, USA) and the amplified nerve traffic was visualized on a Techronic oscilloscope (Beaverton, OR, USA). The output also was led to a nerve traffic analyzer that measured the frequency of spikes that exceeded a selected voltage (just above the noise). Each spike that crossed the threshold generated a voltage step that was independent of spike amplitude. These normalized voltage steps were integrated to determine the number of spikes counted per unit of time. This counting technique is different from integration of the raw voltage signal commonly referred to as integration. The counter is digital in design and counts linearly at instantaneous frequencies up to 10 KHz. The absolute value of the recorded traffic is dependent on the number of active fibers on the recording electrodes and on the level of the window discriminator and thus may have limited meaning. This was particularly true since our nerve recordings were obtained from bundles of fibers of varying size obtained from the renal nerves. Thus, all responses of renal nerve traffic were normalized for their basal values.

In an additional group of 16 experiments, recordings were made from the lumbar sympathetic nerves. The nerves were approached retroperitoneally, and the techniques used for recording and quantitation of traffic were the same as for the renal nerves.

Protocol

Experiments were performed in 10 hypertensive and 10 normotensive control rabbits. Responses of blood pressure and renal nerve traffic to superfusion of the epicardial surface of the heart with saline containing nicotine was determined with all baroreceptor reflexes intact, after sinoaortic denervation, and after vago-
The concentrations of nicotine ranged from 10 to 500 μg/ml. The order of administration of the different concentrations was randomized. The nicotine was washed from the pericardial surface of the heart with normal saline. Nicotine was administered by positioning a catheter underneath the heart but inside the pericardium and by injecting 0.5 ml of solution. This small volume of administration covered the entire heart but was small enough to avoid the passage of nicotine solution to regions outside the pericardial sac. Care was also taken during the washout period to minimize the overflow of nicotine into the thoracic cavity. Responses of arterial pressure and renal sympathetic traffic to epicardial administration of nicotine were recorded with each state of innervation.

Experiments also were performed in eight hypertensive and eight normotensive control rabbits in which lumbar rather than renal nerve traffic was recorded. Epicardial nicotine was applied in concentrations of 10 to 1000 μg/ml. The experiments were done only after sinoaortic baroreceptor denervation.

All experiments were conducted in conformity with the guiding principles of the American Physiological Society for research involving animals.

Data Analysis
Values for control were averaged over 30 to 60 seconds before nicotine administration. The changes in arterial pressure following the administration of nicotine were recorded for each dose administered. The responses of nerve traffic were determined for the first 10 seconds following the administration of each dose of nicotine. The relationship between the dose of nicotine and the increases in renal or lumbar nerve traffic and arterial pressure was determined with baroreceptor reflexes intact (renal) and following sinoaortic baroreceptor denervation (renal and lumbar) by a multivariate analysis of variance as modified by Kleinbaum. Following sinoaortic denervation and vagotomy, only the highest dose of nicotine administered previously was readministered and a comparison of the responses to this dose following sinoaortic denervation alone and with the addition of vagotomy was made using a paired t test. Postvagotomy studies were not done for the lumbar nerves. Probability levels less than 0.05 were considered significant. Results are presented in the text and figures as means ± SE.

Results
Experiments were done in 10 rabbits with renal hypertension 6 to 8 weeks after renal wrapping and in 10 age-matched controls. The basal mean arterial pressures recorded in the conscious hypertensive rabbit (central ear artery) were significantly higher (126 ± 5 mm Hg) than those in the normotensive rabbits (98 ± 6 mm Hg). At the beginning of the protocol, following anesthesia and thoracotomy, the respective pressures for these groups were 99 ± 7 and 77 ± 5 mm Hg (p < 0.05). The renal nerve traffic recorded at the start of the protocol was 64 ± 10 impulses/sec in the hypertensive group and 108 ± 23 impulses/sec in the normotensive group. These values were not significantly different. At the conclusion of the experiments, the hearts were removed and the left ventricles were weighed. Left ventricle to body weight ratio was significantly higher in the hypertensive (1.3 ± 0.1 g/kg) than in the normotensive (1.1 ± 0.1 g/kg) rabbits, although the difference was modest.

Figure 1 illustrates the response of a normotensive rabbit to the epicardial application of 0.5 ml of normal saline containing 100 μg/ml of nicotine. Note the striking fall in arterial pressure and inhibition of renal sympathetic traffic with a latency of just over 1 second. In contrast to this striking response observed in the normotensive rabbit, Figure 2 illustrates the response of a renal hypertensive rabbit to the same dose of nicotine applied to the epicardial surface of the heart. This is a particularly abnormal response but indicates that renal sympathetic traffic was modestly inhibited in this hypertensive rabbit. Figure 3 illustrates the mean responses of mean arterial pressure and renal nerve activity as a function of the concentration of nicotine applied to the epicardial surface of the heart in normotensive and hypertensive rabbits with arterial baroreceptor reflexes intact. Note the dose-dependent inhibition of renal nerve traffic in both groups, which was significantly greater in the normotensive than in the hypertensive rabbits. The absolute responses of arterial pressure in the two groups were not significantly different; however, the decreases in arterial pressure expressed as a percentage of control were significantly smaller in the hypertensive group. Thirty minutes after sinoaortic denervation, the mean arterial pressures were greater in the hypertensive (105 ± 11 mm Hg) than in the normotensive rabbits (69 ± 4 mm Hg) but renal nerve activities were not different (87 ± 15 vs 116 ± 36 impulses/sec).

FIGURE 1. Responses of integrated renal nerve activity, renal electroneurogram, and arterial pressure with renal nerve time-frequency histogram to epicardial application of normal saline containing nicotine, 100 μg/ml, in a normotensive rabbit. Note striking fall in pressure and renal nerve activity.
Integrated Renal Nerve Activity (impulses)

Renal Electroneurogram

Arterial Pressure (mmHg)

Nicoine (100yg/ml)

Figure 2. Responses of a renal hypertensive rabbit to epicardial application of normal saline containing nicotine, 100 μg/ml. The format of the figure is the same as in Figure 1 and should be contrasted with that figure.

Figure 3. Responses (mean ± SE) of renal nerve activity (A) and mean arterial pressure (B) to epicardial application of nicotine in normotensive and hypertensive rabbits with baroreceptor reflexes intact. The changes of renal nerve traffic and mean arterial pressure appear on the ordinate. The responses of renal nerve traffic but not arterial pressure were significantly smaller in the hypertensive rabbits.

Following sinoaortic baroreceptor denervation, epicardial administration of nicotine also resulted in concentration-dependent inhibition of renal nerve traffic and arterial pressure (Figure 4). Note the striking impairment in the hypertensive group compared with the normotensive group. As occurred with the baroreceptor reflexes intact, there also were decreases in arterial pressure that, again, were not significantly different in the two groups.

The effect of vagotomy on the responses to epicardial application of nicotine are illustrated in Figure 5. Following sinoaortic denervation but with vagi intact, epicardial application of nicotine solution, 250 or 500 μg/ml, resulted in large decreases in arterial pressure and renal sympathetic nerve activity. The changes in renal nerve activity were significantly smaller in the hypertensive than in the normotensive group. After vagotomy, the inhibition of renal nerve traffic by epicardial nicotine was markedly reduced in both groups. There were, however, significant decreases in renal nerve traffic even after vagotomy. The decreases in arterial pressure following nicotine were significantly reduced in the normotensive but not in the hypertensive group. The relative preservation of the arterial pressure responses may have been due in part to the fact that postvagotomy epicardial application of nicotine resulted in decreases in heart rate. In the normotensive group heart rate decreased by 50 ± 12 beats/min before and 26 ± 6 beats/min after vagotomy. In the hypertensive group heart rate decreased by 13 ± 4 beats/min before and 10 ± 3 beats/min after vagotomy.

The responses of lumbar nerve traffic and arterial
The major finding of this study is that chemical stimulation with nicotine of chemosensitive sensory endings on the epicardial surface of the heart results in dose-dependent inhibition of renal nerve traffic that is strikingly impaired in rabbits with renal hypertension. If one accepts the assumptions that the behavior of chemosensitive, in contrast to mechanosensitive, endings is not altered in hypertension and that the number of such receptors also is not altered in hypertension, then it is reasonable to conclude from these data that the abnormality in the cardiopulmonary baroreceptor reflex control of renal sympathetic traffic previously reported is most likely due to an abnormality in the central nervous system. As we have reported previously, the impairment in the cardiopulmonary baroreceptor reflex was striking in rabbits with renal hypertension and was evident with an approximately 20% increase in left ventricular to body weight ratio, indicating very modest hypertrophy. This finding suggested the possibility that the central nervous system was the locus for the abnormality in the vagal cardiopulmonary baroreceptor reflex, which we have previously observed in this model of hypertension. The results in the renal nerves reported here are consistent with this view and were obtained in rabbits with very modest cardiac hypertrophy.

The present findings also are consistent with our prior observation that the arterial baroreceptor reflex control of renal nerve traffic is abnormal in renal hypertension and that the abnormality is central in origin. In those experiments we found that electrical stimulation of one aortic depressor nerve (all buffer nerves sectioned) resulted in significantly less inhibition of renal sympathetic traffic in hypertensive than in normotensive rabbits. This was observed principally during activation of medullated baroreceptor afferent fibers. Unfortunately, it is not appropriate to stimulate the afferent vagal nerves electrically (as we have done previously for the aortic depressor nerve) because they contain afferent fibers from the lungs and gut as well as from the heart. Thus, it would be impossible to interpret the responses to afferent electrical stimulation of the vagal nerves in terms of localizing the abnormality in the cardiopulmonary baroreceptor reflex. I believe that the responses to epicardial nicotine are the closest that we can come to providing a controlled stimulus to vagal cardiac afferent fibers that will permit us to differentiate between abnormalities in the afferent limb of the reflex and those that are due to impaired central neural mechanisms.

In the present study, nicotine was used only to stimulate cardiac receptors. Thus, the abnormalities observed are related to reflexes for which the afferent limb originates in the heart. Our earlier studies and those of others using volume expansion stimulated receptors throughout the cardiopulmonary region. This made it difficult to separate the independent influence of receptors in the heart from those in the lung or
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pulmonary circulation. To my knowledge, this is the first study to demonstrate impaired reflex control by cardiac (as opposed to cardiopulmonary) receptors in renal hypertension.

Although these data suggest a central abnormality in the cardiopulmonary baroreceptor reflex control of sympathetic traffic in renal hypertension, the results of studies in other experimental models suggest abnormalities elsewhere in the reflex arc. Ricksten and Thoren2 have demonstrated alterations in the cardiopulmonary baroreceptor reflex in spontaneously hypertensive rabbits. They found a resetting of cardiac C-fibers to a higher pressure threshold.2 The threshold of the reflex also was increased, but the sensitivity of the reflex was not reduced in the hypertensive group.2 This effect appeared to be due to a decreased peripheral venous distensibility that resulted in augmented rather than impaired inhibition of sympathetic nerve traffic for a given amount of volume administered.8,9

Ferrari et al.3 have reported that the cardiopulmonary baroreceptor reflex is impaired in Dahl salt-sensitive rats in the prehypertensive phase when they are fed a low salt diet. They found that for a given increase in cardiac filling pressure there was less inhibition of sympathetic nerve traffic in Dahl salt-sensitive than in Dahl salt-resistant rats. They attributed this defect to abnormalities in the receptors or their coupling elements in the cardiac tissues since it was present in the prehypertensive phase and in the absence of cardiac hypertrophy or reduced atrial distensibility. It is not possible to exclude the presence of a similar defect in the chemosensitive endings of renal hypertensive rabbits.

In the present study, the responses of the lumbar nerves to epicardial nicotine also were markedly impaired in renal hypertension. Thus, the impairment in the control of sympathetic outflow by cardiac receptors is not restricted to the renal nerves. The severity of the hypertension and cardiac hypertrophy was much greater in the rabbits from which lumbar nerve traffic was recorded than was observed in the studies on the renal nerves. The major difference between these two hypertensive groups is the later timing of renal wrapping in the more hypertensive group (7 vs 3 days). I would like to emphasize that markedly abnormal responses to nicotine occurred whether the extent of cardiac hypertrophy was mild (renal nerve experiments) or severe (lumbar nerve experiments). On the basis of this finding, it seems unlikely that the observed abnormal responses were due only to the extent of hypertrophy.

Findings of impaired cardiopulmonary baroreceptor reflexes in experimental models of hypertension stand in contrast with the results of studies by Mark and Kerber,10 who found that the cardiopulmonary baroreceptor reflex control of forearm vascular resistance actually was augmented in young men with borderline hypertension. Preliminary observations from our laboratory indicate that this augmentation of the cardiopulmonary baroreceptor reflex observed in borderline hypertension is lost in the established phase of hypertension.11 This loss may be due to the associated cardiogenic reflexes in renal hypertension. The author thanks David Brands and Steve Yuhi for technical assistance and Ms. Shirley McCray for typing the manuscript.

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