Continuous Inhibition of Renin Release in Dogs by Vagally Innervated Receptors in the Cardiopulmonary Region

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ABSTRACT

Inhibition of the release of renin by vagal afferents from the heart and lungs was studied in 14 dogs with their aortic nerves cut and their carotid sinuses vascularly isolated. The release of renin from one kidney was calculated from the venous-arterial difference in plasma renin activity (radioimmunoassay) and the renal blood flow (electromagnetic flowmeter). Renin release was determined before and during temporary interruption of afferent vagal nerve traffic (bilateral cooling of the cervical vagi). With carotid sinus pressure maintained at 40 mm Hg, vagal cooling increased mean aortic blood pressure (24%), decreased renal blood flow (19%), and increased renin release (241%). With sinus pressure maintained at the mean aortic blood pressure existing during the control period, vagal cooling caused a lesser increase in mean aortic blood pressure (12%), little decrease in renal blood flow (7%), and a marked increase in renin release (522%). The changes in renal blood flow and renin release with vagal cooling were prevented by renal denervation. Thus, vagal afferents from the cardiopulmonary region exert a tonic restraint on the release of renin; this restraint occurs in circumstances in which these afferents cause little change in total renal blood flow.

KEY WORDS renin secretion heart and lung receptors renal circulation vagal block vagal afferents carotid baroreceptors radioimmunoassay for angiotensin I renal sympathetic activity

Methods

These experiments were conducted on 14 dogs (13-26 kg) anesthetized with sodium thiopental (15 mg/kg, iv) and chloralose (80 mg/kg initially and 10 mg/kg hourly, iv), paralyzed with gallamine triethiodide (3 mg/kg hourly, iv), and artificially ventilated with oxygen (12-14 cycles/min, peak inspiratory pressure 10-12 cm H2O). Arterial Po2, Pco2, and pH were measured periodically; Po2 was always greater than 400 mm Hg, Pco2 was between 28 and 42 mm Hg, and pH was between 7.30 and 7.40. All of the dogs were given atropine (0.2 mg hourly, iv) to ensure that the effects of cooling the cervical vagi were not partially due to interruption of efferent vagal nerve traffic.

Carotid and Aortic Baroreceptors and Chemoreceptors.—The carotid sinuses were either denervated by stripping the walls of all of the arteries at the carotid bifurcations, including the common carotid arteries, or vascularly isolated according to the Moisseyjeff technique (11). In the latter case, the sinuses were perfused with oxygenated Krebs-Ringer's-bicarbonate solution and maintained at the desired nonpulsatile...
pressure. The aortic nerve on each side was identified at the junction of the vagus with the laryngeal nerve, traced caudally to its junction with the vagosympathetic trunk, and cut. Several studies have demonstrated that this procedure results in acute loss of the baroreflex and the chemoreflex from the aortic arch and the baroreflex from the major intrathoracic arteries (10, 12).

**Vagal Cold Block.**—The afferent traffic in the cervical vagi was blocked by temporarily cooling the nerves to between 0° and -1°C. This temperature, which was measured at the surface of the nerves by a small thermistor, appeared to block nerve conduction effectively, because, during cooling, no further circulatory effects were observed by vagal section. It was assumed that the circulatory and humoral effects of the vagal block reflected the reflex influence of the vagal afferents prior to the block.

**Hemodynamic Measurements.**—Systemic arterial blood pressure was measured with a strain-gauge transducer via a catheter placed in the abdominal aorta from the left femoral artery. Mean blood pressure was obtained by electronic damping of the pulsatile signal. The left kidney was exposed through a lateral subcostal incision, and renal blood flow was measured with a noncannulating electromagnetic flow probe (10-16 mm in circumference) placed at the origin of the left renal artery. To avoid interruption of renal circulation, the zero-flow signal was determined at the beginning of the experiment with the flow probe placed around a common carotid artery and was checked again at the end of the experiment with the flow probe around the renal artery; there was no difference between the two signals. At the end of the experiment, the flow probe was placed around a common carotid artery and calibrated by delivering known quantities of blood to the artery via a roller pump.

**Renin Measurements.**—Blood samples (4 ml) were withdrawn from the left renal vein through a catheter inserted via the gonadic vein and from the aorta through a catheter inserted via the right femoral artery. This blood loss was replaced by an equal amount of isotonic saline buffered to pH 7.4. The blood samples were collected in cooled heparinized tubes (5-ml Vacutainers), and the tubes were centrifuged at 4°C. After centrifugation, the plasma was separated, and ethylenediaminetetraacetic acid (EDTA) was added to give a final concentration of approximately 190 mg/10 ml. The plasma renin activity was estimated by radioimmunoassay according to the method of Haber et al. (13).

Three determinations were made on each sample, and the mean of these readings was expressed in nanograms of angiotensin I generated in 1 ml of plasma per hour. In 36 replicate determinations on a plasma pool of samples from six dogs, the mean plasma renin activity was 13.0 ng/ml hour⁻¹ with a coefficient of variation of 10%. Because the magnitude of the increases in venous plasma renin activity during vagal cold block frequently was many times that in the arterial samples, excessive substrate consumption could have limited the rate of formation of angiotensin I; if so, the calculated venous plasma renin activity would have been less than the actual concentration of renin. To study this possibility, the formation of angiotensin I was measured after 20, 40, and 60 minutes of incubation in three pairs of arterial and renal venous samples (selected so that each venous sample had a high plasma renin activity). The rate of formation of angiotensin I per unit of time normally was constant up to 60 minutes of incubation for both the arterial and the venous samples (Fig. 1).

**Protocol and Data Analysis.**—Vagal cold block was initially performed 30 minutes after the completion of the surgical preparation and was repeated thereafter at intervals of not less than 12 minutes. The blood samples were withdrawn immediately before the block and at various times during the block. The duration of the withdrawal was usually less than 30 seconds. In each dog, two cold blocks were usually performed for each experimental condition (different carotid sinus pressures and renal denervation), and the responses were averaged. The amount of renin released was calculated as the venous-arterial difference in plasma renin activity multiplied by renal blood flow. When renal blood flow was not constant, the mean value during the time of withdrawal of the sample was taken. The data from each dog were summed to obtain the mean ± se. The statistical significance of the difference in the means was evaluated using Student’s t-test for paired observations.

**Results**

**Carotid Sinuses Denervated.**—In a previous study (10), it has been shown that the reflex circulatory control exerted by the vagal afferents from the cardiopulmonary region is most evident in the absence of any influence from the carotid baroreceptors. Therefore, in the first six dogs the carotid sinuses were denervated. Vagal cold block was maintained for 5 minutes, and samples were taken from the renal vein immediately before the block and during the second and the fifth minute of the block. It was thought that during this period the plasma renin activity in the renal artery would show little change so that the changes in plasma renin activity in the renal vein (corrected by the changes in renal blood flow) would indicate the changes in the amount of renin released.

![Aortic Samples vs Renal Venous Samples](attachment://image.png)  
**FIGURE 1**

Formation of angiotensin I at different incubation times in samples from the aorta (left) and the renal vein (right). At both low and high levels of angiotensin formation, the rate of formation was usually linear up to 60 minutes.  

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In each dog, the vagal cold block caused a significant increase in mean aortic blood pressure, no significant change in pulse pressure, and a significant decrease in renal blood flow. These changes were accompanied by a large increase in both plasma renin activity in the renal vein and renin output (Figs. 2 and 3). The average increases in aortic blood pressure were not different between the second and fifth minutes; however, the decrement in renal blood flow was significantly ($P < 0.05$) greater and the increase in renin output was significantly ($P < 0.01$) less at the fifth minute.

Carotid Sinuses Vascularly Isolated.—In eight dogs, the carotid sinuses were vascularly isolated, and the intrasinus pressure was adjusted to the level of the existing mean aortic blood pressure and kept constant. Vagal cold block was maintained for 3 minutes, and the blood samples were taken simultaneously from the left renal vein and the aorta immediately before and during the third minute of the block. Vagal block caused small increases in mean aortic blood pressure and pulse pressure and a small decrease in renal blood flow; all of these changes were statistically significant. During the block, plasma renin activity in the renal artery increased slightly, and in seven of the eight dogs plasma renin activity in the renal vein and renin release increased markedly (Fig. 4).

In six of these dogs, the effects of vagal cold block when carotid sinus pressure was maintained at the level of the existing mean aortic blood pressure were compared with those when the sinus pressure was maintained at 40 mm Hg. At 40 mm Hg, the carotid baroreceptors are not active (14); therefore, this condition is equivalent to their denervation. Vagal cold block was performed at least 10 minutes after the sinus pressure had been decreased from the level of the existing aortic blood pressure to 40 mm Hg. Samples were taken from the aorta and
Effect of bilateral cervical vagal cold block on aortic blood pressure, aortic pulse pressure, left renal blood flow, plasma renin activity in the aorta and the left renal vein, and renin release (venous-arterial difference in plasma renin activity multiplied by renal blood flow) in eight dogs with their aortic nerves cut and their carotid sinuses vascularly isolated and maintained at a pressure equal to the mean aortic blood pressure during the control period. Arterial and venous samples for measurements of plasma renin activity were taken before the block and during the third minute of the block.

Renal Denervation.—The effect of renal denervation was tested in six dogs with their carotid sinuses either denervated (three dogs) or vascularly isolated and maintained at 40 mm Hg (three dogs). To achieve renal denervation, the kidney was separated from the surrounding tissues, and the renal artery was stripped and painted with 5% phenol solution. After this procedure, vagal cold block caused an increase in aortic blood pressure similar to that found prior to renal denervation, but there was no decrease in renal blood flow and no increase in plasma renin activity in the renal vein (Fig. 6).

Discussion

The present experiments showed that blocking vagal traffic from the cardiopulmonary region caused a marked increase in renin release and that this effect was abolished after renal denervation. Thus, the vagally innervated receptors in the cardiopulmonary region exert a tonic reflex inhibition of renin release by decreasing the sympathetic nerve activity to the kidney.

This conclusion is based on the fact that section of the cervical aortic nerves coupled with denervation or vascular isolation of the carotid sinuses eliminated mechanoreceptors other than those in the cardiopulmonary area as a source of afferent inhibition of the vasomotor center. Studies from this and other laboratories have shown that section of the aortic nerves in the neck abolishes both the reflex hypotension produced by distention of the vascularly isolated aortic arch, brachiocephalic trunk, and right subclavian artery (10, 12) and the hypertensive response to injection of cyanide into the aortic root (10, 12, 15-17). Infrequently, no aortic nerve can be identified (12). When this phenomenon occurs, the experiment has to be abandoned, because the nerve probably lies within the vagal trunk.

In acute studies, Ito and Scher (18) recorded a decrease in aortic blood pressure in response to stimulation of the central end of the sectioned “peripheral aortic nerves” after section of the cervical aortic nerves in one of three dogs and one

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of five cats; the hypotension was 10 mm Hg or less. Because of this finding, they have suggested that section of the aortic nerves in the dog may not abrogate baroreceptor and chemoreceptor reflexes from the aortic arch. However, it should be appreciated that two of the nerves that compose the peripheral aortic nerve—the dorsal and ventromedial cervical cardiac nerves—supply filaments not only to the aortic arch but also to the pretracheal plexus, a primary area of nerve distribution for the heart (19). Öberg and Thörnen (20) have recorded a reflex decrease in blood pressure caused by electrical stimulation of nonmyelinated afferent fibers in the cardiac nerve in the cat. These fibers normally are silent or have a sparse irregular discharge and are not easy to identify by standard electrophysiological techniques (21). Kulaev (22) has shown, in cats and rabbits, that afferent impulses traveling along the nonmyelinated fibers of the vagus and aortic depressor nerves from cardiac receptors to the central nervous system are capable of exerting profound and varied effects on arterial blood pressure. Thus, the evidence to date favors the contention that, after identification and section of both aortic nerves, there is an acute loss of aortic baroreceptor and chemoreceptor reflexes.

The role of the arterial baroreceptors in the control of renin release is debatable. There is no increase in the rate of renin secretion after bilateral carotid occlusion in dogs with their vagi intact or after bilateral vagotomy (23, 24). In the latter circumstance, if the aorta is constricted to decrease renal perfusion pressure and increase the rate of renin secretion, the carotid occlusion decreases the rate of renin secretion. In another study, after the vagosympathetic trunks were cut, both common carotid arteries were occluded for 20–25 minutes; the authors (7) reported that when the abdominal aorta was partially constricted during occlusion of both common carotid arteries, in an effort to maintain a constant renal perfusion pressure, there was a release of renin, but no data were given. By contrast, Hodge et al. (6) have found that, with bilateral carotid occlusion which increases the systemic arterial blood pressure by 10–120 mm Hg, there is no change in the concentration of circulating angiotensin if the occlusion is maintained for 5 minutes or less. However, longer periods of occlusion (7–26 minutes) did cause an increase in concentration in 10 of 17 animals despite the increase in renal arterial blood pressure. This increase is the result of an increase in the rate of generation of angiotensin, presumably as a consequence of an increase in the rate of renin secretion. Recently, Brennan et al. (25) have correlated changes in carotid sinus pressure with changes in plasma renin activity; neither increasing nor decreasing carotid sinus pressure has any demonstrable effect on the arterial plasma renin activity in vagotomized dogs or dogs with their vagi intact.

In the present study, when the vagi were intact a decrease in carotid sinus pressure caused the expected increase in aortic blood pressure but did not result in an increase in the output of renin. By comparison, when the carotid baroreceptors were inactive, the increase in aortic blood pressure caused by vagal cold block was greater during the second minute than it was during the fifth minute of block. This difference probably was not due to decreased sympathetic activity, because the decrease in renal blood flow and the increase in aortic blood pressure were well maintained throughout the block. It may be that the prolonged vagal block caused an increase in the arterial concentration of angiotensin II which in turn partially inhibited the release of
renin (26, 27). It is also possible that the sympathetic nerves have their major effect on the discharge of preformed renin. Thus, the transient effect is greater than the prolonged one.

In a previous study on dogs (10), we have shown that the vasomotor inhibition exerted tonically by the vagally innervated cardiopulmonary receptors decreases as the inhibition from the carotid baroreceptors is increased. Similarly, in a study on cats, Öberg and White (9) have shown that the vasomotor inhibition exerted tonically by the vagal afferents is greater during common carotid occlusion than it is at normal carotid sinus pressures. In the present study, the vagal block caused an increase in arterial blood pressure and a decrease in renal blood flow when the input from the carotid baroreceptors was absent (dogs with sinus denervation or with sinus pressure below the threshold of the baroreceptors). These effects of the block were decreased when the carotid baroreceptor input was increased (pressure maintained at the level of the existing arterial blood pressure). However, in both conditions, the vagal cold block caused a similar marked increase in renin release. Thus, when the input from the carotid baroreceptors is fixed at about its normal value, the cardiopulmonary receptors seem to exert a relatively greater control of the renin secretion than of the circulation.

It has not been established if the sympathetic nerves affect renin release directly through their innervation of the juxtaglomerular apparatus (28, 29) or indirectly through changes in renal hemodynamics. Coote et al. (30) have reported that, in the cat, electrical stimulation of the renal nerves does not cause renin release if the simultaneous decrease in renal blood flow is prevented by an α-adrenergic blocking agent. On the other hand, Johnson et al. (31) and La Grange et al. (32) have described neurally mediated increases in renin release in the absence of a decrease in renal blood flow in the dog. In our experiments with the carotid sinus pressure maintained at the level of the existing arterial blood pressure, vagal cold block caused little decrease in renal blood flow and a marked increase in renin release. Thus, when the neurally mediated release of renin is not necessarily correlated with a decrease in renal blood flow. This finding is in accord with the evidence that the neurally mediated release of renin is not necessarily correlated with a decrease in renal blood flow. This lack of correlation does not necessarily imply that the renal nerves have a direct effect on the juxtaglomerular cells; activation of the sympathetic nerves to the kidney may increase the release of renin through intrarenal hemodynamic changes without a change in total renal blood flow. For example, it is known that sympathetic stimulation causes a redistribution of renal blood flow between the cortical and subcortical regions before the total flow decreases (33).

Thames et al. (34) have found that, after cardiac denervation by surgical division and reanastomosis caudal to the venoatrial junctions in the dog, the increase in renin release in response to hemorrhage is decreased. Because this technique leaves most of the atrial receptors innervated and the ventricular receptors denervated, these authors have suggested that ventricular receptors are implicated in the control of renin release. Brennan et al. (35) have shown that, in the dog, distention of the right atrium causes a decrease in renin secretion, suggesting that atrial receptors are involved.

Our data do not permit localization of the receptors responsible for the tonic control of renin release. Although many of these receptors may be located in the heart, studies (36) on rabbits have shown that the pulmonary receptors have a marked inhibitory influence on renal sympathetic nerve activity and renal circulation. Thus, the possibility exists that both cardiac and pulmonary receptors contribute to the tonic restraint of renin production.

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