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Role of Renal Nerves in Mediating the Increased Renin Secretion during Continuous Positive-Pressure Ventilation[1] (41616)

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Abstract. The effect of renal denervation on the increase in plasma renin levels during continuous positive-pressure ventilation (CPPV) was studied in anesthetized dogs. Renin secretion was measured in a group of dogs with intact renal nerves and a group which had previously undergone bilateral renal denervation. Renal perfusion pressure was maintained constant throughout the experiment. Renin secretion increased significantly during application of CPPV with a positive and expiratory pressure of 10 cm water in the dogs of the intact group but was not altered in dogs of the denervated group. These results support the conclusion that CPPV increases renin secretion by reflexly altering renal nerve activity.

Increasing end expiratory pressure during constant volume ventilation frequently produces reflex alterations in renal function including an antidiuresis and antinatriuresis (1–3). We have published evidence indicating that these alterations in renal function are mediated, primarily, by renal nerves (4, 5). Plasma renin levels may also increase with continuous positive-pressure ventilation (CPPV) (6, 7) but the increase does not occur under all experimental conditions (8). The mechanism by which renin levels increase during CPPV has not been delineated. Available evidence indicates that stimuli which produce hemodynamic changes similar to those observed during CPPV increase plasma renin levels by initiating reflex changes in renal nerve activity (9, 10). It is possible, therefore, that the renal nerves may also be involved in the reflex elevation of plasma renin levels associated with CPPV. In this paper we present the results of a series of experiments designed to examine the role of renal nerves in mediating changes in renin secretion during CPPV.

Methods. Mongrel male dogs weighing an average of 17.5 ± 0.6 kg were divided into two groups of six dogs each; an intact group and a renal denervated group. Bilateral renal denervation was performed under aseptic conditions 8–10 days prior to an experiment. The dogs were anesthetized for the denervation with Thiamylal (18 mg/kg, iv). Anesthesia was maintained by allowing the animals to breathe a gaseous mixture of 59% nitrous oxide, 40% oxygen, and 1.5–2.0% methoxyflurane. Both kidneys were exposed retroperitoneally through separate flank incisions. The renal arteries and renal veins were carefully stripped of all extravascular tissue from their origins to the renal hilum. All visible nerves were sectioned and the arteries and veins were painted with 8% phenol. The incisions were closed and the animals allowed to recover. Prophylactic treatment with a penicillin–streptomyocin combination antibiotic was given for 4 days postoperatively.

For the experiment, dogs from both the intact group and the renal denervated group were subjected to the same protocol. The dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) with additional doses of approximately 1 mg/kg being given every 15 min to maintain anesthesia. Each dog was placed in the supine position and intubated with a cuffed endotracheal tube. The cuff was inflated to a gas-tight fit and the tube was connected to a volume ventilator delivering a tidal volume of approximately 15 ml/kg at a frequency of 12/min. The tidal volume was adjusted at the beginning of the experiment to achieve normal blood values of pH, PCO_2, and PO_2. A rigid catheter was placed in line with the endotracheal tube for continuous monitoring of proximal airway pressure. A femoral vein was cannulated for fluid infusion. Catheters were positioned via the femoral arteries in the abdominal aorta, imme-

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diately distal to the origin of the left renal artery for measuring renal perfusion pressure and in the thoracic aorta for measuring arterial pressure and pulse pressure.

Through a left flank incision an inflatable silastic cuff was positioned extravascularly around the abdominal aorta approximately 4 cm proximal to the origin of the renal arteries. The cuff was inflated momentarily and arterial pressures monitored to confirm the locations of the arterial catheters. During inflation a rise in pressure indicated that the tip of the catheter for measuring arterial pressure was proximal to the cuff and a fall in pressure indicated the tip of the catheter for measuring renal perfusion pressure was distal. A catheter for withdrawing renal vein renin samples was inserted into the spermatic vein with its tip positioned in the left renal vein. An electromagnetic-flow probe was positioned around the renal artery. Zero blood flow was determined after positioning the probe and at the completion of the experiment by occluding the renal artery. During the period of stabilization and the application of CPPV, zero flow was determined using the electronic zero capability of the Biotronex sine wave flowmeter (Model BL-613).

After completion of all surgical procedures the aortic cuff was inflated to produce a renal perfusion pressure of 90–95 mm Hg. This pressure was maintained throughout the experiment by adjusting the degree of cuff inflation. A 1-hr period between cuff inflation and the collection of the first blood sample was allowed. Blood samples (1.0 ml) for determining blood gases and hematocrit and 4-ml samples for measuring arterial and renal vein plasma renin activities were drawn 2 min before application of CPPV, at 5 and 15 min of CPPV, and 30 min after CPPV was removed. CPPV was discontinued immediately after withdrawing the 15-min samples. CPPV using 10 cm water positive end expiratory pressure was accomplished by the partial static inflation of an occlusive balloon manifold incorporated in the expiratory limb of the ventilator system. Blood samples for renin determinations were collected in chilled vacutainer tubes containing EDTA as an anticoagulant. The samples were centrifuged under refrigeration and the plasma separated and stored at −80°C until assayed. Arterial pressures, measured with P23 Db pressure transducers, and left renal blood flow were recorded on an Electronics for Medicine DR-8 recorder for 1 min prior to withdrawing the blood samples. An output signal from each recording channel was averaged over the recording period on an A/D data signal averager to determine mean arterial pressure, mean renal perfusion pressure, and mean renal blood flow. Pulse pressure and heart rate were determined from the arterial pressure recordings. CPPV was applied 14 times in the six dogs of the intact group and 14 times in the dogs of the denervated group. No more than three applications were performed in each dog.

Plasma renin activity was determined using the angiotensin I ^{125}I radioimmunoassay kit manufactured by New England Nuclear. All samples from one experiment were assayed in duplicate in the same assay. Two assays were conducted on each sample. In our laboratory the coefficient of variation of renin activity measurements on three separate pools of dog plasma averaged 10.2% ($n = 8$), 9.0% ($n = 6$), and 6.5% ($n = 17$). Renin secretion rate in nanograms/minute was calculated by multiplying the difference between the renal vein renin activity and arterial renin activity times the renal plasma flow. Renal plasma flow was calculated from the renal blood flow and hematocrit. Within-group comparisons were performed using an analysis of variance for repeated measures. Significant differences from control levels ($P < 0.05$) were determined using the Dunnett procedure.

Results. Application of CPPV with a positive end expiratory pressure of 10 cm water produced similar hemodynamic changes in dogs from both the intact and denervated groups (Fig. 1). Pressure in the thoracic aorta (MAP) was significantly reduced at 15 min of CPPV in both groups and also at the 30-min recovery period in the intact dogs. We were able to prevent, however, any significant change in renal perfusion pressure by using the inflatable cuff. Pulse pressure and heart rate in dogs of both groups were significantly changed at 5 and 15 min of CPPV; returning to control levels during the recovery period.

Renal blood flow to the left kidney during the control period averaged 2.26 ± 0.12 ml/min/g of kidney (mean ± SE) in the intact group and 2.03 ± 0.46 ml/min/g in the de-

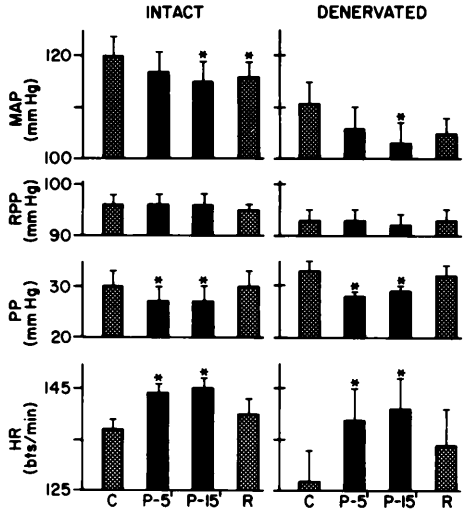

FIG. 1. Hemodynamic changes during the application of CPPV. Values represent mean ± SE for 14 applications of CPPV in six intact dogs and 14 applications in renal denervated dogs. MAP = mean arterial pressure; RPP = renal perfusion pressure; PP = arterial pulse pressure; HR = heart rate; C = control value obtained immediately before application of CPPV; P-5′ and P-15′ = values obtained at 5 and 15 min of CPPV; R = value 30 min after removing CPPV. Asterisk indicates value is significantly different ($P < 0.05$) from control.

nervated group. Blood flow remained unchanged during CPPV in the intact group but in the dogs of the denervated group flow was significantly reduced at 15 min of CPPV and at recovery to 1.91 ± 0.45 and 1.81 ± 0.41 ml/min/g, respectively. Vascular resistance in the left kidney followed a pattern similar to that of blood flow. Resistance averaged 0.988 ± 0.073 mm Hg/ml/min/g during the control period and remained unchanged during the periods of CPPV and recovery in dogs of the intact group. In the denervated group, however, renal resistance which averaged 1.206 ± 0.158 mm Hg/ml/min/g in the control period gradually increased during CPPV and became statistically significant at 1.379 ± 0.195 mm Hg/ml/min/g during the recovery period. Blood gas values were similar in the two groups. Control pH values were 7.34 ± 0.01 and 7.36 ± 0.03 in the intact group and denervated group, respectively, and remained unchanged in both groups during and after application of CPPV. No change in PO_2 values occurred during CPPV in either group. However, in the intact group PCO_2 was significantly increased from 36 ± 1 to 38 ± 1 Torr at both 5 and 15 min of CPPV. Also, in the denervated dogs PCO_2 increased significantly at 15 min of CPPV from 35 ± 1 to 38 ± 1 Torr.

Significant changes in renin activity in both arterial plasma samples and renal vein plasma samples were observed exclusively in dogs of the intact group (Fig. 2). At both 5 and 15 min of CPPV renin activity was elevated above control in arterial and venous samples. After CPPV was removed, arterial and venous plasma renin activities returned toward control levels but were still significantly increased. In contrast, no significant changes in either the arterial or renal vein plasma samples occurred in the dogs of the denervated group.

As shown in Fig. 3 renin secretion was changed at both 5 and 15 min of CPPV only in the intact group of dogs. No significant changes were observed in the denervated animals.

Discussion. The rise in arterial and renal venous plasma renin activities in dogs of the intact group exposed to CPPV confirm the studies of Ziegler *et al.* (6) and Bark *et al.* (7). The increase in renin, however, is in contrast to the results of Kaukinen and Eerola (8) who observed no change in arterial plasma renin activity. There are several reasons which may explain the differences in results. Our study

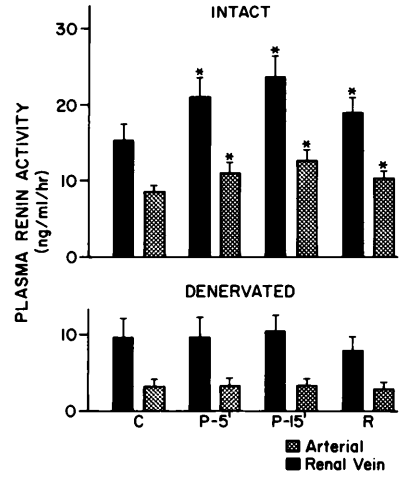

FIG. 2. Arterial and renal vein plasma renin activity during CPPV. Abbreviations are the same as in Fig. 1.

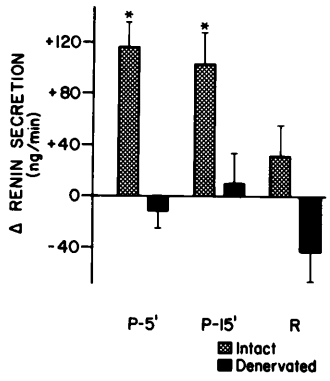

FIG. 3. Change in renin secretion from control values during CPPV. Abbreviations are the same as in Fig. 1.

and the studies of Ziegler *et al.* and Bark *et al.* were performed in anesthetized dogs (6, 7). Kaukinen and Eerola (8) conducted their study on sedated patients of an intensive care unit. The state of anesthesia and species differences, therefore, may have contributed to the difference in results. Kaukinen and Eerola (8) also did not measure renal vein renin activity, only arterial plasma renin activity. It is possible, therefore, that in the patients small changes in renin secretion may have occurred but the changes were not sufficient to increase arterial plasma renin levels by an amount which could be detected by the method used to measure renin activity. This possibility is supported by the authors' statement that peripheral renin activity increased slightly, but not significantly, in most patients. Also, the relatively high renin levels measured by Kaukinen and Eerola (8) in their patients before CPPV was applied may have masked any small but statistically significant changes in renin activity during CPPV. The authors attributed the high renin levels to the trauma and surgical intervention the patients were subjected to before the study.

Renal denervation effectively eliminated any change in renin secretion caused by application of CPPV. This result supports the conclusion that increases in renin secretion during CPPV are mediated, primarily, by alterations in renal nerve activity. Ziegler *et al.* (6) were unable to document the role of renal nerves in the renin increase. They measured arterial plasma renin activity in two dogs each with an autotransplanted kidney and a contralateral nephrectomy. In both dogs CPPV increased renin activity. However, as the investigators suggested their results do not permit making conclusions on the effect of renal nerves on renin secretion because the reduction in renal mass due to the nephrectomy may have influenced the renin–angiotensin system. The results of the study by Bark *et al.* (7) support our conclusion. In anesthetized dogs pretreated with propranolol, CPPV, with a positive end expiratory pressure of 15 cm water, did not increase arterial plasma renin activity.

It is unlikely that the failure of renin secretion to increase in dogs with denervated kidneys was caused by alterations other than the denervation. The changes in blood gases and hemodynamics during CPPV were similar in both intact and denervated dogs. Also, the possible direct effect of changes in renal perfusion pressure on renin secretion was eliminated in our studies by the use of the inflatable aortic cuff to maintain perfusion pressure constant. The fall in renal blood flow at 15 min of CPPV and at recovery in the denervated dogs probably did not contribute significantly to the failure of renin levels to increase. Generally, renin levels increase with a fall in renal blood flow (10). Also, the results obtained at 5 min of CPPV when renal blood flow was not reduced demonstrate that renin secretion in denervated dogs is not increased as it is in intact dogs. Renal blood flow fell in the denervated group of animals primarily in response to an increase in renal vascular resistance. The 10.8% fall in flow during recovery correlates well with the 13.9% increase in renal resistance. The factors responsible for the small increase in resistance are unknown but depth of anesthesia and possible denervation hypersensitivity may have contributed.

We have demonstrated in earlier studies (4, 5) that application of CPPV produces hemodynamic changes, similar to those observed in our current study, which cause alterations in arterial baroreceptor activity that initiate changes in renal nerve activity to produce an antidiuresis and antinatriuresis. We would speculate, therefore, that arterial baroreceptors also serve, at least in part, as the afferent limb of the reflex associated with the rise in renin secretion during CPPV. Whether or not the increased renin levels contribute to

the changes in renal function remains to be determined.

Our study indicates that the renal nerves have a primary role in mediating the increase in renin secretion during CPPV. The mechanism by which the nerves cause the increase remains to be documented. Alterations in renal nerve activity can change renin secretion by influencing intrarenal vascular receptors, the sodium load to the macula densa, and the juxtaglomerular cells themselves (10).

1. Ueda H, Neclerio M, Leather RP. Effects of positive end expiratory pressure on renal function. Surg Forum **23**:209–211, 1972.
2. Kumar A, Pontoppidan H, Baratz RA. Inappropriate response to increased plasma ADH during mechanical ventilation in acute respiratory failure. Anesthesiology **40**:215–221, 1974.
3. Berry AJ. Respiratory support and renal function. Anesthesiology **55**:655–667, 1981.
4. Fewell JE, Bond GC. Role of sinoaortic baroreceptors in initiating the renal response to continuous positive-pressure ventilation in the dog. Anesthesiology **52**:408–413, 1980.
5. Fewell JE, Bond GC. Renal denervation eliminates the renal response to continuous positive-pressure ventilation. Proc Soc Exp Biol Med **161**:574–578, 1979.
6. Ziegler M, Janzik W, Miksche L, Mohring H, Weigand W, Gross F. Effects of positive and negative pressure breathing on plasma renin concentration in the dog. Pflugers Arch **348**:185–196, 1974.
7. Bark H, Le Roith D, Nyska M, Glick SG. Elevations in plasma ADH levels during PEEP ventilation in the dog: mechanisms involved. Amer J Physiol **239**:E474–E481, 1980.
8. Kaukinen S, Eerola R. Positive end expiratory pressure ventilation, renal function and renin. Ann Clin Res **11**:58–62, 1979.
9. Donald DE. Studies on the release of renin by direct and reflex activation of renal sympathetic nerves. Physiologist **22**:39–42, 1979.
10. Davis JO, Freeman RH. Mechanisms regulating renin release. Physiol Rev **56**:1–56, 1976.

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