

Increase in Renin Release after Sinoaortic Denervation and Cervical Vagotomy in Anesthetized Dogs (II) (39903)

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Introduction. Previously we demonstrated that cervical vagotomy, both in intact dogs and in dogs previously subjected to sinoaortic denervation, increases plasma renin activity (PRA) despite high-sodium diets (1, 2). We reported also that this increase in PRA resulting from cervical vagotomy could be blocked by infusion of propranolol or by renal denervation, results that suggest that this increase in PRA is mediated by increased sympathetic discharge to the kidney (1, 2). In the earlier experiments the kidneys were denervated immediately before the measurement of PRA, and PRA usually rose as arterial blood pressure decreased. Thus it was important to explore the mechanism for the increase in PRA after cervical vagotomy in dogs whose kidneys had been denervated for several days. In order to assess the contribution of the adrenal medulla, we did the same experiment in adrenalectomized dogs whose kidneys had been denervated. Renal denervation alone greatly attenuated the increase in PRA that results from cervical vagotomy but not that following sinoaortic denervation, while adrenalectomy with renal denervation abolished the increase in PRA induced by sinoaortic denervation as well. The present series of experiments demonstrates that the increase in PRA produced by cervical vagotomy results largely from increased renal nerve activity, whereas the increase in PRA produced by sinoaortic denervation is a result both of increased renal nerve activity and of catecholamines released from the adrenal medulla.

Materials and methods. (A) *General surgical procedures.* Female mongrel hounds (body weight, 15-25 kg) were maintained on a diet containing 180 mEq of sodium each day for 1 to 2 weeks. The general surgical procedure was essentially the same

as that previously described (2). The animals were anesthetized with 30 mg/kg of sodium pentobarbital and ventilated with a Harvard respirator. Light anesthesia was maintained by intermittent administration of pentobarbital. Renal perfusion pressure (RPP) was kept relatively constant (within ± 3 mm Hg) by means of a suprarenal aortic snare. Systemic arterial blood pressure was measured through a Tygon catheter (Suprenant; o.d., 2.5 mm) inserted in a brachial artery. Renal perfusion pressure was measured through a Tygon catheter, inserted into a femoral artery, with its tip lying below the renal arteries. Another Tygon catheter was placed in a femoral vein for infusion. Arterial blood pressure was measured with Statham transducers (P 23 IA) and recorded on a Brush 440 recorder.

(B) *Chronic renal denervation and adrenalectomy.* Renal denervation with or without adrenalectomy was performed 7-10 days before the experiments were done. All visible nerves along the renal arteries and veins were carefully dissected out and cut and the vessels were painted with 95% alcohol and 5% phenol. At the end of operations, 300-500 ml of saline were infused intravenously. The animals subjected to both renal denervation and adrenalectomy were given 300 mg of cortisone intramuscularly (im) after operation and maintained on 100 mg of cortisone (im) per day for the first 3 days and 25 mg each day until the day of experiment. On the morning of the experiment, 50 mg of cortisone were injected into the stomachs of the animals and 50-100 mg of hydrocortisone were given intravenously, followed by slow infusion of hydrocortisone (0.8-1.6 mg/ml, infused at 1.3 ml/min).

(C) *Experimental procedure.* Each animal was prepared as in (A). Thirty minutes after completion of general surgical procedures,

we obtained an arterial blood sample and started sinoaortic denervation. We performed carotid denervation by dissecting out all the visible nerves and vessels at the carotid bifurcation, and by stripping the adventitia from the internal, external, and common carotid arteries. We then painted 95% alcohol and 5% phenol onto these vessels. Abolition of the increase in systemic arterial blood pressure during carotid occlusion was considered an indication of successful denervation. We performed aortic denervation by the method of Edis and Shepherd (3). The vagal sympathetic trunk was opened and the aortic nerve, located at its junction with the superior laryngeal nerves, was cut. We then separated the main vagal trunks from the sympathetic nerve and placed loose ligatures around the vagal trunks. We then obtained arterial blood samples at intervals of 30 and 60 min. After this we sectioned the vagus nerves about 2 cm below the nodose ganglion and collected arterial blood samples at intervals of 30, 60, and 90 min.

Plasma renin activity (PRA) was determined by a radioimmunoassay method modified from that of Katz and Smith (4) and described in detail in an earlier paper (2). Packed cell volume (PCV) was determined by the microcapillary method. Statistical analysis was done with the use of Student's paired *t* test.

Results. In dogs maintained on a high-salt diet sinoaortic denervation caused increases in mean arterial pressure (MAP), PRA, and PCV, and cervical vagotomy caused further increases in these variables. This confirms an earlier report (2). Figure 1 shows the results from 11 animals. With RPP maintained relatively constant, MAP increased from 141.9 ± 4.2 mm Hg in control periods to reach 180.5 ± 7.5 mm Hg ($P < 0.005$) 60 min after sinoaortic denervation; it increased further to reach 217.7 ± 12.1 mm Hg ($P < 0.005$) 1 min after cervical vagotomy. MAP then gradually declined, and, 90 min after vagotomy, it stabilized at approximately the same level as before vagotomy (167.1 ± 6.8 as compared to 180.5 ± 7.5 mm Hg, $P > 0.5$). Mean PRA increased from 2.09 ± 0.76 ng/ml/hr during control periods to reach 13.09 ± 2.34 ng/

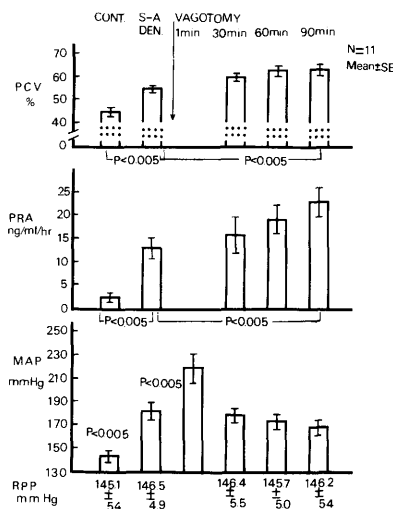


FIG. 1. Effect of sinoaortic denervation (S-A DEN) and cervical vagotomy on packed cell volume (PCV), plasma renin activity (PRA), and mean arterial pressure (MAP) in dogs whose renal perfusion pressure (RPP) was maintained relatively constant. Each column represents mean \pm one standard error of the mean.

ml/hr ($P < 0.005$) 60 min after sinoaortic denervation; it increased further to reach 22.82 ± 3.90 ng/ml/hr ($P < 0.005$) 90 min after vagotomy. Mean PCV also increased from $44.9 \pm 1.3\%$ during control periods to reach $54.8 \pm 1.3\%$ ($P < 0.005$) 60 min after sinoaortic denervation and $61.5 \pm 2.2\%$ ($P < 0.005$) by 90 min after cervical vagotomy.

In the animals whose kidneys had been denervated 7–10 days previously, sinoaortic denervation caused increases in mean MAP from 133.1 ± 5.4 mm Hg during control periods to 181.9 ± 8.0 mm Hg ($P < 0.005$) 60 min after sinoaortic denervation; it increased further to reach 211.2 ± 11.9 mm Hg ($P < 0.005$) by 1 min after cervical vagotomy. Mean PCV increased from $45.4 \pm 1.2\%$ during control periods to reach $52.1 \pm 1.1\%$ ($P < 0.005$) 60 min after sinoaortic denervation; it increased further to reach $55.5 \pm 1.1\%$ ($P < 0.005$) 90 min after cervical vagotomy. Mean PRA increased from 2.09 ± 0.76 ng/ml/hr during control periods to reach 13.09 ± 2.34 ng/ml/hr ($P < 0.01$) 60 min after sinoaortic denervation. In these animals, cervical vagotomy caused only a further slight, but not

statistically significant, increase in PRA. At 90 min after cervical vagotomy, mean PRA was 16.81 ± 3.77 ng/ml/hr ($P > 0.1$) (Fig. 2).

In the animals whose kidneys had been denervated, with adrenalectomy, 7 to 10 days previously, sinoaortic denervation and cervical vagotomy also cause increases in MAP. The degree of increment, however, varied from animal to animal. Figure 3 summarizes the results from six experiments. MAP increased from 140.3 ± 4.6 mm Hg during control periods to reach 159.1 ± 8.3 mm Hg ($P < 0.025$) 60 min after sinoaortic denervation; it increased further to reach 187.8 ± 15.4 mm Hg ($P < 0.025$) 1 min after cervical vagotomy. MAP then gradually declined to reach 140.0 ± 6.9 mmHg 90 min after cervical vagotomy, a value significantly lower than that before vagotomy ($P < 0.005$). The increase in PRA after sinoaortic denervation and cervical vagotomy, as observed in control experiments (Fig. 1), was abolished by renal denervation and adrenalectomy. Mean PRA in these animals was 2.07 ± 0.94 ng/ml/hr during control periods, 1.04 ± 0.36 ng/ml/hr ($P > 0.1$) 60 min after sinoaortic denervation, and 1.81 ± 0.70 ng/ml/hr ($P > 0.1$) 90 min

after cervical vagotomy (Fig. 3). Mean PCV was $40.9 \pm 1.7\%$ during control periods, $44.9 \pm 1.6\%$ ($P < 0.01$) 60 min after sinoaortic denervation, and $45.0 \pm 1.7\%$ ($P > 0.1$) 90 min after cervical vagotomy.

Discussion. The present series of experiments confirms previous findings (2) that sinoaortic denervation and cervical vagotomy cause increases in MAP, PRA, and PCV (Fig. 1). Mancia *et al.* (5) reported that PRA increased upon cooling of the vagi in dogs with aortic arch denervation in which the carotid sinus was perfused at constant pressure. Previously we reported that renal denervation abolishes the increase in PRA that follows cervical vagotomy (1). Mancia *et al.* (5) also reported that renal denervation abolishes the increase in renin release produced by vagal cooling. In our earlier experiments, however, there was usually a large decrease in arterial blood pressure and an increase in PRA immediately after renal denervation. Since it was possible that the high values for PRA that follow renal denervation might obscure the effect of sinoaortic denervation and cervical vagotomy, we performed sinoaortic denervation and cervical vagotomy a week or more after renal dener-

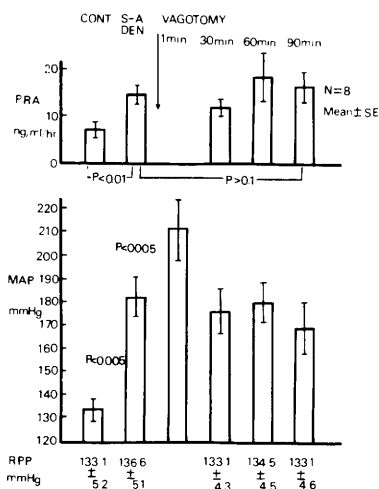


FIG. 2. Effect of sinoaortic denervation (S-A DEN) and cervical vagotomy on plasma renin activity (PRA) and mean arterial pressure (MAP) in dogs whose kidneys had been denervated 8 days previously. Renal perfusion pressure (RPP) was maintained relatively constant. Each column represents mean \pm one standard error of the mean.

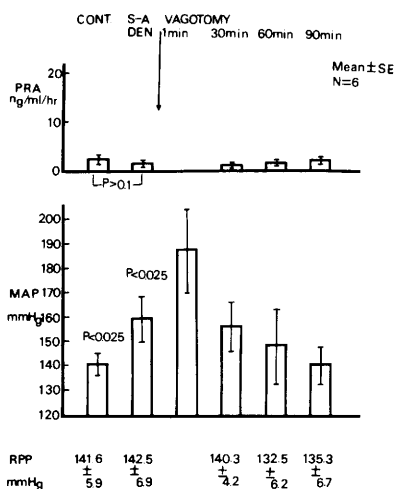


FIG. 3. Effect of sinoaortic denervation (S-A DEN) and cervical vagotomy on mean plasma renin activity (PRA) and mean arterial pressure (MAP) in dogs whose kidneys had been denervated, with adrenalectomy, 7 to 10 days previously. Renal perfusion pressure (RPP) was maintained relatively constant. Each column represents mean \pm one standard error of the mean.

vation in the present series of experiments. We thereby avoided the decrease of arterial blood pressure and the rise of PRA that follow renal denervation.

It is well established that direct renal nerve stimulation causes an increase in renin release (6). It is also well known that such an increase in renin release can be blocked by infusion of propranolol (7). Mancina *et al.* (5) have shown in the dog and Clement *et al.* (8) in the rabbit that cervical vagotomy causes an increase in sympathetic discharge to the renal nerves. We also showed in our earlier report that infusion of propranolol abolishes the increase in PRA that follows cervical vagotomy (2). In the present series of experiments, we show as well that in dogs whose kidneys have been denervated for 7 to 10 days, cervical vagotomy causes only a slight, but not statistically significant, increase in PRA (Fig. 2). Thus it seems reasonable to suggest that the increase in PRA that follows cervical vagotomy, as observed in control animals (Fig. 1), was largely due to increased sympathetic discharge to the kidney.

In control animals, PRA increased significantly after sinoaortic denervation (Fig. 1). Kezdi and Geller (9) have shown that carotid sinus pressure and renal sympathetic nerve discharge bear a reciprocal relationship; that is, a decrease in carotid sinus pressure causes an increase in renal sympathetic nerve discharge. Denervation of the carotid and aortic baroreceptors should thus produce an increase in the sympathetic discharge to the kidney, causing an increase in renin release.

Increase in sympathetic discharge to the kidney may not have been the only factor responsible for the observed increase in PRA after sinoaortic denervation (Fig. 1). This is shown by the statistically significant increase in PRA that follows sinoaortic denervation in animals whose kidneys had been denervated 7 to 10 days before (Fig. 2). It has been shown that intrarenal infusion of catecholamines can cause renin release (6) and that such an increase in renin release can be blocked by propranolol infusion (10). It is possible that catecholamines released from the adrenal medulla may have contributed to the increase in PRA that fol-

lows sinoaortic denervation. Our earlier report showed that the increase in PRA that follows sinoaortic denervation can be blocked by propranolol (2). Thus it appeared likely that sinoaortic denervation produces increases in release of renin both by increase of sympathetic discharge to the kidneys and by release of catecholamines from the adrenal medulla. To test this, we performed sinoaortic denervation and cervical vagotomy on adrenalectomized dogs whose kidneys had been denervated at the time of adrenalectomy. As shown in Fig. 3, the increase in PRA that ordinarily follows sinoaortic denervation and cervical vagotomy (Fig. 1) was abolished in these animals. This supports the view that both the renal nerves and circulating catecholamines contribute to the release of renin in these experiments. These animals received cortisone, approximately 750 mg in all, during the week following adrenalectomy. Whereas this amount of cortisone might well have increased plasma renin substrate concentration (11), and this in turn might lower plasma renin concentration (12), the resultant value for plasma renin activity should not have been lower than normal, as it was in the dogs following renal denervation.

The basal values for PRA in the dogs whose kidneys had been denervated a week before were higher than those in intact dogs fed the same diet ($P < 0.05$) and those in dogs whose adrenals had been removed at the time of renal denervation ($P < 0.05$). It has been reported that the renin content of the kidneys is below control values in dogs whose kidneys had been denervated some days previously (13). We cannot explain the higher plasma values found after renal denervation alone. The animals had slightly lower blood pressure than those in the other two groups (control and renal denervation-adrenalectomy), but the difference was not statistically significant.

The present series of experiments supports the suggestions that the carotid and aortic baroreceptors, as well as the receptors served by the vagus nerves, exert a tonic inhibitory effect on the vasomotor center (2). Removal of this inhibition would lead to an increase in the sympathetic discharge to the kidneys and to release of catechol-

amines from the adrenal medulla; both these factors lead to an increase in renin release.

The physiological significance of the present findings of the role of vagal and sinoaortic baroreceptors in the control of renin release has not been established. There is some evidence that receptors served by the vagal nerves may be located in the heart and the lung (14). It is possible that one of the natural stimuli to these receptors reflects the amount of blood coming back to the heart: venous return. An increase in venous return would cause an increase in the stimulation to these receptors, resulting in more inhibition of the vasomotor center; a decrease in venous return would lower inhibition. It is well known that the upright posture leads to an increase in renin release (15). On the assumption of upright posture, there is a pooling of circulating blood in the lower part of the body, causing a diminution of venous return (16) and a decrease in inhibitory effects on the vasomotor center. The decrease in venous return would lead to a decrease in cardiac output; this would also contribute to the decrease in stimulation of the carotid and aortic baroreceptors, further lowering the inhibitory effects on the vasomotor center. This in turn would lead to an increase in the sympathetic discharge to the kidneys and to release of catecholamines from the adrenal medulla; both factors would lead to an increase in renin release.

Thus the carotid and aortic baroreceptors, working in concert with the receptors served by the vagal nerves, can modulate release of renin with change of posture. It may be that these reflex pathways serve no function under normal conditions. It is perhaps more likely that they mediate the well-known responses of renin release to dietary sodium or to posture. It is well known that subjects maintained on a low-salt diet usually have high PRA values (15). The exact mechanism(s) for this increase in PRA is not known, but it is likely that the sympathetic nervous system is involved (15). Since there is usually a decrease of plasma volume with lowering of the salt intake, it is possible that such a decrease in effective blood volume leads to decreased stimulation of the carotid and aortic baroreceptors and of those recep-

tors served by the vagal nerves. This would lead to an increase in the sympathetic discharge to the kidneys, release of catecholamines from the adrenal medulla, and an increase in renin release. Thus the activity of the renal nerves and the adrenal medulla, indirectly controlled by receptors in both the high- and low-pressure systems, may participate in the regulation of renin release by changes of salt intake.

Conclusions. Studies were done to clarify the mechanism whereby sinoaortic denervation and cervical vagotomy increase plasma renin activity (PRA). Dogs were subjected to renal denervation and some of them to adrenalectomy as well. Seven to ten days later, sinoaortic denervation and vagotomy were done under pentobarbital anesthesia. In intact animals, mean PRA increased after sinoaortic denervation and increased further after cervical vagotomy. In dogs with the kidneys denervated, PRA increased after sinoaortic denervation. Cervical vagotomy in these animals caused a further slight, but not statistically significant, increase in mean PRA. In adrenalectomized dogs with the kidneys denervated, PRA did not change after sinoaortic denervation and cervical vagotomy. These results suggest that the increase in PRA after sinoaortic denervation probably results both from increased sympathetic discharge to the kidneys and from catecholamines released from the adrenal medulla, whereas the increase in PRA after cervical vagotomy is mediated largely by increased sympathetic discharge to the kidney.

1. Yun, J. C. H., Delea, C. S., and Bartter, F. C., *Fed. Proc.* **33**, 287 (1974).
2. Yun, J. C. H., Delea, C. S., Bartter, F. C., and Kelly, G., *Amer. J. Physiol.* **230**, 777 (1976).
3. Edis, A. J., and Shepherd, J. T., *J. Appl. Physiol.* **30**, 294 (1970).
4. Katz, F. H., and Smith, J. A., *Clin. Chem.* **18**, 528 (1972).
5. Mancia, G., Romero, J. C., and Shepherd, J. T., *Circ. Res.* **36**, 529 (1975).
6. Vander, A. J., *Amer. J. Physiol.* **209**, 659 (1965).
7. Loeffler, J. R., Stockieget, J. R., and Ganong, W. F., *Neuroendocrinology*, **10**, 129 (1972).
8. Clement, D. L., Pelletier, C. L., and Shepherd, J. T., *Circ. Res.* **31**, 824 (1972).
9. Kezdi, P., and Geller, E., *Amer. J. Physiol.* **214**, 427 (1968).

10. Assaykeen, T. A., Clayton, P. L., Goldfien, A., and Ganong, W. F., *Endocrinology*, **87**, 1318 (1970).
11. Haynes, F. W., Forsham, P. H., And Hume, D. M., *Amer. J. Physiol.* **172**, 265, (1953).
12. Menard, J., and Catt, K. J., *Endocrinology* **92**, 1382 (1973).
13. Tobian, L., Braden, M., and Maney, J., *Fed. Proc.* **24**, 1524 (1965).
14. Pillsbury, H. R. C., III, Guazzi, M., and Freis, E. D., *Amer. J. Physiol.* **217**, 768 (1969).
15. Gordon, R. D., Kuchel, O., Liddle, G. W., and Island, D. P., *J. Clin. Invest.* **46**, 599 (1967).
16. Vander, A. J., Sherman, J. H., and Lucinao, D. S., "Human Physiology: The Mechanisms of Body Function," pp. 291-295. McGraw-Hill, New York (1970).

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