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Lack of Evidence for a Plasma Humoral Factor of Extrarenal Origin Causing Release of Renin[1] (37866)

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(Introduced by F. M. Bumpus)

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Several hypotheses have been proposed to explain the mechanism of renal renin release. Those theories which seem to be consistent with most experimental findings are the ones which involve the renal baroreceptor, the macula densa, and the sympathetic nervous system (1–3).

Recently, De Vito *et al.* (4) investigated a possibility mentioned by Fasciolo (5), that a humoral factor might act upon intrarenal receptors to control renin release. De Vito's investigation indicated the presence of a nondialyzable plasma humoral factor, of extrarenal origin, that caused renin release in normal animals.

In our study, efforts were made to confirm these results so that subsequent experiments could be performed to determine the nature and origin(s) of the humoral factor. For this, normal dogs were injected with plasma obtained from nephrectomized, hypotensive dogs as in De Vito's experiment; yet plasma renin activity (PRA) did not increase after the injections. Our work, therefore, failed to confirm the results previously reported by the above-mentioned author.

Methods. Normal female mongrel dogs, weighing 13–25 kg and maintained on Purina Dog Chow and tap water *ad lib.*, were used. Surgical procedures and bleedings were performed while the animals were anesthetized with iv sodium pentobarbital (25–30 mg/kg). Mean blood pressures were obtained by means of a polyethylene catheter inserted into the femoral artery and attached to a mercury manometer. All plasma injections in the anesthetized dogs were made by way of a catheter inserted in the femoral vein.

Experimental groups. Protocol 1. Six nonnephrectomized dogs were used to assess the increase in plasma renin release due to hemorrhagic hypotension. Controlled bleeding (25 ml/kg) was performed by way of a catheter placed in the carotid artery. Blood for the determination of PRA was collected from the jugular vein into vacutainer tubes (Becton-Dickenson & Co., Rutherford, NJ) containing ethylenediamine–tetraacetic acid disodium salt. Blood collection was performed before hemorrhage, and at 0.5, 1, 2, and 3 hr after. Blood pressures were recorded as described above at each blood sample collection.

Protocol 2. Six dogs which had been bilaterally nephrectomized 48 hr earlier (donors) and 12 normal dogs (recipients of nephrectomized plasma) were used. The nephrectomized dogs were anesthetized and catheters placed in the carotid and femoral arteries. Fifty milliliters of blood was collected from the carotid artery in a plastic syringe wetted with heparin for plasma injection. Seven additional milliliters of blood for PRA was also collected. Controlled hemorrhage was then performed until mean blood pressure fell to between 50 and 60 mm Hg. One-half hour after the termination of hemorrhage, two samples of blood were collected: one for plasma injection and one for PRA. The blood obtained during the normotensive and hypotensive periods was centrifuged individually at 4° and the plasma labeled normotensive and hypotensive, re-

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spectively. The 12 plasma samples were kept separate for later injection (1–4 hr after withdrawal) into recipient dogs. The plasma was stored at 4°, and 0.5 hr before the injection, it was warmed to room temperature. The 12 recipient, nonnephrectomized animals were divided into two equal groups. The first group served as control, and each dog was injected with one of the 25-ml samples of the plasma obtained from the normotensive, nephrectomized dogs. The other half served as the experimental group, and each dog received one of the 25 ml of plasma obtained from the hypotensive, nephrectomized dogs. Blood from the recipient dogs, for the determination of PRA, was drawn both before plasma injection and at 30, 60, 120, and 180 min after. Blood pressures were recorded at each blood collection.

Protocol 3. Protocol 3 was the same as Protocol 2 except that the recipients were unanesthetized and plasma injections were given by venipuncture of the jugular vein. Blood for the determination of PRA was withdrawn from the femoral artery in three experiments and from the jugular vein in the remaining three.

Plasma renin activity. PRA was measured using a modification of the radioimmunoassay technique of Haber *et al.* (6). In this modification, a nonequilibrium radioimmunoassay is done. Both the samples and the cold angiotensin I tubes (standards) are incubated with antibody for 16 hr at 4°; then the ^{125}I angiotensin I is added and further incubated for 6 additional hr. The free and bound ^{125}I angiotensin I are then separated by dextran-coated charcoal. Dextran is used in a concentration which is ten times higher than the one described by Haber *et al.* (6). The results are expressed in nanograms of angiotensin I per milliliter of plasma per hour of incubation.

Results. Protocol 1. All six normal dogs showed a significant increase in PRA 0.5 hr after they were subjected to hemorrhage. Although blood pressure had returned to normal within 0.5 hr, PRA continued to increase until at least 3 hr after bleeding was terminated (Fig. 1).

Protocols 2 and 3. Neither the anesthetized nor the unanesthetized groups who received injections of plasma from hypotensive or normotensive, nephrectomized animals showed a significant increase in PRA values during the period of time lasting until at least 3 hr after the injections (Fig. 2). Some of the animals in both the control and experimental groups showed wide variations in PRA when drawn at different times. The fact that the variations were found in both the conscious and the anesthetized recipients indicates that the anesthetic used probably was not the cause of the variations.

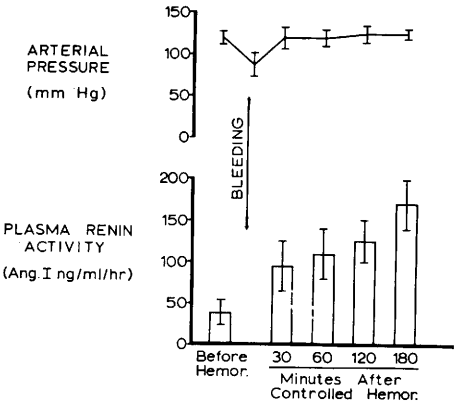

FIG. 1. Mean blood pressure and plasma renin activity in six nonnephrectomized, anesthetized dogs before and at different times after bleeding. Vertical bars indicate SEM.

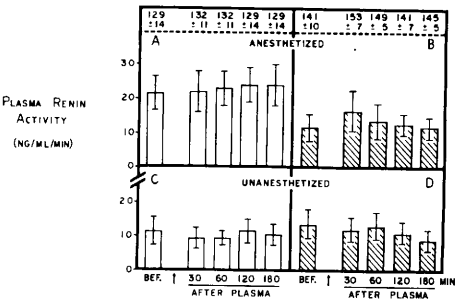

FIG. 2. Plasma renin activity before (BEF.) and at different times after plasma injection (arrow). A and B (top graph) indicates anesthetized dogs. C and D (bottom graph) indicates unanesthetized dogs. White columns (A and C) indicate dogs injected with plasma obtained from normotensive, nephrectomized dogs. Hatched columns (B and D) indicate dogs injected with plasma from hypotensive, nephrectomized dogs. Vertical bars inside of columns indicate SEM. Numbers shown at the top of the graph are the blood pressures of the recipient anesthetized dogs (±SEM).

Anesthetized animals. When the PRA was measured in 11 nonnephrectomized animals before and after the anesthesia, a significant increase in PRA was observed, $P < 0.05$ (one-tail *t*-test for pair of samples).

Nephrectomized animals. The animals nephrectomized 48 hr previously had very low renin, and no changes were observed after 0.5 hr of hemorrhagic hypotension (Fig. 3).

Discussion. The anesthetized, nonnephrectomized dogs (recipients) in our experiments did not show an increase in PRA after the injection of plasma from the nephrectomized, hypotensive dogs. The protocols that we followed for these experiments were the same as the ones reported by De Vito *et al.* (4). The only significant difference is that we measured PRA by the radioimmunoassay. This, however, is unlikely to explain the difference. The normal dogs (Protocol 1) that were bled showed a significant increase in PRA. These results fall in the realm of what was expected according to previous publications (7–9). Also, in 11 animals in which PRA was measured before and after the anesthesia, an increase in PRA was found after the anesthesia. Although some renin activity was detected in the nephrectomized animals, it was very low, 100 times less than in the normal. No changes in the PRA were observed in the nephrectomized dogs 30 min after hemorrhage. These results confirm previous experiments reported by us in which renin was measured by bioassay (10) and differ from those reported by Ganten *et al.* (11). These investigators studied the renin release in nephrectomized dogs after hemorrhagic hypovolemia and found an increase. A possible reason for this difference is that they waited for a longer period of time after hemorrhage (1–6 hr) before measuring the renin. Prolonged hypotension could produce tissue damage and that in itself could produce a spillover into the systemic circulation of extrarenal renin-like enzymes.

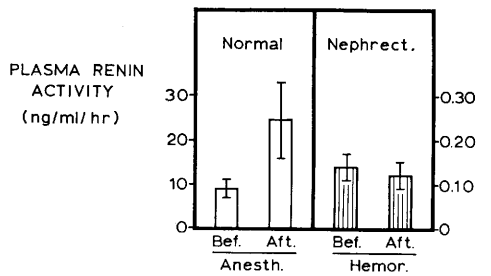

FIG. 3. White columns indicate plasma renin activity before and after anesthesia in nonnephrectomized dogs. Lined columns indicate plasma renin activity in nephrectomized dogs before and after bleeding. Notice that the scale on the right for the nephrectomized dogs is 100 times smaller than the scale on the left for the nonnephrectomized dogs.

One could rule out the possibility that the alleged "renin-releasing factor" was destroyed in our hypotensive plasma since the blood was handled very carefully. The blood was collected in previously chilled polycarbonate tubes, centrifuged at 4°, and kept at this temperature until 0.5 hr before the injection when the plasma was then warmed to room temperature. Furthermore, De Vito *et al.* (4) reported that dialyzing the plasma for 36 hr or using week-old frozen plasma did not remove or alter the ability of plasma from nephrectomized, hypotensive dogs to increase circulating renin-like activity in the recipient dog. This indicates that this hypothetical factor was stable in this condition. One possibility is that the above-mentioned investigators in some way activated the prekallikrein from the plasma, and when it was injected in the recipient dogs, hypotension was produced and renin released. However, one would expect the same results from the hypotensive and normotensive plasma. Furthermore, although these investigators did not report the blood pressure of the recipient dogs, they did mention that no significant change in arterial blood pressure was observed in any of the recipient animals after injection of any of the plasma.

Our experiments failed to indicate the presence of any plasma humoral factor of extrarenal origin which might regulate renal renin release due to hypotension caused by hemorrhage. These results are in contradiction with the ones previously reported by De Vito *et al.* (4). No good explanation was found to explain this discrepancy.

Summary. The suggestion that a plasma humoral factor of extrarenal origin could

cause a release of renin was investigated. For this, normal dogs were injected with plasma obtained from nephrectomized dogs in which hemorrhagic hypotension was produced 0.5 hr before the blood withdrawal. PRA was measured by radioimmunoassay before and after the injection of the plasma from the hypotensive, nephrectomized dogs. PRA did not increase after the injection of plasma. This work, therefore, fails to confirm the results previously reported by another group of investigators (4).

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