# Renal Vascular Response to Plasma and to Known Vasoactive Substances[1] (35173)

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Previous studies have demonstrated that plasma contains some vasoactive substance, or substances (1), not yet identified biochemically. Its presence has been evident in the dose-dependent development of tension by helically cut strips of vascular smooth muscle when plasma is added to the bathing solution. That this tension is not attributable to any known vasoactive agent can be inferred from the use of specific blocking agents and from the individualities of the responses of various vascular beds to plasma and to the several agents (1). Subsequent work with isolated perfused resistance vessels supported these observations (2).

More recently (3), this pressor substance has been obtained in more concentrated form by Sephadex fractionation of protein-free plasma filtrate. Whole plasma and the concentrated plasma factor have been found to evoke similar responses in helical strips from various vascular beds. The contractile effect of plasma factor has also been demonstrated with the isolated perfused dog intestine.

The present study compared the vascular responses of the isolated, perfused rat kidney to rat plasma, to a Sephadex concentrate of hog plasma, and to various known vasoconstrictors.

*Methods. Kidney preparation.* The left kidney was taken from white, male rats anesthetized with sodium pentobarbital (50 mg/kg). The abdominal cavity was entered by the ventral midline approach and all visible branches of the abdominal aorta and inferior vena cava, with the exception of the left renal vessels, were ligated and severed. The aorta was tied just proximal to its bifurcation; a ligature was placed loosely around it proximal to the left renal artery and a polyethylene cannula (PE 50) was inserted into the distal end. Artifical perfusion was begun and the aorta was then ligated proximal to the renal artery. In this way a period of anoxia was avoided. The perfused kidney, together with the aorta and the inferior vena cava, was then removed from the rat and placed in a bath maintained at 37°. To avoid possible loss of perfusate through segmental vessels which might have been overlooked, the cannula was finally tied in position in the renal artery.

*Perfusion apparatus.* A constant perfusion rate was maintained by use of a SigmaMotor pump (Model T-6) which, in all experiments, was adjusted to deliver approximately 3 ml/min of perfusate through polyethylene tubing leading to a cannula in the renal artery. A pair of 3-way stopcocks located proximal to the pump permitted changes in perfusion solution. An injection site just prior to the cannula in the renal artery made possible the rapid administration of a small volume (routinely 0.05 ml) of the test substance.

*Perfusion solutions.* The physiological salt solution (PSS) used as the basic perfusion fluid consisted of (mM): NaCl, 119; KCl, 4.7; $KH_2PO_4 \cdot 7H_2O$, 1.18; $MgSO_4 \cdot 7H_2O$, 1.17; $NaHCO_3$, 14.9; $CaCl_2$, 1.6; glucose, 5.5; sucrose, 50; $CaNa_2$ Versenate, 0.026. The solution was made isoncotic by the addition of 5 g of polyvinylpyrollidone/100 ml. "Ca-free" perfusate was PSS with the $CaCl_2$ omitted; "high-Ca" perfusate was PSS with the $CaCl_2$ increased to 3.2 or 4.8 m*M*. Solutions were aerated by bubbling with a 95% $O_2$, 5% $CO_2$ gas mixture. An alternate perfusion fluid was whole rat plasma.

*Test substances.* A concentrated vasoactive plasma factor was obtained by Sephadex

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fractionation of hog plasma, as previously described (3).

Fresh plasma was prepared from blood obtained from rats on the day of the experiment. A cannula was placed in the abdominal aorta of an anesthetized male rat and the blood was collected in a siliconized glass centrifuge tube containing 500 units of heparin/10 ml of blood.

The known vasoactive substances tested were: epinephrine (Adrenalin chloride solution, Parke-Davis); vasopressin (Pitressin, Parke-Davis); angiotensin (Hypertensin, Ciba); serotonin (5-hydroxytryptamine creatinine sulfate, Nutritional Biochemicals Corp.); and KCl (laboratory reagent).

*Administration of vasoactive agents.* Whole plasma was substituted for PSS as the perfusion fluid. Plasma fraction and the several known vasoconstrictors were administered in 0.05-ml aliquots added rapidly to the perfusate as it passed the injection site just before entering the kidney. This volume was chosen as one small enough to cause little artifact, yet large enough to be measured accurately with a 0.25-ml tuberculin syringe. Injection of a saline blank of this volume showed no response of the vessels to the transient pressure increase associated with the small increase in flow. The amounts of the several substances administered per injection were: epinephrine, 0.5 $\mu$g; vasopressin, 0.01–0.1 U; angiotensin, 0.5–5 $\mu$g; serotonin, 5 $\mu$g; KCl, 0.1 mmole.

*Experimental Procedure.* The isolated kidney in a PSS bath, perfused at 3 ml/min, was allowed a minimum of 30 min for equilibration. At the end of this time a test injection of epinephrine was given. Subsequent injections of the other test substances were made in random order, time being allowed between injections for the pressure to return to base line level. Where effect of changes in the perfusate was to be studied, this change was made after the initial test of the preparation with standard PSS perfusion and epinephrine test injection. Experiments were continued 3 to 5 hr.

*Results.* Close arterial injection of 0.5 $\mu$g of epinephrine produced rapid increase in pressure which reached its maximum within 30 sec, and returned to base line within 4 min (Fig. 1A). The average response to the initial injection, for the 8 kidneys studied, was 111.6 $\pm$ 10.5 mm Hg. Responses to epinephrine diminished markedly with time after start of perfusion (Fig. 2) until, after about 2 hr, there was no longer any response to this dose. Other vasoactive agents, vasopressin (2 expts.), angiotensin (2 expts., Fig. 1B), serotonin (2 expts., Fig. 1C), and KCl (2 expts., Fig. 1D), caused changes in resistance similar to those brought about by epinephrine, with maximum reached in less than 1 min and duration of 4 min or less.

Injection of 0.05 ml of plasma factor also caused an increase in resistance in these 8 kidneys (Fig. 1E). In contrast to responses to epinephrine, these responses developed slowly, 2 to 4 min to maximum resistance,

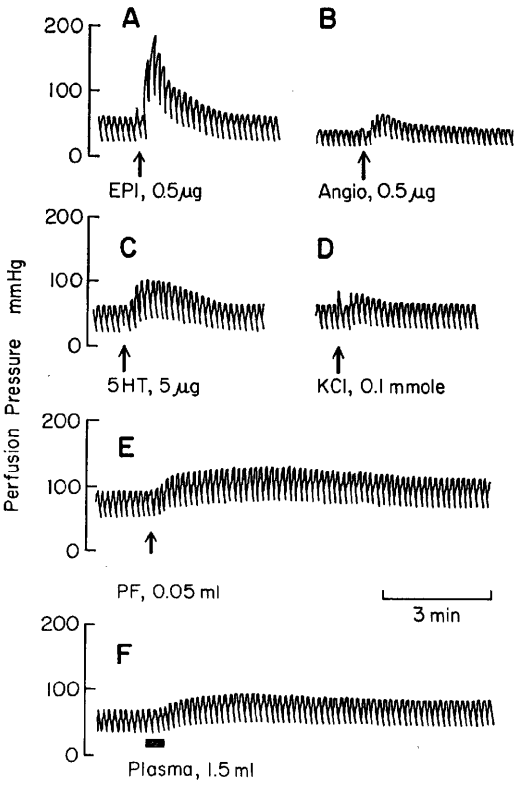


FIG. 1. Pressor responses of isolated perfused (PSS, 3 ml/min) kidney to various agents: (A) epinephrine, 0.5 $\mu$g; (B) angiotensin, 0.5 $\mu$g; (C) serotonin (5HT), 5.0 $\mu$g; (D) KCl, 0.1 mmole in 0.1 ml; (E) plasma fraction, 0.05 ml; (F) rat plasma, 30-sec perfusion (1.5 ml).

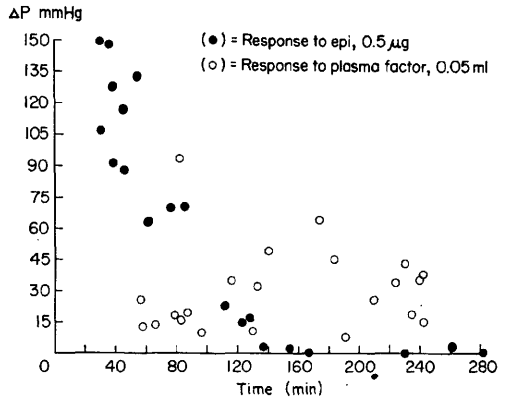


FIG. 2. Responses of isolated perfused (PSS, 3 ml/min) kidney to epinephrine (●); and to plasma factor (○). Sensitivity to epinephrine is lost rapidly with time, sensitivity to plasma factor is maintained 4 hr or more.

and were of long duration, 12 min or more. Response magnitude did not diminish with time (Fig. 2). Perfusion of rat plasma for 0.5 min (5 expts., Fig. 1f) caused a similar increase in resistance, slow in development, prolonged in effect, and not diminishing with time. Thus responses to the plasma factor differ from those caused by the vasoactive agents described above, in time course of the response and stability with time.

The effect of the amount of Ca in the perfusate on the responses to the several vasoactive agents provided still another basis for differentiation of the plasma substance. After 10 min of perfusion with "Ca-free" PSS, injection of plasma factor (3 expts.) produced no increase in resistance (Fig. 3). Ten min after return to normal PSS perfusate, responsiveness to plasma factor had returned. Under these same "Ca-free" conditions responses to epinephrine were diminished, but were not eliminated (1 expt.).

Increase in the Ca content of the perfusate significantly increased the response of the vascular bed to plasma factor (Fig. 3). In 6 expts. the average response to plasma factor in PSS with high Ca was about three times as great as when Ca concentration was 1.6 m*M* (av 84 vs 27 mm Hg). Additional Ca in the perfusate did not change the response to fresh whole plasma or to fresh whole plasma to which processed plasma factor had been added. Nor did increased Ca in the perfusate reverse the trend of diminishing responsiveness of the vascular bed to epinephrine (2 expts.); responses to successive injections were similarly diminished in PSS with 1.6 and 4.8 m*M* Ca.

*Discussion.* The similarities and differences among the responses of the renal vascular bed to the several substances tested add evidence to support the hypothesis that there is a vasoactive substance present in plasma that is distinct from known vasoconstrictors.

Furthermore, the results observed concerning the action of the various agents during perfusion with PSS solutions of low and high Ca concentration suggest that there is a difference in the mechanism of action of the plasma factor and the other agents. Since the response to the plasma factor was eliminated

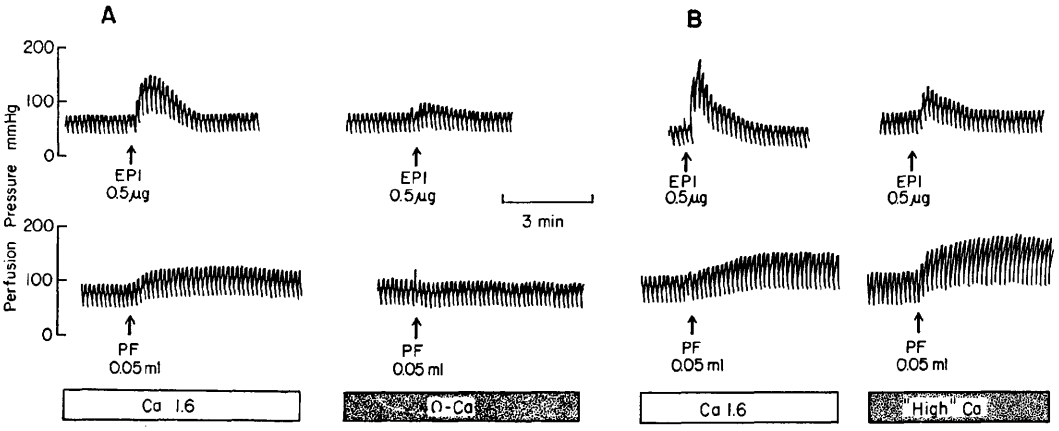


FIG. 3. Effect of "0-Ca" (A); and "high-Ca" (B) in the perfusate on responses to epinephrine and to plasma factor. Test was made 10 min after start of perfusion.

in the absence of Ca and significantly enhanced when the Ca concentration was raised, this resistance change must depend upon Ca from a rapidly exchanging pool. The other agents, not being significantly affected by alterations of Ca concentration of the perfusate, probably evoke increased resistance by utilizing a more stable pool of Ca. The decreasing responsiveness of the preparation with time could be explained by the loss, and lack of replenishment, of Ca in this pool. The differences in the time course of the contractile events evoked by plasma and by the other agents may be associated with such a difference in the Ca source.

The observation that the response to plasma was not enhanced by increased Ca concentration whereas the response to the active Sephadex fraction was, raises the question as to whether it is the same material in these two preparations that is causing vasoconstriction. However, since plasma to which the active Sephadex fraction was added was also unaffected by the Ca concentration, it seems more likely that the failure of the plasma response to be influenced by increased Ca is due to the presence of other substances in plasma. For instance, proteins of plasma may chelate the additional Ca.

It has been observed previously (4) that the perfused kidney continues to autoregulate its flow only if the perfusate contains at least 20% plasma. Since the Sephadex fraction and fresh plasma evoke essentially identical responses, one wonders if this low molecular weight fraction of plasma contains the key to an understanding of renal autoregulation.

*Summary.* The isolated rat kidney was perfused at constant flow rate with physiological salt solution (PSS). Addition of rat plasma or a vasoactive fraction of hog plasma caused an increase in renal vascular resistance. The time course of the responses caused by these two agents was the same, but differed from that of the pressor responses caused by epinephrine, angiotensin, KCl, serotonin, or vasopressin. The ability of the vessels to respond to plasma factor was lost during perfusion with "Ca-free" PSS, whereas the ability to respond to epinephrine was not; the constrictor response produced by the plasma factor was greater during perfusion with "high-Ca" PSS, that produced by epinephrine was not.

1. Bohr, D. F., and Johansson, B., Circ. Res. **19**, 593 (1966).

2. Uchida, E., Bohr, D. F., and Hoobler, S. W., Circ. Res. **21**, 525 (1967).

3. Bohr, D. F., and Sobieski, J., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **27**, 1396 (1968).

4. Waugh, W. H., and Shanks, R. G., Circ. Res. **8**, 871 (1960).

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