Vascular Ionic Effects of Angiotension II in the Rat[1] (35532)

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Angiotensin has been shown to induce vascular ionic changes although the results, obtained in a variety of procedures, are conflicting. Thus, *in vitro,* angiotensin has been reported to increase radioactive sodium efflux from the dog carotid artery (1), to decrease the sodium concentration of the medium incubating the dog femoral artery (2) and to have no effect on the sodium content of the rat aorta (3). Infusion of angiotensin into the artery of a perfused dog limb has been shown to decrease the sodium concentration of the perfusing fluid (4). In a recent study, sustained infusion of angiotensin in dogs for as long as 5 to 6 weeks resulted in an increase in the sodium content of the arterial wall, while the slow phase, as well as the fast phase, of radioactive sodium efflux from these arteries were increased (5). Vasoconstriction produced by angiotensin, in contrast to other vasoactive agents, was not accompanied by any consistent shift of potassium (6).

In the present experiments, the net transfers of sodium and potassium brought about by brief infusion of angiotensin into the isolated vascular bed of the rat tail have been measured by means of ion-selective glass electrodes and compared to those induced by epinephrine or vasopressin.

Methods. Male rats of an inbred Wistar strain (SPF, Woodlyn Farms) weighing 300 g or more and anesthetised by intraperitoneal sodium pentobarbital (33 mg/kg) and subcutaneous sodium phenobarbitone (60 mg/kg) were used. The rat tail vascular bed was isolated and perfused from the artery as described previously (7). The inflow perfusion pressure was recorded with a Statham P 23 pressure transducer. Heparinized blood from a donor rat, previously equilibrated with 95% O_2, 5%CO_2 gas mixture, was infused at the rate of 0.02 ml/min. In a few experiments, Krebs solution was infused in place of blood but the results, qualitatively similar, are not included. The effluent from the vein was collected in a continuous reel of PE 60 polyethylene tubing, and its flow rate was compared to that of a dummy system running in parallel (7). The vascular bed was perfused for 120 min before starting collection. When, after this, 0.6 ml of effluent had been collected, epinephrine,[3] vasopressin,[4] or angiotensin,[5] freshly added to the blood, were infused for 2, 2, and 6 min, respectively. The control blood solution was then again perfused until a final volume of 1.8 ml was obtained.

The record of ionic exchanges occuring during the slow passage of blood through the tail vascular bed, now stored in the reel of polyethylene tubing, was read at the end of the experiment by passing the blood at a constant, faster, flow-rate (0.15 ml/min) through an Na^+ and K^+ capillary glass electrode assembly (8), with an Ag–AgCl electrode as reference. The output of these electrodes was continuously converted by an analog computer to direct readings for Na^+ and K^+ in mEq/liter, and fed into a Grass polygraph. The size of the net ionic transfers was determined by calculating the area of the Na^+ and K^+ displacement from the base line.

Results. The control perfusion pressure of the rat tail vascular bed infused at a rate of 0.02 ml/min was 11 $\pm$ 0.9 mm Hg.

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[3] *l*-Epinephrine bitartrate, Sigma Chemical.

[4] PLV-2, Sandoz.

[5] Isoleucine-5-angiotensin II, Calbiochem.

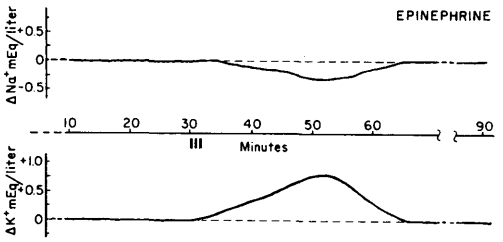

FIG. 1. Sodium and potassium activity monitored in the perfusing medium following infusion of a pressor dose of epinephrine (0.08 μg/min). The bar on the time scale indicates the period of drug infusion. For a peak pressure change of 100 mm Hg, the Na^+ shift was 0.090 μEq and the K^+ shift was 0.250 μEq.

Epinephrine. Infusion of epinephrine at rates of 0.01 to 0.2 μg/min for 2 min ($n = 7$) increased the perfusion pressure by 12 to 200 mm Hg. The increases in perfusion pressure were accompanied by a gain in K^+ in the perfusing blood and a loss in Na^+ (Fig. 1). The mean transfers have been summarized in Table 1. The ionic shifts extended over a period of 35 to 45 min. No ionic transfer was observed after subpressor doses of epinephrine (<0.0002 μg/min). Probably as a consequence of shrinkage of the vascular bed, the apparent effluent flow increased during the rise in pressure. Subsequently this outflow decreased to values always smaller than the inflow rate, probably reflecting an extravasation in the vascular bed.

Vasopressin. Infusion of vasopressin at rates of 2 to 8 mU min for 2 min ($n = 10$) increased the perfusion pressure by 2 to 12 mm Hg. Occasionally, doses of 2 mU/min failed to induce changes in perfusion pressure. As with epinephrine, the increase in pressure brought about by vasopressin was accompanied by a net loss of Na^+ and a gain of K^+ by the perfusing blood (Fig. 2, Table I). No ionic transfer occurred when vasopressin failed to increase the perfusion pressure.

Angiotensin. Angiotensin infused at a rate of 0.01 to 0.2 μg/min ($n = 7$) for 6 min did not increase the perfusion pressure by more than 3 mm Hg. The ionic transfers brought about by angiotensin in these conditions are illustrated in Fig. 3. The perfusing medium constantly gained sodium and lost potassium during infusion of the drug (Table I). The ionic exchange extended over a period of 20 to 30 min. Higher doses of angiotensin of 1.0 to 4.0 μg/min ($n = 7$) produced pressure changes of +6 to +54 mm Hg. These increases in pressure were not accompanied by any significant transfer of potassium. Small Na^+ shifts (<0.020 μEq) could be measured in 3 experiments, but were negligible in others (Fig. 4, Table I).

Discussion. The ionic transfers induced in a vascular bed by pressor doses of epinephrine or vasopressin are similar to previous observations relating to ionic shifts of vasoconstriction (6, 7, 9, 10). The ionic movements are "compatible with a transient increase in passive permeability, during which Na^+ moves into, and K^+ out of, the cell, following their respective downhill gradients" (11).

Angiotensin, in doses inducing no or very small pressure changes in the tail vascular

TABLE I. Ionic Transfers Following Infusion of Epinephrine, Vasopressin, and Angiotensin.

Drug[a]	N[b]	Dose[c]	Δ Pressure[d] (mm Hg)	Δ Na^+[e] (μEq)	Δ K^+[e] (μEq)
Epinephrine	7	0.01–0.2	102 ± 23	−0.090 ± 0.018	+0.071 ± 0.037
Vasopressin	10	2.0 –8.0	6.7 ± 0.4	−0.066 ± 0.010	+0.093 ± 0.016
Angiotensin	7	0.01–0.2	< 3	+0.047 ± 0.006	−0.057 ± 0.01
	7	1.0 –4.0	28 ± 10	+0.019 ± 0.008	< 0.0005

[a] Epinephrine, vasopressin, and angiotensin infused for 2, 2, and 6 min, respectively.
[b] Number of rats.
[c] Epinephrine and angiotensin doses are expressed in μg/min, vasopressin in mU/min.
[d] Maximum increase in perfusion pressure.
[e] Transfer of ions into and out of the perfusing medium. Δ: +, transfer into; −, transfer out of the perfusing fluid.

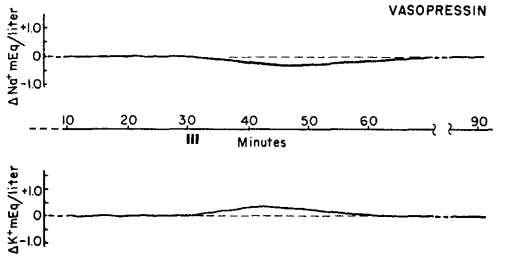

FIG. 2. Sodium and potassium activity following infusion of a pressor dose of vasopressin (6 mU/min). For a peak pressure change of 9.5 mm Hg, the Na^+ shift was 0.074 μEq and the K^+ shift was 0.084 μEq.

bed, consistently shifted the ions in the opposite direction, as compared to pressor doses of epinephrine or vasopressin. The net transfer of Na^+ into the perfusing medium, like the increased radioactive Na^+ efflux from arteries under the action of angiotensin (1, 5), is difficult to explain at the present time. A stimulation by angiotensin of the Na^+–K^+ pump across the smooth muscle cell membrane could account for both the Na^+ and K^+ transfers. Stimulation of membrane ATPase by angiotensin has been suggested by Türker *et al.* (1) to account for the increased Na^+ efflux from incubated arteries. This effect, however, disappeared when vasoconstriction occurred. Thus, when angiotensin significantly increased the perfusion pressure, no or negligible ionic movements were observed. The ionic changes associated with vasoconstriction may well have counterbalanced the direct ionic effects of angiotensin observed with smaller doses

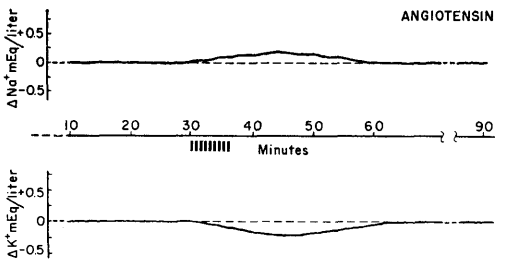

FIG. 3. Sodium and potassium activity following infusion of a subpressor dose of angiotensin (0.2 μg/min). Pressure change was negligible (<2 mm Hg). The Na^+ shift was 0.044 μEq and the K^+ shift was 0.053 μEq.

of this drug. Whatever the correct interpretation of these observations proves to be, it is apparent that angiotensin has at least two effects on vascular tissues, one of which induces a loss of Na^+ and a corresponding gain of K^+ by the tissue, and the other which triggers vasoconstriction. This duality of action may underlie the conflicting observations hitherto reported.

Summary. The net transfers of sodium and potassium ions induced in the rat tail vascular bed by infusion of three vasopressor agents have been measured by means of ion-selective capillary glass electrodes. Increases in perfusion pressure induced by epinephrine

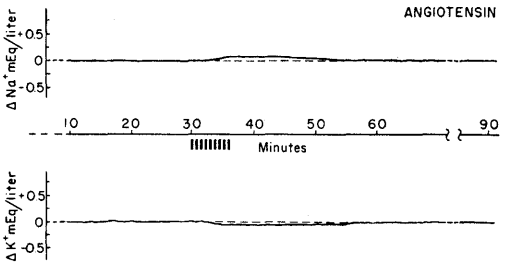

FIG. 4. Sodium and potassium activity following infusion of a pressor dose of angiotensin (1.0 μg/min). For a peak pressure change of 17 mm Hg, the Na^+ shift was 0.010 μEq and the K^+ shift was negligible (<0.0005 μEq).

or vasopressin were accompanied by a consistent loss of sodium and a gain in potassium by the perfusing medium. Pressor doses of angiotensin did not have any significant effect on sodium and potassium movements. At lower, subpressor doses, angiotensin shifted the ions in the opposite direction, resulting in a gain in sodium, and a loss of potassium by the perfusing fluid. Angiotensin thus appears to have at least two effects on vascular tissues, one inducing ionic transfers, the other triggering vasoconstriction.

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