Parasympathetic pathways, renin secretion and vasopressin release

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Summary

1. The interrelationship between parasympathetic neural tone, renin secretion and vasopressin release was examined by observing the effect of bilateral cervical vagotomy on renin secretion in intact and acutely hypophysectomized dogs undergoing a water diuresis.

2. In intact dogs bilateral cervical vagotomy decreased the mean renin secretion from 1245 to 682 units/min ($P<0.01$) as urinary osmolality increased from 95 to 414 mosmol/kg ($P<0.001$). In contrast, in acutely hypophysectomized dogs cervical vagotomy failed to alter renin secretion significantly (834 to 893 units/min) and urinary osmolality was also unchanged (78 to 71 mosmol/kg).

3. The results suggest that a diminution in vagal tone may significantly alter renin secretion by stimulating vasopressin release. Exogenous vasopressin was associated with changes in urinary osmolality and renin secretion which were qualitatively similar to those seen after cervical vagotomy.

4. We suggest that there is a neurohumoral reflex mechanism by which a fall in parasympathetic tone increases the release of vasopressin, which, in turn, suppresses renin secretion. The results are also compatible with the hypothesis that vasopressin inhibits renin release by a direct effect on the juxtaglomerular cells.

Key words: angiotensin, parasympathetic system, renal water excretion, renin, vagus, vasopressin.

Introduction

There is considerable evidence that the autonomic nervous system is involved in the regulation of renin secretion during haemorrhage (Blaine, Davis & Witty, 1970), carotid occlusion (Bunag, Page & McCubbin, 1966), renal nerve stimulation (Vander, 1965) and hypoglycaemia (Otsuka, Assaykeen, Goldfien & Ganong, 1970). In these circumstances, however, sympathetic rather than parasympathetic pathways seem to be of primary importance. The role of the parasympathetic pathways in the regulation of renin secretion is less clear. Conflicting results have been reported with regard to the effect of atrial distension on renin secretion (Brennan, Martin, Jochim & Donald, 1971; Genest, Granger, de Champlain & Boucher, 1968), even though this manoeuvre is known to increase consistently parasympathetic activity (Gauer & Henry, 1964).

Although Hodge, Lowe, Ng & Vane (1969) have reported that cervical vagotomy increases renin secretion in hydropenic animals, the effect of vagotomy might be different in circumstances where vagotomy significantly increases the release of vasopressin (Schrier & Berl, 1972). Since the exogenous infusion of vasopressin has been reported to suppress renin release (Bunag, Page & McCubbin, 1967; Tagawa, Vander, Bonjour & Malvin, 1971), the effect of vagotomy during the state of water diuresis could be to suppress rather than stimulate renin release. Such a finding would be important, since a role of endogenous vasopressin in the control of renin secretion has not been documented. In an effort to establish such a role of endogenous vasopressin, the present studies were...
undertaken to examine the effect of bilateral cervical vagotomy on renin secretion in intact and acutely hypophysectomized dogs with steroid replacement undergoing a water diuresis.

Methods

Experiments were performed on mongrel dogs of either sex weighing 20–30 kg. Food was withheld for 18 h and water was allowed ad libitum. On the day of the study the animals were anaesthetized with pentobarbital (30 mg/kg given intravenously), intubated and ventilated with a Harvard respirator. Light anaesthesia was maintained throughout the experiment by the intermittent administration of pentobarbital. Fourteen animals underwent a transbuccal hypophysectomy via a 2 cm hole in the hard palate on the morning of the experiment and were otherwise treated in the same manner as the remainder of the animals. Before further surgery, all animals received 15 μmol (5 mg) of deoxycorticosterone intramuscularly and the hypophysectomized animals also received dexamethasone 2·6 μmol (1 mg) intramuscularly and 1 mg intravenously.

At the time surgical procedures were started, a solution of 600 ml of 0·139 mol/l (2·5%) dextrose was infused intravenously at 20 ml/min for 30 min. Through bilateral retroperitoneal flank incisions catheters were placed in both ureters and renal veins. An adjustable Blalock clamp was placed around the aorta above renal arteries for use in controlling renal perfusion pressure. Catheters were also placed in the brachial and femoral arteries for continuous measurement of arterial pressure with Statham transducers (Statham Instruments Inc., Oxnard, Calif., U.S.A.). A jugular vein catheter was placed in the right atrium for injection of Indocyanine Green to measure cardiac output by the dye-dilution method (Schrier, Humphreys & Ufferman, 1971). In all but the eight animals infused with vasopressin, the vagus nerve was isolated bilaterally below the level of the carotid body and loose ligatures were placed around the nerve to allow later section in the neck. At the completion of surgery, which lasted approximately 1 h, an intravenous infusion of 0·154 mmol/l sodium chloride solution (0·5 ml/min) was started which contained sufficient inulin and p-aminohippuric acid to maintain concentrations between 15 and 20 mg of inulin/100 ml and 51 and 153 μmol of p-aminohippuric acid/l (1–3 mg/100 ml). At the same time a second infusion of 600 ml of 0·139 mol/l dextrose was given over 30 min and thereafter the infusion rate was decreased to 4 ml/min above urine flow. Experiments were not performed unless the urine osmolality was below 150 mosmol and urine flow rate was stabilized for 40 min. Urine collection periods were 5–10 min in duration throughout the experiment. Arterial and renal venous blood samples were obtained at the mid-point of alternate urine collections for measurements of renal blood flow and inulin clearance. Samples of arterial and venous blood were drawn every third period for renin measurements; measurements of cardiac output were made every third period.

The effects of cervical vagotomy on renin secretion were studied by using each animal as its own control with the following protocols.

Bilateral cervical vagotomy in intact and hypophysectomized dogs

Fifteen experiments were performed in nine intact and six hypophysectomized dogs. After three control periods the previously exposed vagi were sectioned. After an equilibrium period of 30 min three urine collections were made. The duration of these experiments ranged from 1 to 1·5 h. In six of these animals with cervical vagotomy (four intact and two hypophysectomized) eight experiments were performed in which isoproterenol was infused intravenously at a rate of 0·085–0·170 nmol (0·018–0·036 μg) kg/min. After a 30 min equilibration period three collection periods were made and the drug infusion was discontinued. After another 20–30 min equilibration period three post-control collection periods were made. The duration of these experiments ranged from 3½ to 4 h, including both the vagotomy and isoproterenol infusion.

Infusion of exogenous vasopressin in hypophysectomized dogs

In eight acutely hypophysectomized dogs three to five control collections of urine were obtained, and then vasopressin (Pitressin USP, Parke Davis Co.) was infused intravenously at a rate of 30–80 μunits min⁻¹ kg⁻¹. This dosage range of vasopressin was used to obtain an antidiuresis which was similar to that observed after cervical vagotomy. After 30 min of the vasopressin infusion three urine collections were made.
The analytical procedures and calculations used in the present study have been referred to elsewhere (Schrier & Earley, 1970). Renal plasma flow was calculated from the clearance and extraction of PAH. Plasma renin activity (PRA) was measured by using an immunoassay for angiotensin I without addition of renin substrate and was expressed as picamol (pmol) of angiotensin I formed per ml of plasma during a 3 h incubation (Stockigt, Collins & Biglieri, 1970). The rate of renin secretion (RSR) in units/min was calculated from the renal arteriovenous difference in PRA and renal plasma flow and expressed as units of PRA/min. All results are expressed as mean ± SE. Statistical significance was determined by using Student's t-test for paired data from the same animals. Other abbreviations used are: glomerular filtration rate (GFR), renal plasma flow (RPF), free water clearance (C\text{water}) and urine osmolality (U\text{osm}).

Results

Effect of cervical vagotomy on renin secretion in intact and hypophysectomized dogs (Table 1)

The effect of bilateral cervical vagotomy on renin secretion rate (RSR) and plasma renin activity (PRA) during water diuresis was quite different in the intact and in the hypophysectomized animals (Table 1). In the intact dogs vagotomy decreased RSR from a mean of 1245 ± 286 units/min to 682 ± 165 units/min (P < 0.01) and PRA from a mean of 8.7 ± 1.2 to 6.5 ± 1.5 pmol 3 h$^{-1}$ ml$^{-1}$ (P < 0.02). These changes in RSR and PRA were accompanied by an antidiuresis as the mean U\text{osm} increased from 95 ± 12 to 414 ± 62 mosmol/kg, an effect which has been suggested to be mediated by the release of vasopressin (Schrier & Berl, 1972). The fall in RSR and PRA, and the rise in U\text{osm}, were observed immediately after the equilibration period. In the hypophysectomized animals bilateral cervical vagotomy was not associated with significant changes in either renal water excretion or the release of renin. Hypophysectomy also influenced the effect of vagotomy on cation excretion. The excretion of sodium and potassium increased after vagotomy in the intact animals but not in the hypophysectomized animals. In both the intact and hypophysectomized dogs, cervical vagotomy was associated with an immediate but transient rise in arterial blood pressure (5–15 mmHg), which had returned to the control value before collections in the experimental periods. Cardiac output did not change in either group. The changes in glomerular filtration rate (GFR), although small, were statistically significant. This small effect of vagotomy on filtration rate is not a consistent finding since it was not observed in another group of animals studied with the same experimental protocol (Schrier & Berl, 1972).

In the six vagotomized animals that received intravenous isoproterenol, PRA increased from 12.1 ± 3.8 to 21.0 ± 5.0 pmol 3 h$^{-1}$ ml$^{-1}$ (P < 0.01) during the infusion and decreased to 13.0 ± 3.4 pmol 3 h$^{-1}$ ml$^{-1}$ (P < 0.02) after the infusion. Concomitantly mean RSR increased from 1530 ± 492 to 2260 ± 536 units/min (P < 0.02) and returned to 1792 ± 469 (P < 0.1) after isoproterenol. As previously reported in intact animals, the effect of intravenous isoproterenol in stimulating renin secretion occurred despite constancy of renal perfusion pressure, GFR, electrolyte excretion and renal denervation (Reid, Schrier & Earley, 1972). These results thus indicate that intact vagal fibres are not necessary for the effect of intravenous isoproterenol in stimulating renin secretion. The reversibility of this effect of isoproterenol also attests to the stability of the experimental model. This stability of the experimental model is important since post-control periods could obviously not be obtained after cervical vagotomy. Another three animals were examined as ‘sham’ vagotomy studies to investigate further the stability of the PRA over the same period of time as the vagotomy experiments. Eight sequential mean PRA values (pmol 3 h$^{-1}$ ml$^{-1}$) over the duration of the three experiments were as follows: 12 ± 3, 12 ± 4, 12 ± 4, 10 ± 2, 11 ± 2, 11 ± 2, 11 ± 2, 12 ± 2. Thus, in neither these ‘sham’ experiments nor in the isoproterenol studies was there evidence that the duration of the experiment or some other aspect of the model accounted for the highly consistent fall in PRA and RSR after vagotomy in intact animals. Moreover, any such effect might be expected in all animals studied and yet the hypophysectomized animals did not demonstrate such a fall in PRA and RSR after vagotomy.

Effect of exogenous vasopressin on renin secretion in hypophysectomized dogs (Table 2)

Exogenous vasopressin was demonstrated to suppress renin secretion in acutely hypophysecto-
TABLE 1. Effect of cervical vagotomy on systemic haemodynamics, solute and water excretion, and renin secretion in intact and hypophysectomized dogs

Results are mean values for the indicated number (n) of experiments. Values for renal haemodynamics, water excretion, electrolyte excretion and renin rates are expressed per kidney. In each animal the results are means of three to five collection periods. C = mean values for control periods; E = mean values for periods after cervical vagotomy; N.S. = not significant.

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<th>Cardiac output (l/min)</th>
<th>Systemic arterial pressure (mmHg)</th>
<th>Renal perfusion pressure (mmHg)</th>
<th>Glomerular filtration rate (ml/min)</th>
<th>Renal plasma flow (ml/min)</th>
<th>Sodium excretion (μmol/min)</th>
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<th>Free water clearance (ml/min)</th>
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<td>Vagotomy in hypophysectomized dogs</td>
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TABLE 2. Effect of vasopressin infusion on systemic haemodynamics, renal haemodynamics, solute and water excretion and renin secretion in hypophysectomized dogs

Results are mean values for the indicated number (n) of experiments. The values of renal haemodynamics, electrolyte excretion, water excretion and renin secretion rates are expressed per kidney. In each animal the results are means of three to five collection periods; C = mean values for control periods; E = mean values for periods after exogenous infusion of vasopressin. N.S. = not significant.

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<th>Plasma renin activity (pmol 3 h⁻¹ ml⁻¹)</th>
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mized animals. During the infusion of vasopressin in eight hypophysectomized animals undergoing a water diuresis there was a significant diminution in PRA from 31·1 ± 9·4 to 18·5 ± 5·4 pmol 3 h⁻¹ ml⁻¹ (P < 0.0025) and in RSR from 2177 ± 255 to 886 ± 246 units/ml (P < 0.005). These decreases in PRA and RSR occurred in the absence of significant changes in cation excretion and in spite of concomitant decreases in GFR and RPF. Although arterial pressure rose, renal perfusion pressure was controlled throughout the experiments. There was no ready explanation for the higher mean control renin values in this group of hypophysectomized animals. The effect of exogenous vasopressin in suppressing renin secretion, however, was observed in each individual experiment and thus occurred over a wide range of control values of PRA and RSR.

Discussion
Although the influence of the adrenergic nervous system in the regulation of renin secretion is well established (Ganong, 1972), the importance of the parasympathetic nervous system has not been clearly demonstrated. Increases in atrial pressure have been reported both to increase (Genest et al., 1968) and to decrease (Brennan et al., 1971) renin secretion and there are no apparent reasons for these discrepancies. Both groups of investigators, however, suggested that alterations in vagal tone are involved in these effects on renin secretion. These results are not only contradictory but in neither instance was an efferent limb demonstrated whereby alterations in vagal afferent tone might account for changes in renin secretion. Hodge et al. (1969) have interrupted cervical vagal pathways and reported increases in circulating concentrations of angiotensin II as measured by the blood-bath technique. However, studies have not been performed with more sensitive methods of measuring renin or angiotensin to confirm this observation. Since cervical vagotomy has been shown to increase the release of vasopressin (Schrier & Beri, 1972), a suppression of renin secretion by vasopressin might be proposed. The basis for such a possibility is the finding by several investigators that an exogenous infusion of vasopressin is associated with suppression of renin release (Bunag et al., 1967; Tagawa et al., 1971). These findings with exogenous vasopressin infusion, however, do not demonstrate a physiological role of endogenous vasopressin in the regulation of renin secretion. Rather, factors other than vasopressin release may predominate in the regulation of renin release, and the results of Hodge, et al. (1969) suggested that this might be the circumstance during bilateral cervical vagotomy. For example, vagotomy may increase sympathetic tone, which in various circumstances is known to increase renin secretion (Ganong, 1972).

In the present investigation further studies were performed to examine the role of the parasympathetic nervous system in the regulation of renin secretion. In order to demonstrate any role of increased release of vasopressin during cervical vagotomy, the initial studies were performed in animals undergoing a water diuresis. Although arterial pressure rose, renal perfusion pressure was controlled throughout the experiments. There was no ready explanation for the higher mean control renin values in this group of hypophysectomized animals. The effect of exogenous vasopressin in suppressing renin secretion, however, was observed in each individual experiment and thus occurred over a wide range of control values of PRA and RSR.

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In the present investigation cervical vagotomy was also demonstrated not to abolish the effect of systemic beta-adrenergic stimulation with intravenous isoproterenol in increasing renin secretion in either the intact or hypophysectomized dog. Moreover, the infusion of exogenous vasopressin into these hypophysectomized animals was found to suppress renin secretion. The mechanism whereby vasopressin suppresses renin secretion seems to be independent of alterations in sodium delivery to the macula densa, since this effect has been recently demonstrated to occur in the non-filtering kidney (Shade, Davis, Johnson, Gotshall & Spielman, 1973). Our results are also consistent with this interpretation since, if anything, vasopressin infusion appeared to diminish the filtered load of sodium since a small fall in GFR occurred. Based on the macula densa theory, such an effect might be expected to increase, rather than decrease, renin secretion (Vander, 1967).

Taken together, the present data therefore indicate that alterations in parasympathetic tone are involved in the regulation of renin secretion. In the neurohumoral reflex defined in the present investigation, a diminution in vagal afferent discharges is associated with increased release of vasopressin, which in turn circulates to the kidney and suppresses the release of renin. The data of Hodge et al. (1969) suggest that, in the hydropenic state, in which concentrations of endogenous vasopressin are high, cervical vagotomy may actually stimulate rather than suppress renin secretion. We have, however, failed to find support for this possibility in the present study, since with bilateral cervical vagotomy in hypophysectomized animals, in which vasopressin was presumably absent, no consistent effect on renin secretion was observed. It is possible, however, that hypophysectomized animals do not increase their sympathetic tone after vagotomy to a degree comparable with that of intact animals after vagotomy; thus an increase in renin secretion may not be observed in such animals. In any case, on the basis of the present results we conclude that a major effect of alterations in parasympathetic tone on renin release is indirectly mediated by altering the release of vasopressin, an effect which is most apparent with low endogenous amounts of vasopressin. Since alterations in systemic haemodynamics frequently alter parasympathetic tone in both low- and high-pressure cardiovascular sites (Gupta, Henry, Sinclair & Von Baumgarten, 1966) these pathways may be frequently activated and thereby enhance or compete with other stimuli which influence renin secretion.

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