Vasopressin effects on plasma renin activity in male and female rats

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Summary
1. The influence of gonadal and pituitary factors on the plasma renin response to exogenous vasopressin was examined in anaesthetized rats.

2. Plasma renin activity (PRA) was measured in Brattleboro rats (with and without hypothalamic diabetes insipidus) and Long–Evans male and female rats, before and after single intravenous injection of antidiuretic hormone (ADH) or saline.

3. Control saline injections did not change PRA. ADH reduced PRA in male, but increased PRA in female rats. Rats with diabetes insipidus displayed the greatest changes and were used in subsequent experiments. Extrarenal renin activity (nephrectomized rats) gave qualitatively similar responses to ADH. Plasma renin concentration, which was also measured with PRA in intact and nephrectomized male and female rats with diabetes insipidus, increased in the females and decreased in the males after ADH.

4. Castration, 24 h before study, abolished the ADH-induced fall in PRA, and testosterone implanted before castration did not restore the response. Cyproterone acetate reversed the ADH effect in males, so that PRA then rose after ADH. Hypophysectomized male rats, with depressed basal plasma renin activities, also showed a reduced PRA after ADH.

5. Ovariectomy had little effect on the ADH-induced renin release and the response was similar at oestrus, metoestrus and dioestrus. In hypophysectomized female rats ADH reduced PRA; a male pattern of response was seen in hypophysectomized female rats.

6. In both sexes PRA responses to ADH were blunted but not abolished by β-adrenergic blockade (propranolol). α-Adrenergic blockade (phenoxybenzamine) had little influence on the male response but in females the typical increase disappeared so that ADH reduced PRA.

7. It is concluded that pituitary hormones, including gonadotrophins and gonadal factors as well as adrenal sex steroids, appear to affect significantly the interplay between antidiuretic hormone and the renin–angiotensin system.

Key words: antidiuretic hormone, cyproterone acetate, hypophysectomy, ovaries, plasma renin activity, testes, testosterone.

Abbreviations: ADH, antidiuretic hormone; DI, diabetes insipidus; PRA, plasma renin activity; PRC, plasma renin concentration.

Introduction
Vasopressin (antidiuretic hormone) and the renin–angiotensin system are potent factors in the regulation of water balance, and at the same time have secondary influences upon sodium metabolism (Laragh & Cannon, 1962; Andersson & Erikson, 1971; Fitzsimons, 1972; Laragh & Sealey, 1973). Exogenous antidiuretic hormone (ADH) is generally considered to diminish plasma renin activity (PRA) in dogs (Buñag, Page & McCubbin, 1967; Vander, 1968), rats (Gutman & Benzakein, 1974) and man (Khokhar, Slater, Forsling & Payne, 1976; Joppich & Weber, 1976). On the other hand, stimulation of renin release or administration of exogenous angiotensin tend to
increase plasma ADH titres (Bonjour & Malvin, 1970; Malvin, 1973).

In man, the marked variations in the activity of the renin–angiotensin system related to the menstrual cycle, to pregnancy and to the use of orally active contraceptive steroids, have been associated with modified cardiovascular and adrenocortical function (Laragh & Sealey, 1973). The interaction between the changes in the renin–angiotensin system, gonadal steroids and vasopressin require further elucidation in both man and experimental animals.

Investigations into the effects of exogenous ADH on Brattleboro rats with hypothalamic diabetes insipidus revealed that the homozygous form (DI) had higher plasma renin activities than the heterozygous genotype (non-DI) (Gutman & Benzakein, 1971; Gross, Dauda, Kazda, Kynčl, Möhring & Orth, 1972; Möhring, Möhring, Dauda & Haack, 1974; Balment, Henderson & Oliver, 1975a). In male DI rats held in metabolism cages and given daily ADH injections, PRA decreased to non-DI values, whilst female DI animals showed a slight elevation (Balment et al., 1975a). In subsequent acute studies (Balment et al., 1975b) a qualitatively similar pattern of response to ADH given intravenously was found in pentobarbitone-anaesthetized rats, and although the sexual differences were more marked in the Brattleboro rat they were not unique to this strain. Gonadal and especially testicular factors also influenced the eventual PRA responses to ADH (Oliver, Balment & Henderson, 1976). This sexual dimorphism in the renin–angiotensin system with respect to ADH is the subject of the present studies.

Materials and methods

Animals

Brattleboro rats homozygous (DI) and heterozygous (non-DI) for the trait of hypothalamic diabetes insipidus, and normal Long–Evans rats (the strain from which Brattleboro rats were derived; Valtin, Schroeder, Benirschke & Sokol, 1962) were bred in the Department of Zoology, University of Sheffield. Animals were sexually mature; males ranged from 200 to 350 g body weight and females from 150 to 280 g. Rats were provided with tap water and a diet (B.P. Nutritional Ltd., Witham, Essex, U.K.; R. & M no. 1) containing (mmol/g): Na, 0.119; K, 0.213; Ca, 0.139 and Cl, 0.182. Nephrectomies, hypophysectomies and gonadectomies were performed under ether anaesthesia. The stages of the oestrous cycle were assessed by vaginal smears taken immediately before the acute study.

Experimental procedure

Under pentobarbitone anaesthesia (Nembutal, Abbott Laboratories; 60 mg/kg body weight, intraperitoneally) cannulae were placed in the urinary bladder (Clay Adams, Intramedic Catheter, PE90), the left carotid artery (PE50) and the right jugular vein (PE50). Animals were placed on a beam balance; body temperature and weight were kept constant throughout the experiment, fluid losses (blood specimens and urine) being replaced with intravenous saline (NaCl, 155 mmol/l). Mean arterial pressure was recorded with a Statham P23 Db transducer coupled to a Beckman Dynograph.

Thirty minutes after completion of preparative surgery, designated time zero, 7.5 μl of NaCl solution (155 mmol/l) was injected into the jugular vein, and carotid arterial specimens (0.3 ml) were withdrawn 5 and 12 min later. At time 20 min, 75 μunits of ADH were injected intravenously in 7.5 μl of saline. Further arterial specimens were collected at 25, 32 and 42 min. This protocol was rigidly adhered to. Any animals in which deviations likely to affect the experiment (excessive blood losses, extreme hypotension of unexplained cause etc.) occurred, were discarded.

Experimental groups

(i) DI male and female rats were given saline (7.5 μl) at 0 and 20 min as described above, to serve as controls for the following studies with ADH.

(ii) Intact male and female DI, non-DI and Long–Evans rats were given saline at time zero and ADH at 20 min. As DI rats consistently gave the greatest PRA responses to ADH, these animals were used in all subsequent experiments.

(iii) Testicular influence was examined in three groups of DI male rats: one group was castrated 24 h before study; a second group, which received a testosterone implant (10 mg) on day 1, were castrated on day 2 and studied on day 3; a third group were injected subcutaneously with cyproterone acetate (5 mg/day) for 2 days before study.

(iv) Ovarian influences were examined in DI females ovariectomized 24 h before study, and also females in dioestrous, metoestrus and oestrus.

(v) Adrenergic receptor mechanisms were studied in DI male and female rats given phenoxybenzamine (2 mg/kg body weight) or propranolol (1.25 mg/kg). The blocking agents were injected...
intravenously immediately on completion of preparative surgery. Preliminary experiments established the effectiveness of these doses at the beginning and end of the experimental periods: adrenaline and isoprenaline injected in doses that affected blood pressure of unblocked anaesthetized rats were ineffective, so verifying adequate α- and β-adrenergic blockade respectively. In the experimental animals completeness of the blockade was checked by injecting adrenaline and isoprenaline at the end of the study.

Analysis

Plasma renin activity (PRA) was determined indirectly by radioimmunoassay of angiotensin I generated during a 16 h incubation of thawed plasma in the presence of inhibitors at pH 5.5. Plasma renin concentration (PRC) was measured by addition of excess renin substrate to the incubation mixture (Stockigt, Collins & Biglieri, 1971).

Drugs and hormones

The following were used: ADH (Pitressin, Parke Davis), 1-deamino-8-arginine vasopressin (DDAVP; Ferring Pharmaceutical), testosterone (testosterone propionate; Organon Laboratories), cyproterone acetate (CHG 15076; Schering AG, Berlin), phenoxybenzamine hydrochloride (Dibenylene; Smith, Kline and French), propranolol (Inderal; I.C.I. Ltd). Pitressin, standardized in our laboratory against the 3rd International Standard by using the rat antidiuretic assay, had an antidiuretic potency of 1.031. Angiotensin I standard (Spectrum Medical Laboratories, Los Angeles, Calif., U.S.A.) and 125I-labelled angiotensin I (NEN, New England Nuclear, Boston, Mass., U.S.A.) were used in the PRA and PRC radioimmunoassay. The iodinated angiotensin was purified by gel chromatography on Sephadex G-25. Renin substrate was prepared from plasma taken from male rats 24 h after bilateral nephrectomy (Boucher, Ménard & Genest, 1967).

Statistics

The mean arterial blood pressures, taken 30 min after completion of surgery, were compared by unpaired t-tests. The changes in PRA induced by ADH injections were tested for statistical significance by the paired t-test. Measurements during control periods (at 5 and 12 min) were compared with those after ADH injections (at 25, 32 and 42 min): each animal thus served as its own control. P values of less than 0.05 are quoted in Tables and text.

Results

Basal blood pressure (Table 1)

Basal blood pressures of male and female rats, in the various groups, were similar. Moreover, the

<table>
<thead>
<tr>
<th>Type of rat</th>
<th>Arterial blood pressure (mmHg)</th>
<th>P (vs DI male)</th>
<th>Female</th>
<th>P (vs DI female)</th>
<th>P (male vs female)</th>
</tr>
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<tbody>
<tr>
<td>DI</td>
<td>126.5 ± 7.5 (5)</td>
<td></td>
<td>111.9 ± 6.1 (7)</td>
<td>-</td>
<td>n.s.</td>
</tr>
<tr>
<td>Non-DI</td>
<td>140.4 ± 5.5 (6)</td>
<td>n.s.</td>
<td>123.3 ± 5.1 (6)</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Long-Evans</td>
<td>140.0 ± 8.9 (5)</td>
<td>n.s.</td>
<td>121.7 ± 4.7 (6)</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>DI, 24 h gonadectomy</td>
<td>118.0 ± 11.3 (5)</td>
<td>n.s.</td>
<td>140.4 ± 12.8 (4)</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>DI, 24 h nephrectomy</td>
<td>92.5 ± 7.5 (6)</td>
<td>&lt;0.05</td>
<td>85.0 ± 5.5 (7)</td>
<td>&lt;0.05</td>
<td>n.s.</td>
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<tr>
<td>DI, 24 h hypophysectomy</td>
<td>89.2 ± 8.8 (6)</td>
<td>&lt;0.05</td>
<td>81.3 ± 6.5 (5)</td>
<td>&lt;0.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>DI + propranolol</td>
<td>94.0 ± 7.5 (5)</td>
<td>&lt;0.05</td>
<td>96.0 ± 6.8 (5)</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>DI + phenoxybenzamine</td>
<td>86.0 ± 6.9 (5)</td>
<td>&lt;0.02</td>
<td>80.0 ± 5.5 (5)</td>
<td>&lt;0.02</td>
<td>n.s.</td>
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<tr>
<td>DI + cyproterone acetate</td>
<td>138.3 ± 4.4 (6)</td>
<td>n.s.</td>
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<tr>
<td>DI, 24 h castrate + testosterone</td>
<td>120.8 ± 4.5 (6)</td>
<td>n.s.</td>
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</table>
artrial blood pressures observed in the presence of diabetes insipidus (DI group) were similar to those of both non-DI and Long–Evans rats. Gonadectomy had no effect, and nephrectomy, hypophysectomy and treatment with propranolol or phenoxybenzamine reduced blood pressure of both male and female DI rats, although the difference was not statistically significant in propranolol-treated female DI rats.

Responses to ADH

The dose of ADH given (75 μunits/rat, equivalent to 2–4.5 munits/kg body weight) occasionally produced mild pressor responses, but in most experiments blood pressure remained constant throughout. Changes in blood pressures and urine flows, where they occurred, were not related to the sex of the animal. Saline injections given at times zero and 20 min and the serial collection of blood specimens in the protocol had no effect on PRA.

The maximal PRA response to ADH occurred 10 min after injection, with return to control values within 25 min (Tables 2 and 3).

Male rats (Table 2). PRA fell in all intact male rats after ADH; on average DI animals showed greater reductions than non-DI and Long–Evans rats, but in all cases there was a return towards normal after about 25 min. In animals given only two saline injections PRA remained constant.

Castration 24 h previously prevented the fall in PRA induced by ADH, and although PRA fell after ADH in testosterone-implanted castrates, this fall was not statistically significant. Cyproterone acetate given to intact male DI rats completely reversed the response to ADH, and in this group PRA rose after the single ADH injection. Although basal PRA was reduced after hypophysectomy, the overall PRA responses to ADH were unaffected. In DI male rates extrarenal PRA, present after nephrectomy, was reduced by ADH. Propranolol had no obvious effect on the response to ADH, and phenoxybenzamine had no significant effect on the fall in PRA. Plasma renin concentrations (expressed in pmol of angiotensin I equivalents h⁻¹ ml⁻¹) in intact male DI rats (30.7 ± 2 SE 0.8, n = 5) and nephrectomized DI male rats (0.9 ± 0.1, n = 6) fell after ADH injections to 27.6 ± 1.0 and 0.7 ± 0.1 (P < 0.05 for both) respectively.

Female rats (Table 3). In DI and Long–Evans female rats there was a statistically significant rise in plasma renin activities after ADH administration, but this was not significant in non-DI females. The stage of the oestrous cycle had little influence either on the basal renin values or upon the response to ADH. Ovariectomized DI rats showed very large responses and the effect of ADH appeared more prolonged. Hypophysectomy of female DI rats reversed the normal female response. ADH given to hypophysectomized female rats caused a reduction in PRA. This latter
Gonadal factors and ADH actions on plasma renin

TABLE 3. Effects of antidiuretic hormone on plasma renin activities of anaesthetized female rats

Control values are compared with those taken 5 and 12 min after injection (Experimental) and with those after 22 min (Recovery). Mean values ± sem are shown with the number of experiments given in parentheses. (a) Comparison: Control vs Experimental; (b) comparison: Control vs Recovery. n.s., Not significant.

<table>
<thead>
<tr>
<th>Type of rat</th>
<th>Plasma renin activity (pmol of angiotensin I equivalents h⁻¹ ml⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>DI (two saline injections) (6)</td>
<td>5.31 ± 0.51</td>
</tr>
<tr>
<td>DI (6)</td>
<td>5.86 ± 0.53</td>
</tr>
<tr>
<td>Non-DI (6)</td>
<td>6.15 ± 0.23</td>
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<tr>
<td>Long-Evans (6)</td>
<td>7.67 ± 0.96</td>
</tr>
<tr>
<td>DI dioestrus* (5)</td>
<td>4.45 ± 0.54</td>
</tr>
<tr>
<td>DI oestrus* (5)</td>
<td>5.19 ± 0.37</td>
</tr>
<tr>
<td>DI metoestrus* (5)</td>
<td>4.86 ± 0.31</td>
</tr>
<tr>
<td>DI, 24 h ovariectomy (5)</td>
<td>5.11 ± 0.53</td>
</tr>
<tr>
<td>DI, 24 h hypophysectomy (5)</td>
<td>3.90 ± 0.31</td>
</tr>
<tr>
<td>DI, 24 h nephrectomy (7)</td>
<td>1.77 ± 0.18</td>
</tr>
<tr>
<td>DI + propranolol (5)</td>
<td>5.31 ± 0.24</td>
</tr>
<tr>
<td>DI + phenoxybenzamine (5)</td>
<td>5.93 ± 0.28</td>
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* Given I-deamino-8-D-arginine vasopressin (DDAVP) and not pitressin.

The response was similar to that of male rats, both intact and hypophysectomized. In the phenoxybenzamine-treated rats ADH caused PRA to decline; propranolol on the other hand, although blunting the typical PRA increase after ADH, did not abolish it. Extrarenal renin activity, as reflected in the nephrectomized series, also increased after ADH in female rats.

Plasma renin concentrations (in pmol of angiotensin I h⁻¹ ml⁻¹; mean value ± se) of intact (22.9 ± 0.8, n = 5) and nephrectomized (1.1 ± 0.1, n = 7) female DI rats were increased by ADH to 27.7 ± 1.2 (P < 0.05) and 1.5 ± 0.2 (P < 0.02) respectively. These changes in PRC followed those seen in PRA after vasopressin injections.

Discussion

There is considerable evidence of feedback between vasopressin release and angiotensin production; angiotensin may stimulate the release of ADH, and vasopressin inhibits the release of renin (Nash, 1971; Davis & Freeman, 1976). This scheme accounts for many aspects of body water homeostasis, in that angiotensin is potently dipsogenic whereas vasopressin regulates the renal output of water. Such interaction between angiotensin and vasopressin has not been established in all experiments, however, and it seems that extracellular fluid volume, sodium balance and overall endocrine status impinge on direct negative feedback relations (Claybaugh, Share & Shimizu, 1972; Shade, Davis, Johnson, Gotshall & Spielman, 1973; Goetz, Bond & Smith, 1974; Share, 1974; Claybaugh, 1976).

The many physiological and pharmacological actions of vasopressin and angiotensin such as those on vascular and nonvascular smooth muscle, sodium metabolism, release of adrenocorticotrophic hormone and adrenocortical function, pre-empt a simple and direct feedback between the two hormones. Our study suggests that other factors, related to the sex of the animal, influence vasopressin-induced changes in plasma renin activity. Plasma renin concentrations changed in parallel with plasma renin activity, so that a genuine release of renin occurred after ADH injections. Male animals showed a depressed PRA after ADH injections, and females displayed a very marked increase. The residual, extrarenal renin responses to ADH also displayed a sexual difference; the mechanism whereby ADH exerts its actions on renin release is thus shared by renal and extrarenal renin production sites. Such responses argue against a macula densa mechanism being solely responsible for antidiuretic hormone actions...
on renin release, a conclusion also reached by Shada et al. (1973) from studies on non-filtering kidneys of sodium-depleted female dogs.

β-Adrenoreceptors have minimal qualitative influences on vasopressin-induced changes in PRA, since the changes in males and females were unaffected by propranolol. α-Adrenoreceptor blockade, however, blunted the fall in PRA unaffected by propranolol. α-Adrenoreceptor mechanisms are reduced by oestrogens. Gonadal, especially testicular, factors influence the eventual change in PRA after ADH injections. Thus castration abolished the response completely, and testosterone only partly restored it, whereas the anti-androgen, cyproterone acetate, induced a female-type reaction in PRA after ADH. Ovariectomy had a slight effect, marginally enhancing the degree to which PRA increased after ADH. The complex endocrine changes of the oestrous cycle had little effect on the overall actions of vasopressin on PRA.

Hypophysectomized female rats failed to show the usual increase in PRA after ADH, displaying a male-type response; hypophysectomized males showed the usual fall in PRA after ADH. The pituitary factor responsible for these effects is not known. Studies on interactions of pituitary hormones on the renin–angiotensin system and on electrolyte balance have so far failed to elucidate primary mechanisms (Goodwin, Kirshman, Sealey & Poulsen, 1973; Reid, 1977). In addition, oestrogens, progestogens, gluco- and mineralocorticoids, by affecting electrolyte and water balances (Johnston, Davis, Baumber & Schneider, 1970; Oparil, Ehrlich & Lindheimer, 1975; Johnson, Davis, Brown, Wheeler & Witty, 1972; Deis, Lloyd & Pickford, 1963; Laragh & Sealey, 1973) alter synthetic and release mechanisms, which may themselves change in sensitivity to ADH. These considerations, together with known male–female differences in cardiovascular reactivity, sympathetic tone (Albrecht, 1974; Buñag, Walaszek & Mueting, 1975; Buñag, 1976), and adrenocortical function, in particular aldosterone metabolism (Morris, Caron, Graham, Silverman & De Conti, 1975; Morris, Berek & Davis, 1973), clearly allow considerable sexual variation in the properties of renin-release mechanisms.

Although the present results cannot be readily extrapolated to humans, it would be surprising if the sexual divergence of the renin response to exogenous vasopressin were unique to the rat. Many clinical and experimental studies in this area have made little reference to sex, and indeed, data from males and females have frequently been pooled. The water and electrolyte balances of humans and experimental animals are known to be modified during pregnancy or after gonadal steroid therapy, and variations such as water retention, natriuresis, hyperaldosteronism and hypertension may reflect a qualitative sex difference in the reactivities of the renin–angiotensin and hypothalamo–neurohypophysial systems.

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References


