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BLOOD PRESSURE AND RENAL FUNCTION IN A NOVEL VASOPRESSIN-DEFICIENT, GENETICALLY HYPERTENSIVE RAT STRAIN

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SUMMARY

1. Hereditary hypothalamic diabetes insipidus was introduced into the New Zealand genetically hypertensive (NZGH) rat and its normotensive substrain (NZN) by cross-breeding males with female Brattleboro diabetes insipidus (DI) rats.

2. Selective breeding of the resultant DI/hypertensive (DI/H) rats on the basis of maximum systolic blood pressure and vasopressin deficiency produced animals in the F_6 generation with blood pressures at 10 weeks of age higher than in DI/normotensive rats (DI/N), but much lower than in age-matched NZGH animals. Age-matched NZN and DI/N rats had comparable blood pressures.

3. Fluid turnover was far greater in DI/N and DI/H rats than in NZN and NZGH rats. Although comparable in DI/N and NZN rats, water balance (intake − urinary loss) was reduced in DI/H rats by comparison with NZGH rats.

4. Sodium balance was lower in DI/N rats compared with NZN rats but did not differ between DI/H and NZGH animals. Both DI groups had lower potassium balances.

5. Basal plasma vasopressin was elevated in NZGH rats compared with NZN rats, while vasopressin was undetectable in DI animals. Plasma aldosterone levels did not differ between groups, but corticosterone was lower in DI/N and DI/H rats by comparison with NZN and NZGH rats.

6. Replacement of vasopressin to achieve physiological plasma hormone levels restored normal fluid management in DI animals and was associated with a modest increase in systolic blood pressure in DI/N animals, compared with sham-treated rats. A much larger increase in blood pressure was observed in AVP-treated DI/H animals, but blood pressure remained below that in NZGH rats.

7. It is apparent that vasopressin may contribute to the hypertension of the NZGH rat and that it may be required from an early age. The mode of this contribution is unclear, but abnormal renal responses have been identified.

INTRODUCTION

The role of arginine vasopressin (AVP) in the development and maintenance of genetic hypertension in the rat remains controversial. Elevated plasma concen-

trations of AVP have been reported in young spontaneously hypertensive rats (SHR) during the development of hypertension (Crofton, Share, Shade, Allen & Tarnowski, 1978), and a positive correlation between plasma AVP levels and the severity of hypertension has been demonstrated in the stroke-prone SHR (Möhring, Kintz & Schoun, 1979). Intravenous injections of specific vasopressin antisera have lowered blood pressure in the SHR (Möhring, Kintz & Schoun, 1978) and the stroke-prone SHR (Möhring *et al.* 1979). However, the administration of V1 (pressor) antagonists both acutely (Lang, Ganten, Ganten, Rascher & Unger, 1984) and chronically (Sladek, Blair & Mangiapane, 1987) had no effect on blood pressure in the SHR.

There is little published information concerning the potential role of AVP in the other major genetic model of hypertension, the New Zealand genetically hypertensive (NZGH) rat. Crofton, Share, Baer, Allen & Wang (1981) reported that NZGH rats, between the ages of 4 and 11 weeks, had similar urinary AVP excretion rates to those measured in control New Zealand normotensive (NZN) rats. At the age of 13–14 weeks plasma AVP concentrations were also similar in NZGH and NZN animals. However, NZGH rats show an exaggerated pressor response to AVP, such that normal plasma concentrations may exert a hypertensive influence (Phelan, Simpson & Smirk, 1976; Crofton *et al.* 1981). Indeed, preliminary observations indicate that chronic administration of a mixed V1–V2 (pressor and antidiuretic) antagonist lowers blood pressure in NZGH rats (Ashton & Balment, 1986).

In order to investigate the contribution of vasopressin to the development and maintenance of hypertension in the NZGH rat, we have cross-bred NZGH rats with Brattleboro diabetes insipidus (DI) rats. The resulting offspring have enabled the description of the development of hypertension and associated renal function in animals in the complete absence of vasopressin.

A preliminary report of some of these data was made at the First European Congress of Endocrinology (Ashton & Balment, 1987).

METHODS

Animals

The animals employed in this study were obtained from colonies in the Department of Physiological Sciences at the University of Manchester. A 12 h light:12 h dark photoperiod was maintained and animals were allowed free access to food (Labsure PMD diet) and water.

Breeding strategy

Congenital vasopressin deficiency was introduced into the hypertensive rat strain by pairing male New Zealand genetically hypertensive rats (NZGH, $n = 3$) with female Brattleboro rats homozygous for the diabetes insipidus trait (DI, $n = 3$). Offspring from the F_1 generation were selected and paired ($n = 5$ pairs) at random, since, as expected from its recessive form, no animals expressed the DI characteristic in this first generation. The resulting offspring were screened at 7–9 weeks for diabetes insipidus (24 h water intake > 65% body weight) and hypertension (systolic blood pressure > 130 mmHg by tail cuff plethysmography, under light ether anaesthesia, Narco Bio-Systems, USA; Phelan, 1968; Crofton *et al.* 1981). In subsequent generations, matings ($n = 3$–4 pairs), both brother–sister and across litters, employed DI males and non-DI females (animals heterozygous for diabetes insipidus) with the highest blood pressures.

A control strain was also produced by the same breeding strategy. Male New Zealand normotensive (NZN) rats ($n = 3$) were paired with female DI rats ($n = 3$) and the resultant offspring were paired at random. DI male and non-DI female rats ($n = 4$–5 pairs) were subsequently employed in each generation to produce vasopressin-deficient normotensive animals (DI/N) as controls for the vasopressin-deficient hypertensive rats (DI/H).

Metabolic studies

Blood pressure and renal function were monitored in adult male DI/N and DI/H rats from the F_6 generation for comparisons with measures in male NZN and NZGH rats. Following a 7 day equilibration period water and electrolyte turnover were assessed over 9 consecutive days in DI/N ($n = 7$), DI/H ($n = 5$), NZN ($n = 5$) and NZGH ($n = 5$) rats housed individually in metabolism cages (Jencons). Blood pressure was measured indirectly (tail cuff) on two separate occasions.

To investigate the effects of vasopressin replacement on renal function and blood pressure, 2 cm lengths of sealed polypropylene Accurel-PP-tubing (Enka AG, Obernburg, FRG) containing arginine vasopressin (6·47 μg/30 μl 0·154 M-NaCl, grade VI, Sigma) were prepared according to the method of Boer, Kruisbrink & Van Pelt-Heerschap (1983) and implanted subcutaneously, under ether anaesthesia, in DI/N ($n = 7$) and DI/H rats ($n = 5$). Animals were maintained in metabolism cages for 9 days post-implantation and blood pressure was measured. A sham-treatment procedure was performed, in parallel groups of animals, by implanting rats (DI/N, $n = 7$; DI/H, $n = 5$) with Accurel tubing loaded with 30 μl 0·154 M-NaCl.

Parallel groups of AVP-treated animals (DI/N, $n = 7$; DI/H, $n = 5$) were prepared as for the metabolic study in order that trunk blood samples could be taken by decapitation of unanaesthetized rats on the third post-implant day. Blood was similarly collected from untreated rats (DI/N, $n = 7$; DI/H, $n = 5$; NZN, $n = 7$; NZGH, $n = 7$). The separated plasma was stored at -20 °C prior to analysis for arginine vasopressin, aldosterone and corticosterone concentrations by specific radioimmunoassays. Urinary sodium and potassium concentrations were measured by flame photometry (Corning 455 flame photometer).

Arginine vasopressin radioimmunoassay

Plasma AVP concentration was measured by a modification of the method described by Robertson, Mahr, Athar & Sinha (1973). Plasma was deproteinized by adding 1 ml acetone to 0·5 ml plasma, and following centrifugation (1000 $g \times 5$ min) the supernatant was removed. Three millilitres of petroleum ether was added and the resultant organic phase removed. The aqueous phase was frozen to -70 °C in liquid nitrogen and lyophilized. Dried extracts were resuspended in 300 μl of phosphate buffer (pH 7·4). Extraction efficiency was considered to be $58·8 \pm 1·6$% ($n = 10$). No corrections for extraction losses have been made.

Vasopressin standard (triplicate 0–50 pg (100 μl)$^{-1}$ Arg^8-vasopressin, Cambridge Research Biochemicals Ltd) and plasma extracts (duplicate) were incubated with antisera (raised in rabbits to Arg^8-vasopressin, Calbiochem, Behring Diagnostics, USA) and ([3-^{125}I]iodotyrosyl^2) vasopressin [Arg^8] (Amersham International) at 4 °C overnight. Free and antibody-bound ^{125}I-labelled AVP were separated with 2% w/v bovine γ-globulin (Cohn Fraction II, Sigma) and 25% w/v polyethylene glycol 4000 (BDH Ltd). The pellets (bound fraction) were counted on an LKB 1275 mini γ-counter (Wallac, Finland).

The intra-assay coefficient of variation was 17·1% ($n = 10$) and the inter-assay coefficient of variation was 14·6% ($n = 12$). The minimum detectable plasma vasopressin concentration was 0·1 μU ml^{-1} (1 μU = 2·2 pg).

Aldosterone and corticosterone radioimmunoassays

Plasma aldosterone was measured by radioimmunoassay following initial separation from other steroids by LH20 chromatography (Milne, Balment, Henderson, Mosley & Chester-Jones, 1982). Inter- and intra-assay coefficients of variation were 12·7% ($n = 42$) and 14·4% ($n = 10$) respectively. Plasma corticosterone was measured by radioimmunoassay of ethanol-extracted samples as described by Kime (1977). Inter- and intra-assay coefficients of variation were 10·1% ($n = 30$) and 13·6% ($n = 10$) respectively.

Statistical analysis

All values are presented as the mean $\pm$ S.E. of the mean. Statistical comparisons were by Student's t test. A χ^2 analysis was also performed to examine the relationship between the incidence of the DI homozygote condition and sex of the animals.

RESULTS

Genealogy of the vasopressin-deficient hypertensive (DI/H) rat

The incidence of the DI characteristic and the level of blood pressure varied over the first six generations of both DI/N and DI/H strains. Approximately half of the hypertensive cross animals showed the DI trait in the later generations (F_5 49%, F_6 42%; Fig. 1) and there was no significant association between the DI condition and sex (F_2–F_6, $\chi^2 = 0{\cdot}001$–$1{\cdot}01$). A similar pattern of distribution was observed in

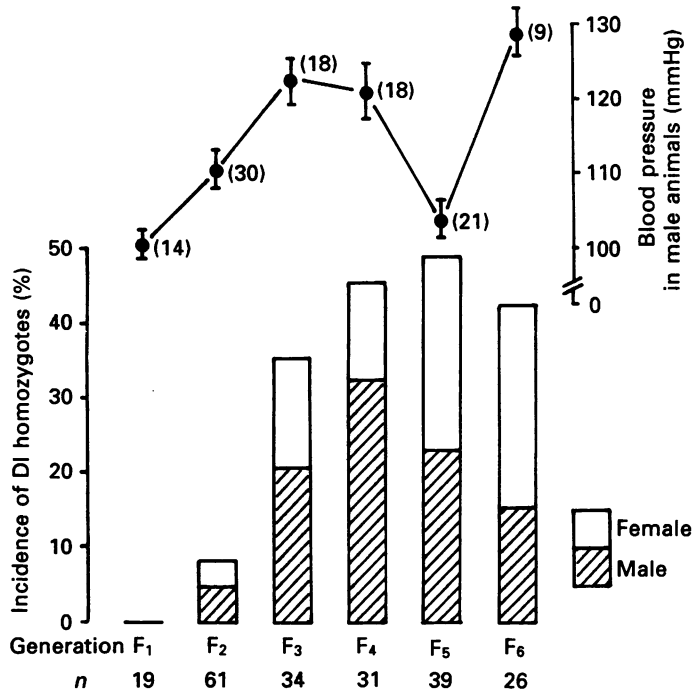

Fig. 1. Incidence of diabetes insipidus and blood pressure levels in the DI/hypertensive cross-breed strain for the first six generations. Upper panel shows systolic blood pressure in all male animals (n values in parentheses); lower panel shows the incidence of diabetes insipidus in both male and female animals (n = total number of animals in each generation).

the normotensive cross strain. The frequency of the DI characteristic was not significantly different from that observed in the hypertensive cross strain (F_5 42%, $\chi^2 = 0{\cdot}09$; F_6 31%, $\chi^2 = 0{\cdot}47$) and again there was no significant association with sex (F_2–F_6, $\chi^2 = 0{\cdot}04$–$1{\cdot}95$).

Following the initial DI/hypertensive cross a rise in mean systolic blood pressure was evident in the next five generations and is detailed for male DI/H rats in Fig. 1. Blood pressure continued to rise, though more slowly, in subsequent generations such that by the F_{11} generation this had reached $144{\cdot}0 \pm 3{\cdot}2$ mmHg ($n = 20$) in 11 to 14-week-old animals, compared with $154{\cdot}3 \pm 3{\cdot}7$ mmHg ($n = 7$, $P < 0{\cdot}05$) in age-matched NZGH rats. Neither male nor female DI/H rats achieved the levels of blood pressure observed in NZGH rats of equivalent age but were considerably

hypertensive with respect to DI/N and NZN animals. When animals exhibiting AVP deficiency are considered separately, at the age of 10 weeks male DI/H rats of the F_6 generation had blood pressures of $127{\cdot}5 \pm 2{\cdot}5$ mmHg ($n = 4$) compared with $142{\cdot}1 \pm 3{\cdot}4$ mmHg ($n = 7$) in male NZGH rats ($P < 0{\cdot}05$). Female DI/H animals also exhibited lower blood pressures than female NZGH rats ($102{\cdot}9 \pm 1{\cdot}8$, $n = 7$, *vs.* $133{\cdot}3 \pm 1{\cdot}7$ mmHg, $n = 6$, $P < 0{\cdot}001$). Male animals had consistently higher blood pressures than their female litter-mates (DI/H, $P < 0{\cdot}001$; NZGH, $P < 0{\cdot}05$). By contrast, DI/normotensive cross rats exhibited relatively stable blood pressures; F_6 male DI/N rats had blood pressures similar to those in previous generations and in NZN males ($102{\cdot}5 \pm 1{\cdot}4$, $n = 4$, *vs.* $95{\cdot}0 \pm 4{\cdot}5$ mmHg, $n = 8$, n.s.) at the age of 10 weeks. Female DI/N animals, however, had higher blood pressures than NZN females ($106{\cdot}9 \pm 3{\cdot}3$, $n = 8$, *vs.* $92{\cdot}2 \pm 4{\cdot}0$ mmHg, $n = 9$, $P < 0{\cdot}05$), though neither group differed from males.

Metabolic studies

Twenty-four hour renal water and electrolyte management in the DI/H and DI/N strains is compared with that of NZGH and NZN rats in Table 1. Values have been standardized in relation to body weight, where appropriate, since NZGH and DI/H rats were lighter than age-matched (20–25 weeks) normotensive animals (NZGH, $n = 5, 246{\cdot}2 \pm 12{\cdot}4$ g *vs.* NZN, $n = 5, 319{\cdot}0 \pm 7{\cdot}6$ g, $P < 0{\cdot}01$; DI/H, $n = 5, 370{\cdot}6 \pm 6{\cdot}4$ g *vs.* DI/N, $n = 7$, $387{\cdot}4 \pm 4{\cdot}7$ g, $P < 0{\cdot}05$). Although systolic blood pressures (mmHg) were similar in these older DI/N and NZN rats ($98{\cdot}7 \pm 1{\cdot}8$, $n = 12$, and $100{\cdot}5 \pm 1{\cdot}7$, $n = 10$), the blood pressure of DI/H animals was still somewhat lower than that of NZGH rats ($127{\cdot}0 \pm 1{\cdot}9$, $n = 10$ *vs.* $152{\cdot}0 \pm 3{\cdot}4$, $n = 10$; $P < 0{\cdot}001$).

As anticipated, fluid turnover in both DI/H and DI/N rats was far greater than in their AVP-replete counterparts. Both hypertensive groups (NZGH and DI/H) also produced significantly ($P < 0{\cdot}01$) more urine than respective normotensive animals. However, while water balance (water intake − urine volume) was greater ($P < 0{\cdot}05$) in NZGH than in NZN rats, DI/H animals maintained a smaller ($P < 0{\cdot}05$) water balance than DI/N rats. Urine osmolality was lower in both DI groups compared with AVP-replete rats, but urine osmolalities were comparable for hypertensive and normotensive groups both in the presence and absence of vasopressin.

The similar rates of food intake (g (100 g body wt)$^{-1}$ (24 h)$^{-1}$) in DI/N and DI/H rats ($7{\cdot}3 \pm 0{\cdot}2$ and $7{\cdot}2 \pm 0{\cdot}2$) were comparable with those observed in NZGH animals ($7{\cdot}2 \pm 0{\cdot}1$), while NZN rats exhibited a lower rate of food intake ($6{\cdot}0 \pm 0{\cdot}1$) than all other groups ($P < 0{\cdot}001$). Sodium balance did not differ between hypertensive and normotensive animals, nor between NZGH and DI/H rats. AVP-deficient DI/N rats, however, exhibited a lower sodium balance than NZN animals. NZGH rats maintained a larger ($P < 0{\cdot}01$) potassium balance than NZN rats, but this difference was not apparent in the DI substrains, both of which had lower potassium balances than the AVP-replete animals (Table 1).

The plasma concentrations of vasopressin, aldosterone and corticosterone in AVP-replete and AVP-deficient normotensive and hypertensive rats are shown in Fig. 2. Plasma AVP was significantly higher in NZGH rats than in NZN rats, and AVP was undectectable in plasma obtained from DI/N and DI/H rats.

TABLE 1. Water and electrolyte management in New Zealand genetically hypertensive (NZGH) and normotensive (NZN) rats and their vasopressin-deficient substrains (DI/H, DI/N). Balance values were calculated as the dietary intake minus urinary excretion

	n	Urine volume (ml (100 g body wt)$^{-1}$ (24 h)$^{-1}$)	Urine osmolality (mosmol kg^{-1})	Water balance (ml (100 g body wt)$^{-1}$ (24 h)$^{-1}$)	Sodium balance (μmol (100 g body wt)$^{-1}$ (24 h)$^{-1}$)	Potassium balance (μmol (100 g body wt)$^{-1}$ (24 h)$^{-1}$)
NZN	5	2·9 ± 0·1 ***	2098·5 ± 76·2 ***	11·2 ± 0·4	412·4 ± 20·4 **	1053·5 ± 33·5 ***
DI/N	7	51·5 ± 1·7	264·2 ± 9·3	10·8 ± 0·7	313·3 ± 20·3	720·7 ± 55·7
NZGH	5	3·8 ± 0·2 ***	1908·8 ± 106·7 ***	14·3 ± 0·9 ***	459·0 ± 44·5	1291·6 ± 48·2 ***
DI/H	5	67·6 ± 1·6	250·2 ± 9·9	7·1 ± 0·8	361·4 ± 19·9	725·9 ± 58·8

Statistical comparisons (*t* test) between AVP-replete and AVP-deficient animals of the normotensive and hypertensive strains are shown (** $P < 0·01$, *** $P < 0·001$).

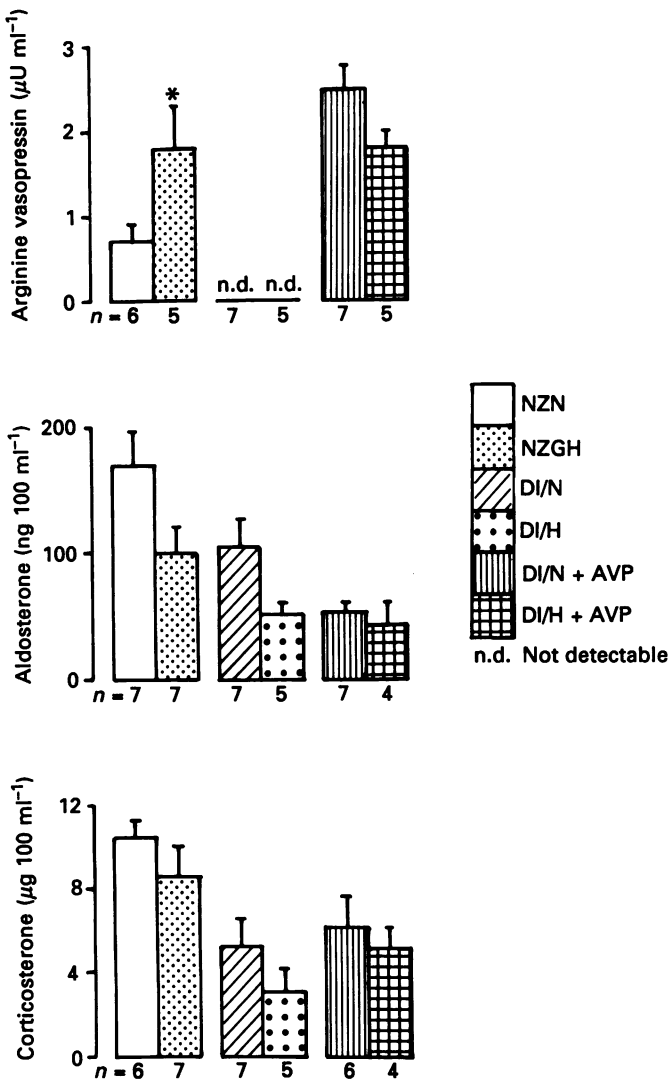

Fig. 2. Plasma hormone concentrations in AVP-replete (NZN, NZGH), AVP-deficient (DI/N, DI/H) and AVP-deficient rats in receipt of exogenous vasopressin (DI/N+AVP, DI/H+AVP) of the normotensive and hypertensive substrains. Statistical comparisons (*t* test) between normotensive and hypertensive animals are shown (*$P < 0.05$).

There were no statistically significant differences in the plasma concentrations of aldosterone and corticosterone between normotensive and hypertensive animals in either the presence or absence of vasopressin (Fig. 2). Corticosterone levels were lower in the vasopressin-deficient DI/N ($P < 0.01$) and DI/H rats ($P < 0.05$) compared respectively with NZN and NZGH animals, though aldosterone levels were not significantly altered.

Effects of vasopressin replacement

The circulating levels of exogenous AVP measured on the third post-implant day (Fig. 2) in DI/H rats closely matched the basal concentration of the peptide observed in untreated NZGH rats, while for DI/N rats the induced plasma AVP level was significantly greater ($P < 0.001$) than that observed in NZN rats. In both groups, however, the induced AVP levels were still within the physiological range. AVP replacement in DI/N rats was associated with lower ($P < 0.05$) aldosterone levels than in vasopressin-deficient DI/N rats. Corticosterone concentrations were not significantly altered, remaining lower ($P < 0.01$) than those observed in NZN animals. Plasma steroid concentrations in AVP-replete DI/H rats did not differ significantly from those observed in DI/H and NZGH animals.

Data from sham-implanted animals indicated that changes in water and electrolyte turnover over the first two post-operative days might reflect the effects of stress associated with implantation procedures. Accordingly, only measures on subsequent days have been taken to be representative of the potential effects of AVP replacement, in AVP-implanted rats. The characteristic polyuria and polydipsia of DI/N and DI/H rats were ablated by AVP replacement. Urine output (Fig. 3) remained significantly reduced ($P < 0.01$) over the first 5 days following AVP implantation but the antidiuretic action of exogenous AVP was clearly declining over this period. Accordingly, measures for the third day post-implantation have been taken to be representative of adequate AVP replacement, and free of sham-operative disturbance, for the consideration of the remaining parameters of fluid and electrolyte management.

The decline in urine flow was associated with increased urine osmolality (sham *vs.* experimental, DI/N 279.3 ± 11.8, $n = 7$, *vs.* 1014.9 ± 72.9 mosmol kg^{-1}, $n = 7$, $P < 0.001$; DI/H 281.4 ± 13.8, $n = 5$, *vs.* 946.2 ± 91.8 mosmol kg^{-1}, $n = 5$, $P < 0.01$). Drinking rates fell appropriately to match the decline in urine flow such that water balances remained stable (sham *vs.* experimental DI/N 6.5 ± 1.3 *vs.* 3.7 ± 0.6 ml (100 g body wt)$^{-1}$ (24 h)$^{-1}$, n.s. DI/H 6.6 ± 1.2 *vs.* 6.7 ± 0.7 ml (100 g body wt)$^{-1}$ (24 h)$^{-1}$, n.s.) as did body weights (sham *vs.* experimental, DI/N 405.6 ± 13.0 *vs.* 387.4 ± 4.7 g, n.s.; DI/H 363.9 ± 26.2 *vs.* 370.6 ± 6.4 g n.s.).

Neither AVP replacement nor sham operation significantly altered food intakes in either DI/N or DI/H rats. Sodium and potassium management appeared to be largely unaffected by AVP replacement in DI/N rats, which maintained stable sodium and potassium balances (sham *vs.* experimental, Na^+ 233.5 ± 48.6 *vs.* 236.1 ± 42.8 μmol (100 g body wt)$^{-1}$ (24 h)$^{-1}$, n.s.; K^+ 785.2 ± 116.2 *vs.* 719.0 ± 143.3 μmol (100 g body wt)$^{-1}$ (24 h)$^{-1}$, n.s.). The DI/H rats, however, showed modest sodium retention following AVP replacement, though potassium balance was unaltered (sham *vs.* experimental, Na^+ 176.6 ± 38.2 *vs.* 306.8 ± 30.0 μmol (100 g body wt)$^{-1}$ (24 h)$^{-1}$, $P < 0.05$; K^+ 639.6 ± 88.9 *vs.* 870.5 ± 112.0 μmol (100 g body wt)$^{-1}$ (24 h)$^{-1}$, n.s.).

On the third post-operative day systolic blood pressure was modestly elevated in AVP-treated DI/N rats by comparison with sham-operated controls (94.3 ± 2.3 *vs.* 105.0 ± 2.6 mmHg, $P < 0.01$), though this was still within the normal range for NZN rats. A much larger rise in blood pressure was evident in AVP-treated DI/H animals

in which systolic blood pressure rose to $133{\cdot}5 \pm 2{\cdot}9$ mmHg, compared with $127{\cdot}0 \pm 1{\cdot}6$ mmHg ($P < 0{\cdot}05$) prior to AVP and $113{\cdot}0 \pm 3{\cdot}7$ mmHg ($P < 0{\cdot}001$) in sham-treated animals. The raised blood pressure of AVP-treated DI/H rats, however, remained below that seen in NZGH animals.

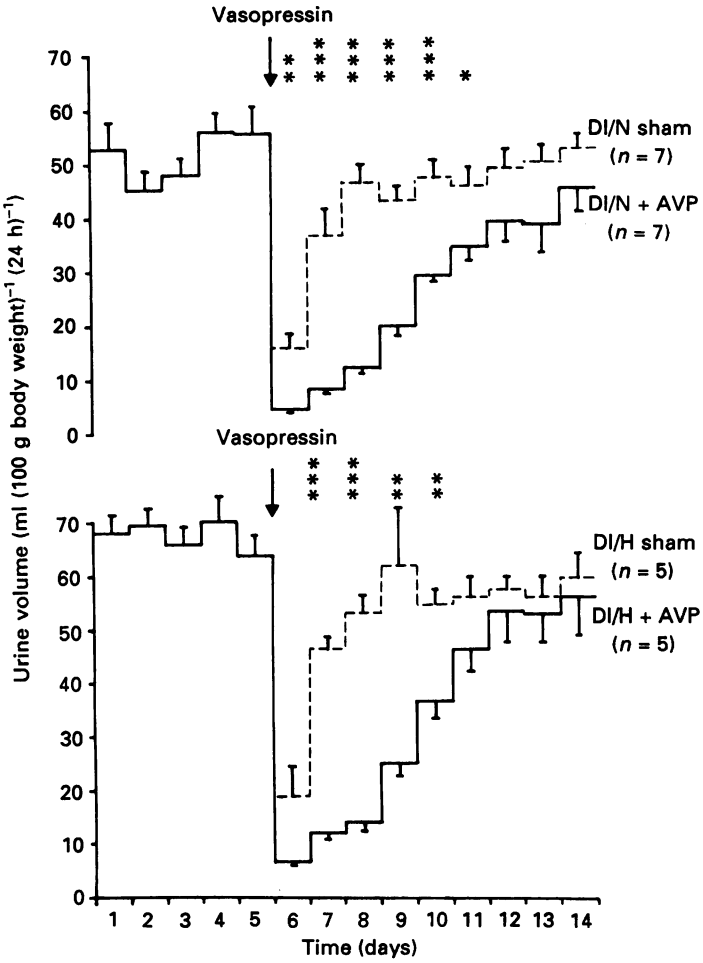

Fig. 3. The effect of vasopressin replacement and sham implantation on urine output in hypertensive (DI/H) and normotensive (DI/N) rats with diabetes insipidus. Statistical comparisons (*t* test) between sham and treated animals are shown (*$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$).

DISCUSSION

It was clear from the high fluid turnover rate and the absence of detectable AVP in plasma, that the DI gene had been successfully introduced into both NZN and NZGH strains. The reduction in fluid turnover and increased urinary osmolality achieved upon AVP replacement indicates that these animals responded normally to the peptide, supporting the concept that they exhibit an inability to secrete vasopressin, rather than a renal insensitivity to its actions. In this respect both

DI/N and DI/H rats resemble the parent Brattleboro DI strain (Rosenbloom & Fisher, 1975).

Some interesting changes in fluid management were associated with AVP deficiency in hypertensive rats. The AVP-replete hypertensive rat maintained a greater water balance than normotensive animals, but the reverse was observed in the DI animals, DI/H rats showing a 50% lower water balance than the DI/N group. The retention of water by NZGH rats is consistent with their elevated basal AVP levels, but the relative renal wasting of water by hypertensive animals in AVP deficiency suggests that fluid management may be substantially altered in the hypertensive rat. AVP replacement did not fully restore the water balance of DI/H rats to that seen in NZGH animals. Longer-term AVP replacement may be required to overcome the altered renal fluid handling resulting from chronic vasopressin deficiency (Lee & Williams, 1972).

Simpson, Phelan, Jones, Butt, Young & Ledingham (1979) have reported a reduced ability of NZGH rats, compared with NZN rats, to excrete an intravenous isotonic saline load. We did not observe such renal water retention upon hypotonic saline infusion, but the effects of acute AVP administration on both water and sodium excretion were altered. In particular, NZGH animals exhibited a blunted renal natriuretic response to vasopressin (Ashton & Balment, 1988). Although NZGH rats have reduced total carcass sodium, plasma sodium concentrations are normal (Simpson, Phelan, Clark, Jones, Gresson, Lee & Bird, 1973; Simpson *et al.* 1979; Crofton *et al.* 1981). An elevation in plasma aldosterone concentration has been reported in the adult NZGH rat (Carretero, Polomski, Hampton & Scicli, 1976) but this was attributed to secondary renovascular damage. Indeed, lower circulating levels of aldosterone might be expected as the activity of the renin–angiotensin system (RAS) is reduced in NZGH rats (Gresson, 1972; Simpson *et al.* 1973, 1979) in association with the high blood pressure and also the inhibitory effect of elevated vasopressin levels (Fyhrquist, Tikkanen & Linkola, 1981; Schwartz & Reid, 1986). However, circulating aldosterone levels in NZGH rats were not significantly different from those in normotensive animals in this study. Quantitatively, the levels of aldosterone in NZN and NZGH rats were much higher than those currently and previously measured in this laboratory for Sprague–Dawley, Long–Evans and Brattleboro rats (Milne *et al.* 1982). This elevation appears to represent a strain difference and is not related to the expression of hypertension. Exogenous AVP administration was associated with reduced plasma aldosterone concentration in DI/N animals, possibly reflecting an inhibitory effect of AVP on the renin–angiotensin–aldosterone axis (Fyhrquist *et al.* 1981; Schwartz & Reid, 1986), though no such effect was evident in DI/H rats. Circulating corticosterone levels were lower in both vasopressin-deficient hypertensive and normotensive groups, consistent with previous observations in the vasopressin-deficient Brattleboro rat (Buckingham & Leach, 1980; Milne *et al.* 1982). In the Brattleboro rat this is largely attributed to the reduced activity of the hypothalamic–pituitary–adrenal axis which results from the absence of a vasopressin component in the hypothalamic stimulation of pituitary corticotrophin secretion (Buckingham & Leach, 1980; Gillies & Lowry, 1980).

Following the cross of hypertensive and Brattleboro rats the initial offspring exhibited systolic blood pressures in the normal range. As might be expected from the additive multiple gene-based hypertension of the NZGH rat (Phelan, 1968),

blood pressure fluctuated but rose in subsequent generations as the genetic base was re-established. This rapid rise in blood pressure in initial generations of the new strain is comparable with that described for the early stock of the parent NZGH strain (Phelan, 1970). The fluctuation in blood pressure observed between early generations of the new strain was probably exacerbated by the routine screening of animals in weeks 7–9 of age, which spans the period of rapid blood pressure rise (25–30 mmHg) in these hypertensive animals (Crofton *et al.* 1981; Phelan & Simpson, 1987). The older DI/H animals (*ca.* 20 weeks) used in the metabolic studies exhibited systolic blood pressure levels greater than those normally observed in age-matched NZN and DI/N animals, but remained lower than those commonly shown by NZGH rats. Brattleboro rats maintain relatively normal arterial blood pressures, which are supported in part by an increased activity of the RAS (Balment, Henderson & Oliver, 1975; Gardiner & Bennett, 1983, 1985). The normal blood pressure of DI/N and the mild hypertension of DI/H rats may accordingly rely in part upon an activated RAS, though it would appear that the development of full hypertension requires the presence of vasopressin.

Previous reports in another vasopressin-deficient hypertensive rat model, a result of crossing Brattleboro rats with the stroke-prone SHR to produce an SHRDI strain (Ganten, Rascher, Lang, Dietz, Rettig, Unger, Taugner & Ganten, 1983), indicated that blood pressure in this type of hypertension did not rely upon vasopressin. These vasopressin-deficient animals had blood pressures comparable with those of the parent SHR animals, leading Ganten and co-workers to the conclusion that AVP was not required to maintain the elevated blood pressure in this type of rat. However, the pathogenesis of hypertension clearly differs in NZGH and SHR animals. Of particular note is the difference in the genetic regulation of hypertension in these two strains, which is less complex in the SHR, involving three gene loci compared with at least five loci in the NZGH animal (Okamoto & Aoki, 1963; Phelan, 1970). NZGH rats have an elevated blood pressure from the age of at least 2 days (Jones & Dowd, 1970), in contrast to the SHR which has a distinct pre-hypertensive stage (Grollman, 1972). Differences in salt and water regulation have also been reported. Extracellular fluid volume is reduced in the NZGH rat (Simpson *et al.* 1979) which has been shown to retain both water and sodium upon isotonic saline loading (Simpson *et al.* 1979) by comparison with normotensive controls. In contrast, the SHR has normal extracellular fluid volume (Trippodo, Walsh & Frohlich, 1978) and showed an exaggerated diuresis and natriuresis upon saline loading (Willis, McCallum & Higgins, 1976; DiBona & Rios, 1978). The blood pressure response to volume and salt loading also differed, blood pressure remaining stable in the NZGH rat but increasing in the SHR (Yamori, 1983). These contrasting renal and haemodynamic responses suggest that volume and salt regulation may differ between the SHR and NZGH rat, and therefore it is perhaps not too surprising that AVP deficiency had differing effects on the development of hypertension in SHRDI (Ganten *et al.* 1983) and DI/H animals. Humoral factors, possibly including AVP, may be responsible for the early development of hypertension in the NZGH rat, since the other major contributor to the elevated blood pressure in later life, the sympathetic nervous system, is not fully functional over the first week of life (Iverson, de Champlain, Glowinski & Axelrod, 1967).

Replacement of AVP in the DI/H animals resulted in a substantial increase in

systolic blood pressure, but not to the levels observed in NZGH rats, despite the induction of circulating AVP concentrations comparable with those of the NZGH rat. This suggests that AVP may make a short-term contribution to the hypertension expressed in the NZGH rat, but that the presence of AVP may be required over a longer period for the normal and full development of hypertension in this model. Long-term AVP administration, possibly at the developmental stage prior to the increase in blood pressure to adult levels at 7–10 weeks (Phelan, 1968; Crofton *et al.* 1981; Phelan & Simpson, 1987), may help clarify the role of vasopressin in the development of severe hypertension. This concept is supported, in part, by the observation that young (38 day), vasopressin-deficient Brattleboro rats have lower blood pressure than Long–Evans rats (Laycock & Obika, 1988), though this difference is corrected in the adult Brattleboro animal, which shows normal blood pressure (Gardiner & Bennett, 1983).

In conclusion, it appears that severe hypertension does not develop in the NZGH rat in the absence of vasopressin. Comparatively short-term administration of exogenous vasopressin was associated with an increase in blood pressure, but levels did not approach those normally seen in the NZGH rat. Fluid and electrolyte regulation in AVP-deficient hypertensive animals resembled that seen in Brattleboro DI rats, and was restored to normal by physiological replacement of vasopressin.

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REFERENCES

ASHTON, N. & BALMENT, R. J. (1986). Effect of vasopressin antagonism in established genetic hypertension. *Journal of Endocrinology* **111**, suppl., abstract 111.

ASHTON, N. & BALMENT, R. J. (1987). Renal function and blood pressure in a novel strain of genetically hypertensive rat with diabetes insipidus. *First European Congress of Endocrinology, Copenhagen 1987*, abstract 4–171, p. 46.

ASHTON, N. & BALMENT, R. J. (1988). Neurohypophysial hormone influence on renal function in the New Zealand genetically hypertensive rat. *Acta endocrinologica* **118**, 422–428.

BALMENT, R. J., HENDERSON, I. W. & OLIVER, J. A. (1975). Vasopressin induced changes in plasma renin activity in the Brattleboro rat. *Journal of Endocrinology* **67**, 61–62 *P*.

BOER, G. J., KRUISBRINK, J. & VAN PELT-HEERSCHAP, H. (1983). Long-term and constant release of vasopressin from Accurel tubing: implantation in the Brattleboro rat. *Journal of Endocrinology* **98**, 147–152.

BUCKINGHAM, J. C. & LEACH, J. H. (1980). Hypothalamo-pituitary-adrenocortical function in rats with inherited diabetes insipidus. *Journal of Physiology* **305**, 395–404.

CARRETERO, O. A., POLOMSKI, C., HAMPTON, A. & SCICLI, A. G. (1976). Urinary kallikrein, plasma renin and aldosterone in New Zealand genetically hypertensive (GH) rats. *Clinical and Experimental Pharmacology and Physiology* **3**, suppl. 3, 55–59.

CROFTON, J. T., SHARE, L., BAER, P. G., ALLEN, C. M. & WANG, B. C. (1981). Vasopressin secretion in the New Zealand genetically hypertensive rat. *Clinical and Experimental Hypertension* **3**, 975–989.

CROFTON, J. T., SHARE, L., SHADE, R. E., ALLEN, C. & TARNOWSKI, D. (1978). Vasopressin in the rat with spontaneous hypertension. *American Journal of Physiology* **235**, H361–366.

DIBONA, G. F. & RIOS, L. L. (1978). Mechanism of exaggerated diuresis in spontaneously hypertensive rats. *American Journal of Physiology* **235**, F409–416.

FYHRQUIST, F., TIKKANEN, I. & LINKOLA, J. (1981). Plasma vasopressin concentration and renin in the rat: effect of hydration and haemorrhage. *Acta physiologica scandinavica* **113**, 507–510.

GANTEN, U., RASCHER, W., LANG, R. E., DIETZ, R., RETTIG, R., UNGER, T., TAUGNER, R. & GANTEN, D. (1983). Development of a new strain of spontaneously hypertensive rat homozygous for hypothalamic diabetes insipidus. *Hypertension* **5**, 119–128.

GARDINER, S. M. & BENNETT, T. (1983). The effects of Captopril on blood pressure, urinary water and electrolyte excretion and drinking behaviour in Brattleboro rats. *Clinical Science* **65**, 589–597.

GARDINER, S. M. & BENNETT, T. (1985). Interactions between neural mechanisms, the renin–angiotensin system and vasopressin in the maintenance of blood pressure during water deprivation: studies in Long Evans and Brattleboro rats. *Clinical Science* **68**, 647–657.

GILLIES, G. & LOWRY, P. J. (1980). Corticotrophin releasing activity in extracts of the stalk median eminence of Brattleboro rats. *Journal of Endocrinology* **84**, 65–73.

GRESSON, C. R. (1972). Suppressed renin levels in the genetically hypertensive rat. *Proceedings of the University of Otago Medical School* **50**, 51–52.

GROLLMAN, A. (1972). The spontaneous hypertensive rat: an experimental analogue of essential hypertension in the human being. In *Spontaneous Hypertension, its Pathogenesis and Complications*, ed. OKAMOTO, K., pp. 238–242. Tokyo: Igaku Shoin Ltd.

IVERSON, L. L., DE CHAMPLAIN, J., GLOWINSKI, J. & AXELROD, J. (1967). Uptake, storage and metabolism of norepinephrine in tissues of the developing rat. *Journal of Pharmacology and Experimental Therapeutics* **157**, 509–516.

JONES, D. R. & DOWD, D. A. (1970). Development of elevated blood pressure in young genetically hypertensive rats. *Life Sciences* **9**, 247–250.

KIME, D. E. (1977). Measurement of 1-αhydroxycorticosterone and other corticosteroids in elasmobranch plasma by radioimmunoassay. *General and Comparative Endocrinology* **33**, 344–351.

LANG, R. E., GANTEN, D., GANTEN, U., RASCHER, W. & UNGER, T. (1984). Pathogenesis of hypertension in spontaneously hypertensive rats: definite evidence against a pressor role of vasopressin. *Clinical and Experimental Hypertension* **6**, 121–138.

LAYCOCK, J. F. & OBIKA, L. F. O. (1988). Young anaesthetized and conscious Brattleboro rats have lower blood pressures than their Long Evans controls. *Journal of Physiology* **403**, 78*P*.

LEE, J. & WILLIAMS, P. G. (1972). The effect of vasopressin (pitressin) administration and dehydration on the concentration of solutes in renal fluids of rats with and without hereditary diabetes insipidus. *Journal of Physiology* **220**, 729–743.

MILNE, C. M., BALMENT, R. J., HENDERSON, I. W., MOSLEY, W. & CHESTER-JONES, I. (1982). Adrenocortical function in the Brattleboro rat. *Annals of the New York Academy of Sciences* **394**, 230–240.

MÖHRING, J., KINTZ, J. & SCHOUN, J. (1978). Role of vasopressin in blood pressure control of spontaneously hypertensive rats. *Clinical Science and Molecular Medicine* **55**, 247–250s.

MÖHRING, J., KINTZ, J. & SCHOUN, J. (1979). Studies on the role of vasopressin in blood pressure control of spontaneously hypertensive rats with established hypertension (SHR, Stroke-prone strain). *Journal of Cardiovascular Pharmacology* **1**, 593–608.

OKAMOTO, K. & AOKI, K. (1963). Development of a strain of spontaneously hypertensive rats. *Japanese Circulation Journal* **27**, 282–293.

PHELAN, E. L. (1968). New Zealand strain of rats with genetic hypertension. *New Zealand Medical Journal* **67**, 334–344.

PHELAN, E. L. (1970). Genetic and autonomic factors in inherited hypertension. *Circulation Research* **26** & **27**, suppl. 2, II65–74.

PHELAN, E. L. & SIMPSON, F. O. (1987). The New Zealand strain of genetically hypertensive rats. *Hypertension* **9**, suppl. I, I15–17.

PHELAN, E. L., SIMPSON, F. O. & SMIRK, F. H. (1976). Characteristics of the New Zealand strain of genetically hypertensive (GH) rats. *Clinical and Experimental Pharmacology and Physiology*, suppl. 3, 5–10.

PHELAN, E. L. & SMIRK, F. H. (1960). Cardiac hypertrophy in genetically hypertensive rats. *Journal of Pathology and Bacteriology* **80**, 445–448.

ROBERTSON, G. L., MAHR, E. A., ATHAR, S. & SINHA, T. (1973). Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. *Journal of Clinical Investigation* **52**, 2340–2352.

ROSENBLOOM, A. A. & FISHER, D. A. (1975). Radioimmunoassayable AVT and AVP in adult mammalian brain tissue: comparison of normal and Brattleboro rats. *Neuroendocrinology* **17**, 354–361.

SCHWARTZ, J. & REID, I. A. (1986). Role of the vasoconstrictor and antidiuretic activities of vasopressin in inhibition of renin secretion in conscious dogs. *American Journal of Physiology* **250**, F92–96.

SIMPSON, F. O., PHELAN, E. L., CLARK, D. W., JONES, D. R., GRESSON, C. R., LEE, D. R. & BIRD, D. L. (1973). Studies on the New Zealand strain of genetically hypertensive rats. *Clinical Science and Molecular Medicine* **45**, suppl. 1, 15–21s.

SIMPSON, F. O., PHELAN, E. L., JONES, D. R., BUTT, T. J., YOUNG, P. L. & LEDINGHAM, J. M. (1979). Pathogenesis of hypertension in the New Zealand strain of genetically hypertensive (GH) rats. *Japanese Heart Journal* **20**, suppl. 1, 58–60.

SLADEK, C. D., BLAIR, M. L. & MANGIAPANE, M. (1987). Evidence against a pressor role for vasopressin in spontaneous hypertension. *Hypertension* **9**, 332–338.

TRIPPODO, N. C., WALSH, G. H. & FROHLICH, E. D. (1978). Fluid volumes during onset of spontaneous hypertension in rats. *American Journal of Physiology* **235**, H52–55.

WILLIS, L. R., MCCALLUM, P. W. & HIGGINS JR, J. T. (1976). Exaggerated natriuresis in the conscious spontaneously hypertensive rat. *Journal of Laboratory and Clinical Medicine* **87**, 265–272.

YAMORI, Y. (1983). Physiopathology of the various strains of spontaneously hypertensive rats. In *Hypertension: Physiopathology and Treatment*, 2nd edn, ed. GENEST, J., KUCHEL, O., HAMET, P. & CANTIN, M., pp. 556–681. New York: McGraw-Hill.