Familial hypothalamic diabetes insipidus in rats (Brattleboro strain)

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VALTIN, H., AND H. A. SCHROEDER. Familial hypothalamic diabetes insipidus in rats (Brattleboro strain). Am. J. Physiol. 206 (2): 425-430. I g6+-Familial hypothalamic diabetes insipidus (DI) has arisen as an apparently spontaneous mutation from a strain of Long-Evans hooded rats being bred for unrelated researches not involving radioactivity. The DI rats decrease water intake and urine flow, and increase urine osmolality in response to injected vasopressin. They concentrate their urine only minimally or not at all in response to dehydration, hypertonic saline, nicotine, or stress, and their serum osmolalities and sodium concentrations are significantly higher than those of normal animals. They show marked diminution of neurosecretory material in the neurohypophysis and supraoptic nucleus. The data suggest that the deficiency causing DI in these rats is a lack or dearth of synthesis of vasopressin or its carrier protein, or both.

METHODS

All experiments were done on unanesthetized rats. For studies, rats were housed in metabolism cages (Acme Metal Products, Inc., Chicago) using ground Wayne Lab Blox for food. Urine was collected under mineral oil and blood taken from the tails of unanesthetized animals directly into Beckman/Spinco polyethylene microtest tubes of 400 μl capacity, without anticoagulant. Only nonhemolyzed samples were analyzed. Osmolalities were determined on a Fiske osmometer, sodium and potassium on a Baird internal standard flame photometer, whole blood hematocrit by the micro method, and glucose by the Glucostat method (Worthington Biochemical Corp.). P values were computed by Student’s t test.

Water intake, urine flow, urine osmolality, urinary excretion of sodium and potassium, and body weight were measured individually on 25 rats of both sexes, 30-33 days of age, representing the total of three litters. Response to subcutaneously injected vasopressin (0.5 pressor unit of Pitressin Tannate in oil, Parke, Davis & Co.) was ascertained in five adult DI rats of both sexes. Multiple measurements were made on a single adult female with DI, either in the untreated state or from one to five consecutive days after injection of vasopressin. Dehydration was studied in the same rat during 6-hr intervals at the same time of day. Pneumonia was precipitated inadvertently in this rat when the trachea instead of the stomach was intubated in the course of an experiment. The animal received penicillin intramuscularly and its fluid balance was followed with consecutive 24-hr collections beginning with the 1st hr of illness.

The response to hypertonic saline was ascertained on two groups of rats consisting of four normal adult female animals and three adult females with DI, by the method of Friedmann et al. (6). Each rat received 3 ml 3% NaCl by stomach tube, and spontaneously voided, pooled urine was collected at 30 min and at hourly intervals thereafter up to 4½ hr. Six identical experiments were run on each group.

The effect of nicotine was studied on the same animals given hypertonic saline. Since the predicted effect of nicotine is the interruption or delay of water diuresis, both groups were studied for their response first to a water load and then to a water load plus nicotine. Three milliliters of tap water were given to each animal by stomach tube and collections made at 30 min and at
hourly intervals thereafter up to 4½ hr. In subsequent experiments each rat received water and 500 μg nicotine base in 0.25 ml normal saline subcutaneously. Six experiments with water only and six experiments with water plus nicotine were made on each group. Animals were given no food or water during the course of the studies with hypertonic saline and nicotine.

Whole-blood hematocrit and serum osmolality and sodium were measured on 15 adult DI rats (6 females, 9 males) and on 16 normal rats (7 females, 9 males) matched for age.

RESULTS

Fluid balance and electrolyte excretion. Figure 1 shows water intake, urine flow, urine osmolality, excretion of urinary sodium and potassium, and body weight of 16 normal (9 females, 7 males) and 9 DI rats (5 females, 4 males), representing the total of three litters. They are easily segregated into normal and DI groups on the basis of urine flow, the range being 2.4–6.7 ml/100 g 24 hr in the former and 54.1–101.6 ml/100 g 24 hr in the latter. The severity of the disease is reflected in the fact that the 24-hr urine flow averages about 70% of body weight. All of the differences in Fig. 1 except those of urinary electrolytes have undoubted statistical significance, while excretion of sodium and potassium was probably higher in the DI animals. The greater excretion of potassium than sodium in both groups probably results from the fact that Wayne Lab Blox contain twice as much potassium as sodium on a molar basis. Adult DI rats of both sexes also weighed significantly less (P < 0.01) than their normal litter mates.

Vasopressin. Figure 2 presents data in one adult diseased female rat (DI 6), both untreated and after injections of vasopressin tannate in oil. The graphs are composites of several experiments on the same animal, and presumably reflect progressively diminishing blood titers of vasopressin as the curves go from left to right.

![Graph 1](image1.png)

**Fig. 1.** Mean data on 16 normal and 9 DI rats. Brackets indicate ± 1 se. Note that the scales vary.

![Graph 2](image2.png)

**Fig. 2.** Urine flow and osmolality, water intake, and urinary sodium excretion of a single adult female rat with diabetes insipidus, both untreated and in response to injections of vasopressin tannate in oil and to the stress of pneumonia. Each point represents a 24-hr collection period, either in the untreated state (control points on the extreme right) or from 1 to 5 days after a single subcutaneous injection of 0.5 pressor unit. Thus, as the curves proceed from left to right, blood titers of vasopressin presumably diminished. The 1st day of pneumonia is represented at the extreme left, and subsequent days of illness by succeeding points to the right. No vasopressin was given while the rat had pneumonia.
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In the untreated state, urine flow and water intake reached peaks of 250 and 300 ml/24 hr, respectively, with urine osmolality as low as 125 mosmoles/kg. With maximum vasopressin effect urine flow and water intake were 8 and 31 ml/24 hr, respectively, and urine osmolality 2,450 mosmoles/kg. The mean body weight of the rat was 260 g. Similar responses to injection of vasopressin were obtained in one other adult female and three adult males with DI.

Infection. The development of pneumonia in rat DI 6 afforded the opportunity to observe the response to the stress of infection (Fig. 2). A drastic reduction in urine flow subsided by the 4th day of illness. Although the urine was slightly concentrated on the 1st day (380 mosmoles/kg), all the points fell below the control curve. We interpret these results to mean that the mechanism of antidiuresis was not primarily an increase in the blood titer of vasopressin. The data, however, do not exclude the possibility that small amounts of vasopressin were secreted. The illness was accompanied by reduction in sodium excretion. Potassium excretion (not shown in the figure) was unaltered.

Dehydration. Figure 3 shows the response of a single adult female rat (DI 6) to dehydration. While 6 hr of dehydration caused a reduction of about 50% in urine flow, urine osmolality did not exceed that of normal plasma. Therefore the decreased urine flow probably was not the result of a rise in the blood titer of vasopressin, although a small rise has not been ruled out. The periods of dehydration were accompanied by a mean loss of 7.5% of body wt. Comparable results were obtained on a second adult female rat.

Hypertonic saline. The response to the ingestion of a solution of 3% NaCl is shown in Table 1 and Fig. 4. Slight diureses associated with increased sodium excretion occurred in both groups. Peak diuresis and natriuresis occurred 1 hr later in DI than in normal animals. The most striking difference between the two groups is in their urine osmolalities. In normal rats urine osmolality rose by 706 mosmoles/kg to a mean high of 1,993 mosmoles/kg, while in DI rats it rose by only 144 mosmoles/kg to a high of 405 mosmoles/kg. These results suggest that the blood titer of vasopressin increased in the normal group but only slightly or not at all in DI animals.

Nicotine. Reactions to a simple water load and water load plus nicotine are illustrated in Table 1 and Fig. 5. The normal animals showed the expected response (Fig. 5A) with maximal diuresis occurring 90 min after the instillation of water only, and an osmotic diuresis at 90 min with delay of the water diuresis when water plus nicotine was used (2, 3). In the DI rats, a similar small load of water also caused a diuresis which, however, was not associated with further dilution of the urine, and which reached its peak at 150 instead of at 90 min. When nicotine was added, as was shown by Burn et al (3) in hypophysectomized rats, a single diuretic peak occurred 1 hr earlier, again associated with only minimal changes in urine osmolality.

Figure 5B shows that nicotine produced a natriuresis in normal and perhaps also in DI rats, and water alone caused natriuresis in DI rats only. We interpret these data to mean that the diureses due to nicotine in both groups and that due to water in DI rats may have resulted from increases in glomerular filtration rate (GFR) and not from decreased titer of vasopressin.

Blood values. Figure 6 shows that serum osmolality and sodium are significantly higher in DI than in normal rats. No difference could be detected in the hematocrits.

Carbohydrate metabolism. Urines of the diseased animals were free of reducing substances, and nonfasting blood sugars determined on two adult female rats with DI had normal values of 107 and 98 mg/100 ml.

Anatomical studies. A preliminary report has been published (11) and details will follow in a separate paper. The diseased animals, both male and female, showed marked reduction of neurosecretory material of the posterior pituitary glands when stained with either aldehyde fuchsin or chrome alum hematoxylin. The supraoptic nuclei of DI rats also showed reduction of neurosecretory material, especially along the axons, and the cells appeared active and hypertrophied. The paraventricular nuclei appeared normal.

Gross and light microscopic examination of all other organs, including the genitourinary system, revealed no abnormalities. Possible differences in the size of organs have not been determined.

Genetics. The mode of inheritance of the disorder will be the subject of a separate report. The disease has been carried into the fifth generation, and of 55 diseased rats raised, 31 were females and 24 males. There is a high incidence of semisterility, fetal deaths, stillbirths, and runts. Although the pattern of inheritance has not been fully clarified, the pedigree is most compatible with an autosomal recessive trait controlled by two gene loci; homozygosity of recessive genes at either locus will cause the disease. Neither genetic analysis nor chromosome mapping of blood and tissues has shown evidence for translocation heterozygosity.

Albinism is an associated mutation in this strain, and
### TABLE I

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<th>Urine Flow, $\mu l$/kg min</th>
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**Response to hypertonic saline**

- Normal rats
- Rats with DI

**Response to water load**

- Normal rats
- Rats with DI

**Response to water load plus nicotine**

- Normal rats
- Rats with DI

Figures in parentheses indicate the average values during two or more collection periods. For example, in the second experiment on normal rats given hypertonic saline no urine was voided spontaneously at 30 and 90 min, and the urine volume obtained at 150 min was divided by 150 to yield the figure given in the table.

is inherited as an autosomal recessive. There are albino rats with or without DI, as well as DI rats with or without albinism.

**DISCUSSION**

The classical response of the urinary volumes of these rats to exogenous vasopressin, the failure to obtain an appreciable increase in urinary concentration in response to dehydration, stress, hypertonic saline, or nicotine, and the pathological changes in the hypothalamic-neurohypophysial system leave no doubt that the rats have hypothalamic diabetes insipidus. The severity of the disease is manifest by the very high intakes of water, which may equal or exceed the total body weight in 24 hr (Fig. 1). Nevertheless, these animals, like dogs with surgically produced DI (15), have a fairly sensitive mechanism for decreasing the flow of
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FIG. 4. Response to hypertonic saline in 4 normal and 3 DI rats. Three milliliters of 3% NaCl were given to each rat by stomach tube at 0 time. Each point is the mean of those figures in Table 1 which are not in parentheses. Mean values for urine osmolality have been weighted for urine flow.

FIG. 5. Response to a small water load, and to a small water load plus nicotine in 4 normal and 3 DI rats. Three milliliters of tap water were given to each rat by stomach tube at 0 time. Nicotine, 500 μg of base in 0.25 ml normal saline, was injected subcutaneously immediately after the instillation of water. Each point represents the mean of those figures in Table 1 which are not in parentheses. Mean values for urine osmolality have been weighted for urine flow.

Increased GFR in response to loads of water also occurs in various species, both normal (5, 14) and DI (9). This mechanism probably accounts for the very slight urinary concentration during dehydration and stress (1, 4). The reduction in urinary sodium excretion which occurred during pneumonia is consistent with such a mechanism.

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A slight, steady increase of urine osmolality to a point above plasma osmolality occurred in DI rats following water and saline loads and following a water load plus nicotine. It is probable that the rats became dehydrated, as their cumulative urine output during 4 hr exceeded the oral intake by a factor of at least 5. Therefore, reduced GFR may account for the urinary concentration.

If the saline load was distributed throughout the extracellular fluid volume, the calculated increase in serum osmolality was not enough to cause the rise in urine osmolality to 405 mosmoles/kg.

Our data do not explain why DI rats excrete more sodium and potassium in the urine than do their normal littermates (Fig. 1). Since the rats were not pair fed and food intake was not measured, the differences in electrolyte excretion may simply reflect differences in intake. Other possible explanations include high flow rates through the nephron, endocrine abnormalities other than vasopressin, and differences in GFR, but elucidation awaits further study.

The fact that serum osmolality and sodium con-
centration are higher in DI than in normal rats (Fig. 6) suggests that diseased animals might have long periods of mild dehydration interspersed with brief periods of normal hydration or overhydration immediately after drinking. Dogs with surgically produced DI have shown wider fluctuations in total electrolyte content of serum than normal dogs (15); our DI rats show larger variations in serum osmolality and sodium than do normal controls. Such a fluctuating state would be expected to result in prolonged stimulation of osmoreceptors and might account for the observed hypertrophy of neurons in the supraoptic nuclei. These neurons may be the osmoreceptors (7). No change was found in the hematocrit probably because the micro method is not sufficiently accurate to detect slight risks.

Our physiological studies suggest that DI rats have a dearth or absence of vasopressin in the bloodstream. This deficiency could result either from lack of release of this hormone from the neurohypophysis or from insufficient synthesis of hormone. In the former event, neurosecretory material should accumulate in the neurohypophysis and supraoptic nucleus. Therefore, the defect probably lies in the synthesis of vasopressin or its carrier substance, or both. Our data do not show whether such synthesis is wholly absent or just greatly reduced. In fact, the mode of inheritance in this strain permits both possibilities in different animals. Thus, whenever we have invoked a decrease in GFR as a possible explanation for the slight concentration of urine in DI rats, small increases in blood titer of vasopressin cannot be ruled out as an alternate or additional mechanism. Bio-assays for vasopressin, now being done, should settle this question.

So far as we know, this is the first recorded instance of familial hypothalamic diabetes insipidus in an animal other than man. In the strain of mice reported by Silverstein et al. (10), polydipsia is the primary abnormality and the hypothalamo-neurohypophysial system is normal histologically. Since our animals were discovered in laboratories at Brattleboro, Vermont, we propose that they be known as the "Brattleboro strain."

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REFERENCES