THE USE OF RATS WITH LESION-INDUCED OR HEREDITARY HYPOTHALAMIC DIABETES INSIPIDUS FOR EVALUATING PROLONGED RESPONSES TO ANTIDIURETIC HORMONE PREPARATIONS

By

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ABSTRACT

The diuresis and the urinary excretion of ions by rats with hereditary and with lesion-induced hypothalamic diabetes insipidus and the change in these parameters in response to lysine vasopressin have been found to be practically identical. The time course of the antidiuretic response can be followed in detail over long periods; it has been found to be sensitive to the nature of the antidiuretic hormone preparation and to the dose level. It is concluded that rats with hypothalamic diabetes insipidus are useful models for studying the time course of the antidiuretic response, in particular to long-acting preparations.

Methods for the measurement of the antidiuretic activity of the antidiuretic hormones (ADH) and related peptides in the rat have been greatly improved in recent years. It is now possible to follow the response of the ethanol-anaesthetised, water-loaded rat to intravenous injections of these peptides practically continuously and to obtain information not only about the total magnitude, but also about the time course of the antidiuretic effect on a time scale of minutes or even hours. The anaesthetised and hydrated rat is less satisfactory for following the longer and more variable responses to intraperitoneal, subcutaneous or intramuscular injections of antidiuretic peptides, and for the assay of depot preparations of ADH it is useful only in as far as the hormone content of the preparation can be determined after separation of the peptide from the depot material (tannic acid, oil etc.); the duration of the response to
such preparations – which, to be useful in human therapy, should be several days – cannot be measured in this assay system.

Since the persistence of the effect of a single dose of an ADH preparation is an important feature for its value in the therapy of human diabetes insipidus, an assay system in which this persistence could be studied on the proper time scale would be of some value. Wilson & McGinty (1951) have used conscious, normal rats which were intermittently water-loaded at intervals of up to 60 hours after administration of the ADH preparation. This method, while useful, has some disadvantages. It does not allow the time course of the response to be plotted in detail, large numbers of animals are required and the repeated manipulation involved in water loading is a disturbing factor. To develop a model free from these drawbacks we have used rats with hypothalamic diabetes insipidus (DI), a pathological condition very similar to the ADH-sensitive human DI.

Hypophysectomised rats (Vogel et al. 1966) are not suitable for this purpose because hypophysectomy is known to cause only transitory DI in rats. However, a permanent DI can be induced by bilateral electrolytic lesions placed stereotactically in the hypothalamus of young rats (Alexander 1958; Mikulôš 1964) and those animals which develop DI following such lesions can be used for the remainder of their lives. An attractive alternative is provided by the Brattleboro strain of rats with hereditary diabetes insipidus (Valtin et al. 1962; Valtin & Schroeder 1964; Valtin 1967).

A comparison of the concentrating activity of the kidneys of the two kinds of animals with DI (hereditary and induced by lesions) in response to single intramuscular doses of lysine vasopressin (LVP) is described here. The time course of the response to LVP, to a synthetic analogue with prolonged action and to two depot preparations of ADH at two dose levels has been followed in order to establish the value of these animals for the purpose discussed above.

**MATERIALS AND METHODS**

**Animals**

Wistar rats with fully developed DI resulting from bilateral electrolytic lesions placed stereotactically in the hypothalamus about 2 months after birth (Mikulôš 1964) were kindly supplied by Dr. I. Mikulôš, of the Institute for the Care of Mother and Child, Prague, Czechoslovakia. Long-Evans hooded rats of the Brattleboro strain were bred from stock generously presented by Dr. H. Valtin, of Dartmouth Medical School, Hanover, New Hampshire, U.S.A. All animals were about 12 months old. Rats of either sex were used; the average weights are noted for each experiment.

**Measurements**

The rats were kept in metabolic cages with food (pelleted laboratory diet) and water *ad libitum*. The urine was collected continuously in test-tubes placed in a chromatographic fraction collector actuated by a timer at 2-hour intervals. For each 2-hour
fraction the volume was noted and the conductivity measured with an OK-102 conductivity meter (Radelkis, Hungary); where appropriate the sodium and potassium were determined with a Zeiss (Jena, G. D. R.) Model III flame photometer and chloride by potentiometric titration with silver nitrate. The ADH preparations were injected intramuscularly in 0.1 ml solvent or suspension medium, each injection being preceded by a 12- or 24-hour control period. All numerical results recorded represent means of 5 or 6 separate experiments and are given with the standard error of the mean (± s_x).

**Materials**

Lysine vasopressin (LVP) and Na₂-glycyl-glycyl-glycyl-8-lysine vasopressin (GGG-LVP) were synthetic preparations purified by ion exchange chromatography (Kasafirek et al. 1966). »Vasopressin depot« was a suspension of lysine vasopressin tannate in olive oil prepared by Miss M. Síslerová at the Research Institute for Pharmacy and Biochemistry. Pitressin® tannate in oil was a commercial preparation (Parke, Davis Co., batch no. LFD 469). The doses are given in International Units of rat pressor activity as determined in the Dekanski (1952) preparation.

**RESULTS**

*Renal excretory response of the two types of DI rats to LVP*

The rate of diuresis and the urine conductivity recorded during a 48-hour control period for a group of rats with lesion-induced DI and a group of Brattleboro DI rats (Fig. 1) shows diurnal variations, similar for both groups, with minimal diuresis (maximal conductivity) in the early morning and maximal diuresis (minimal conductivity) in the evening. The mean values of urine flow for each group over 24 hours are given in Table 1.

In response to LVP there is a sharp decrease in urine flow (with practically complete cessation during the first 2-hour period following injection) and a corresponding sharp increase in conductivity. The return of the two parameters to control values follows a similar course for both groups of DI rats (Fig. 1).

The urinary concentration of sodium, potassium and chloride during a 24-hour control period and during 24 hours following injection of LVP follows the same time course as the conductivity (Fig. 2). In the control period, the total excretion of each ion slightly higher for the rats with lesion-induced DI than for the Brattleboro rats. After the injection of LVP the excretion of all

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* The pressor response to GGG-LVP is much more prolonged than that to LVP (Kynčel et al. 1966); the pressor activity was evaluated from the maximal rise in blood pressure recorded in response to a given dose (see Plíška & Krejčí 1966; Plíška 1966).

**Because of the small volume of urine collected, no values for conductivity or ion concentrations can be given for this period.
Urine flow and urine conductivity during a control period and following injection of LVP. Measurement at 2-hour intervals beginning at 8 a.m.; at arrow, LVP (1 IU) in aqueous solution injected intramuscularly. Full line: rats with lesion-induced DI (3 ♂, 2 ♀, average weight 180 g); dashed line: Brattleboro DI rats (3 ♂, 2 ♀, average weight 191 g). Vertical bars represent ± sₓ.

ions is decreased in both groups (except for potassium in the group with lesion-induced DI) (Table 1).

Response of DI rats to vasopressin preparations with prolonged action

The antidiuretic response of a mixed group of rats to Pitressin tannate is shown in Fig. 3. The prolonged response to this hormone preparation is clearly evident in the record of both urine flow and urine conductivity (plotted here as the reciprocal, i.e. the resistivity). Both parameters appear to be equally useful for following the time course of the response. This is confirmed by the results of a second experiment (Fig. 4), using Brattleboro rats, in which the rate of urine flow and the reciprocal of the conductivity (means over 24-hour periods) are expressed as percentages of the values for the control period. The time course of the return to control values is seen to be closely similar for both
Table 1.
Diuresis and excretion of sodium, potassium and chloride ions during 24-hour periods preceding and following injections of LVP. Same experiment as in Fig. 1; figures give means ± sX.

<table>
<thead>
<tr>
<th></th>
<th>Lesion-induced DI (weight 180 ± 36 g)</th>
<th>Brattleboro strain DI (weight 191 ± 30 g)</th>
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<tr>
<td>Urine (ml)</td>
<td>control</td>
<td>154 ± 31</td>
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<tr>
<td></td>
<td>after LVP</td>
<td>121 ± 25</td>
</tr>
<tr>
<td>Sodium (meq.)</td>
<td>control</td>
<td>2.10 ± 0.29</td>
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<tr>
<td></td>
<td>after LVP</td>
<td>1.77 ± 0.18</td>
</tr>
<tr>
<td>Potassium (meq.)</td>
<td>control</td>
<td>1.78 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>after LVP</td>
<td>1.91 ± 0.19</td>
</tr>
<tr>
<td>Chloride (meq.)</td>
<td>control</td>
<td>2.43 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>after LVP</td>
<td>1.87 ± 0.26</td>
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parameters, the results from the conductivity measurements being somewhat more homogeneous.

The rather small scatter of the results for the mixed group of DI rats as defined by the standard error plotted in Fig. 3 indicates that this group is homogeneous in its response. Direct comparison of two groups of rats with hereditary and lesion-induced DI, respectively, confirms that the response of both types of DI animals to the tannate preparations is identical (Fig. 5).

The value of the DI rats in defining the time course of prolonged antidiuretic responses is demonstrated by an experiment (Fig. 6) in which a group of rats with hypothalamic lesions was treated with three different hormone preparations in equipressor doses. The results show that the response to GGG-LVP, a »hormonogen« type of vasopressin derivative (see Beránková-Ksandrová et al. 1966) is distinctly more prolonged than the response to LVP itself, in agreement with the results obtained using ethanol-anaesthetised water-loaded rats (Kynčl et al. 1966; Kynčl, unpublished results). The response to LVP tannate (Vasopressin depot) is still more prolonged, though the maximal intensity of the response does not develop until 10–12 hours after injection (Fig. 6).

Finally, the results in Table 2 show the response to two doses of each of two preparations of vasopressin tannate (Pitressin tannate and Vasopressin depot), summed over 24-hour periods after injection. The difference in response to the two dose levels is quite evident. In this experimental design, the two
Fig. 2.
Urinary concentrations of sodium, potassium and chloride during control period and following injection of LVP. The same experiment as in Fig. 1.
Changes in urine flow and urine resistivity following injection of a depot preparation of ADH. Mean $\pm s_x$ for 6 rats (2 $\delta$ and 1 $\Omega$ each of the Brattleboro strain and with lesion-induced DI, average weight 260 g). Measurement at 2-hour intervals beginning at 8 a.m.; at arrow, intramuscular injection of Pitressin tannate in oil (0.5 IU). $R$ resistivity in ohm/cm².

preparations appear equivalent in their effect on both diuresis and urine concentration.

**DISCUSSION**

Apart from their obvious and proven value for physiological studies (*Valtin* 1967), rats with hypothalamic DI and in particular animals of the Brattleboro strain with hereditary DI have, in addition, a number of advantages for the assay of antidiuretic preparations. Their enhanced sensitivity to ADH has already been noted (*Yamane & Kunishige* 1966; *Sawyer & Valtin* 1967; *Vierling et al.* 1967; *Jones & Lee* 1967). Furthermore there is little danger of interference from endogenous ADH, no anaesthesia or surgery is required and the permanent diuresis and polydipsia obviates artificial hydration and the manipulation connected with it. The animals can be kept essentially undisturbed in their accustomed environment over the course of the experiment. The high rate
Fig. 4.
Time course of the antidiuretic response to a depot preparation of ADH. Brattleboro DI rats (3♂, 3♀, average weight 290 g). Urine flow (shaded columns) and urine resistivity (white columns), ± s_r, for 24-hour intervals following intramuscular injection of Pitressin tannate in oil (0.5 IU), expressed as percentages of the control values.

of urine flow makes it possible to collect samples sufficient for analysis during 1 or 2 hours, an operation which can be made automatic by the use of a fraction collector. This arrangement in turn makes it possible to follow water and electrolyte excretion in such animals continuously over a period or days or even weeks. Several doses of preparation and standard can thus be tested in the same animal even when the response is prolonged as a result of intramuscular administration or the use of long-acting ADH preparations, and statistically valid results can therefore be obtained with only 5 or 6 animals.

The relatively short time intervals of measurement in our experimental arrangement have clearly revealed diurnal variations in urine flow (Fig. 1) for both groups or rats; the source and nature of these variations are under further study*. Our results for the 24-hour urine output and ion excretion are

* Differences in diuresis during the day and night have already been noted by Valtin (personal communication; see Fig. 9 in Valtin 1967).
Antidiuretic response of rats with hereditary and lesion-induced DI during 48 hours after injection of a depot preparation of ADH. B: Brattleboro DI rats (as for experiment in Fig. 4) and L.: rats with lesion-induced DI (3♂, 3♀; average weight 314 g) injected with Pitressin tannate in oil (0.5 IU) intramuscularly. Shaded columns urine flow, white columns urine resistivity in percentages of control values; vertical bars represent ± s_x.

in good agreement with the previous finding of Valtin & Schroeder (1964) for the Brattleboro strain and Friedman & Friedman (1965) for both types of DI rats. We failed to find the difference between the excretion of sodium and potassium noted by the earlier authors but this may have been affected by the composition of the diet, which was not controlled in our experiments. Like Friedman & Friedman (1965) we found that the rate of urine excretion is somewhat higher, and of ion excretion somewhat lower in the Brattleboro rats than in animals with DI induced by lesions, though the differences are not statistically significant. We also found that over a 24-hour period following injection of LVP, ion excretion in both groups was decreased as against the control period; the change is not statistically significant but this may merely be a reflection of the small number of animals used. The changes in urine flow, urine conductivity and ion excretion in response to LVP (Figs. 1 and 2; Table 1) appeared to be identical, within the fiducial limits, for both groups, as were the antidiuretic responses to a depot preparation of ADH (Fig. 5).
**Fig. 6.**
Time course of the antidiuretic responses to LVP, GGG-LVP and LVP tannate. Rats with lesion-induced DI (as for experiment in Fig. 5). Resistivity of 2-hour urine samples as percentages of control values following injection (at 8 a.m.) of 0.5 pressor units each of LVP (●), GGG-LVP (◯) and LVP tannate in oil (○). Points represent three-interval moving averages.

**Table 2.**
Time course of the antidiuretic response to two depot preparations of ADH. Animals as in the experiment in Fig. 5. Urine flow (roman type) and urine resistivity (italic type) expressed as percentages of control values, ± s̄, for 24-hours periods following intramuscular injection of Pitressin tannate or Vasopressin depot in two doses.

<table>
<thead>
<tr>
<th></th>
<th>Pitressin tannate</th>
<th>Vasopressin depot</th>
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<tr>
<td></td>
<td>0.5 IU</td>
<td>1 IU</td>
</tr>
<tr>
<td>0–24 hours</td>
<td>28.5 ± 6.3</td>
<td>28.8 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>28.5 ± 3.0</td>
<td>29.9 ± 1.9</td>
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<tr>
<td>24–48 hours</td>
<td>57.6 ± 8.0</td>
<td>38.9 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>40.9 ± 2.5</td>
<td>37.3 ± 3.1</td>
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<tr>
<td>48–72 hours</td>
<td>78.2 ± 5.4</td>
<td>55.0 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>59.9 ± 1.7</td>
<td>41.4 ± 2.4</td>
</tr>
<tr>
<td>72–96 hours</td>
<td>63.6 ± 4.0</td>
<td>50.3 ± 3.1</td>
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Comparison of the response to a solution of LVP, to a long-acting synthetic analogue (GGG-LVP) which is thought to exert its prolonged effect by releasing the hormone through enzymatic action in vivo (Beránková-Ksandrová et al. 1966), and to a depot preparation of LVP tannate, showed that the time course of the response to the three preparations could be clearly differentiated and that each time course could be followed in considerable detail (Fig. 6). As a result it was possible to show that the response to this preparation of LVP tannate only reaches maximal intensity after 10–12 hours, an observation which could hardly have been made by any of the assay methods previously used. The clear difference in the response to two doses of the same preparation (Table 2) makes it possible to obtain a numerical expression for the persistence independent of dose (Pliska 1966).

These results confirm that rats with DI constitute a useful model for the study of ADH preparations.

REFERENCES

Beránková-Ksandrová Z., Bisset G. W., Jošt K., Krejčí I., Pliska V., Rudinger J.,
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