PITUITARY-ADRENAL ACTIVATION IN RATS WITH HEREDITARY HYPOTHALAMIC DIABETES INSIPIDUS

By
Akira Arimura, Takashige Saito, Cyril Y. Bowers and Andrew V. Schally

ABSTRACT

Pituitary adrenal activation in rats with hereditary hypothalamic diabetes insipidus (DI) and normal control rats (Brattleboro strain) was studied under various conditions. Plasma corticosterone concentration was essentially the same in resting DI and control rats. No significant difference in plasma corticosterone was observed after giving the animals ether, histamine, vasopressin or acetylcholine. Since DI rats lack vasopressin, these findings provide some evidence that vasopressin is unlikely to be the physiological corticotrophin releasing factor (CRF). Epinephrine induced a smaller increase in plasma corticosterone in DI rats than control animals, but the difference was not statistically significant. However, intraperitoneal injection of 0.9% saline resulted in significantly less elevation of plasma corticosterone in DI rats than normal. This suggests certain differences in responses between normal and DI rats depending upon the intensity or nature of the stress. Hypothalamic CRF in control and DI rats was determined using rats treated with chlorpromazine, morphine and Nembutal®. The CRF of hypothalami of DI rats was about half of that of control animals. In the extracts of posterior pituitary lobes, corticotrophin (ACTH) activity was found in almost the same amount in DI and control rats. The posterior pituitary lobe of DI rats lacked CRF.

This investigation was supported by Public Health Service Research Grants No. AM 09094 and AM 07467.
activity when tested in neurohypophysectomized rats. Histological examination of the adrenals of DI rats revealed normal structure, suggesting normal ACTH secretion at rest.

The relationship of corticotrophin releasing factor (CRF) to vasopressin has been extensively studied. McCann & Brobeck (1954) were the first to demonstrate corticotrophin releasing activity of vasopressin in rats with lesions in the median eminence of the hypothalamus. They reported that rats with diabetes insipidus caused by placing lesions in the hypothalamus failed to release corticotrophin (ACTH) in response to stress and postulated that vasopressin may be the physiological releaser of ACTH. A number of papers have appeared to date to describe corticotrophin releasing activity of vasopressin (McCann & Fruit 1957; Jørgensen & Nielsen 1958; Leeman & Munson 1958; Dasentini et al. 1959; Kwaan & Bartelstone 1959; Martini et al. 1959; Anderson et al. 1962; Martini & Pecile 1962).

On the other hand many reports clearly demonstrated the existence of a corticotrophin releasing factor different from vasopressin in the neurohypophysis and the hypothalamus (Saffran et al. 1955; Porter & Jones 1956; Guillemin 1957; Royce & Sayers 1960; Schally et al. 1962). This subject has been reviewed by Nichols (1961), Guillemin & Schally (1963) and de Wied et al. (1964). Recently McCann et al. (in press) reported that in rats with hereditary hypothalamic diabetes insipidus (DI), plasma corticosterone rose in response to etherization and to the mild stress of restraint. They also reported that the hypothalamic tissue of DI rats contained CRF in almost the same amount as the control rats. We also had an opportunity to study the pituitary adrenal activation in the DI rats of the same strain which were supplied by Dr. H. Valtin. Although we confirmed that DI rats released ACTH in response to various stresses, the data which we obtained was different in some respects from those reported by McCann et al. (in press). The present paper describes this study in detail and debates the relationship between vasopressin and CRF.

MATERIALS AND METHODS

Animals

The animals studied were hereditary hypothalamic DI homozygous rats and normal rats of the Brattlebro strain, kindly supplied by Dr. H. Valtin. Each rat was kept in an individual cage.

Determination of stress response

ACTH release in 4-5 month old DI and control rats was evaluated by increase in plasma corticosterone 15 minutes after stress. In order to test the responses to various types of stress in the 12 animals in each group at our disposal, several experiments had to be performed using the same animal. These experiments, except for the last one in which the animals were sacrificed in the laboratory, were performed in the
animal room. Each rat was transferred to an anaesthesia jar containing ether vapor. As soon as the rat was anaesthetized, approximately 0.5 ml of blood was collected in a heparinized syringe from the jugular vein. The blood collection was completed within 2 minutes of the beginning of ether inhalation so that the effect of ether itself on the plasma corticosterone was considered to be negligible. Usually an interval of 1 week elapsed between each experiment. In the last experiment, for convenience, the rats were moved from the animal house to the laboratory where they were decapitated by a guillotine without anaesthesia, and blood samples were collected from the trunk into heparinized test tubes. Animals were handled as carefully as possible to avoid unnecessary stimulation. After centrifugation of the blood, 0.2 ml of plasma was used for corticosterone determination done by the method of Silber et al. (1958) as described by Guillemin et al. (1959). The difference in response between the DI and the control groups was tested by Student’s t test. In order to make up for the blood loss by repeated blood collections, 2 to 3 ml of heparinized blood from normal intact rats of Sprague-Dawley strain was transfused into the experimental animals every 2 weeks.

Measurement of hypothalamic CRF

Rat hypothalamic tissue extending from the optic chiasma to the mammillary body was dissected out immediately after the animals were sacrificed by decapitation. Twelve hypothalami of rats of the same group were pooled and homogenized in 2 ml of 0.1 M ice cold HCl. The extract was then centrifuged in a refrigerated centrifuge at 15,000 rpm for 5 minutes. The supernatant was diluted with 0.9% saline. Acid extracts of the posterior pituitary lobes were made in a similar way.

CRF assays were performed using chlorpromazine-morphine-Nembutal-treated male rats (CPZ-M-N preparations) of Sprague-Dawley strain weighing 240–280 g. The acid extracts of hypothalami or the posterior lobes were injected into the jugular vein. Plasma corticosterone concentration 15 minutes after the injection was used as the index of CRF activity. This assay is superior to morphine-Nembutal or dexamethasone-morphine-Nembutal tests (in preparation for publication).

Comparison of CRF activity in the hypothalamic tissue between control and DI rats was made at 2 dose levels and the result was subjected to factorial analysis (Bliss 1952).

Determination of CRF activity of neurohypophysial extracts

CRF activity in the extracts of the posterior pituitary glands was also tested in neurohypophysectomized male rats from Charles River Breeding Laboratories Inc., Boston, Mass., because these rats were found to be partially refractory to vasopressin (Arimura et al. 1965). The neurohypophysectomized rats were used 1 week after the operation (Arimura et al. 1965). The extracts to be tested were injected into the jugular vein of these rats under Nembutal anaesthesia. Five minutes later, blood was collected from the trunk after decapitation and immediately centrifuged. The separated plasma was stored at –5°C until assayed for ACTH activity.

ACTH activity of plasma

ACTH activity of the plasma was determined by a micromethod described by Lipscomb & Nelson (1959) with our modification which simplified the original technique. Assays were performed in male rats 3 to 6 hours after transauricular hypophysectomy (Tanaka 1955). Samples were given into the jugular vein and immediately
followed by 0.1 ml heparin solution (1000 U/ml). Adrenal venous blood collected from the 7th to 10th minute after the injection was transferred into a graduated centrifuge tube which contained approximately 2.5 ml of 0.9% saline. After the blood was gently mixed with saline, the mixture was centrifuged for 30 minutes at 2000 rpm. The volume of clear supernatant usually ranged from 2.7 to 3.2 ml, a volume large enough to be measured with fair accuracy. Thus measuring haematocrit to obtain plasma volume can be omitted. This dilution of blood brought the concentration of the supernatant into a similar range with corticosterone levels in the peripheral plasma, thus enabling us to use the routine determination in measuring corticosterone in the adrenal effluent. Total corticosterone secreted into the adrenal venous blood for a period of 3 minutes was calculated. ACTH activity of the test plasma was expressed in terms of corticosterone secretion rate (ng/min). Using this method, we obtained an excellent linear relationship between log responses and log doses. The following formula of the dose-response curve with the precision index, \( \lambda = 0.112 \), was obtained with 6 determinations at each dose between 0.02 and 0.18 mU of ACTH USP.

\[
y = 3.514 + 1.115 \times \\
\text{where,}
\]

\[
y: \text{log response (ng/min)} \\
x: \text{log dose (mU)}
\]

**ACTH and pressor activities of hypothalamic and neurohypophysial tissues**

ACTH activity of the extracts of tissues were assayed in hypophysectomized male rats of Sprague-Dawley strain 24 hours postoperatively as described by Guillemin et al. (1958). The body weight of these assay rats ranged from 140 to 180 g. Pressor activity was measured according to the method of Dekanski (1952).

**Histological examination**

The anterior and posterior pituitary lobes, the testes, the prostate, and the adrenals were dissected out and weighed soon after the animals were sacrificed. These organs, except the pituitary glands, were fixed in Bouin's solution, embedded in paraplast, cut in 5 \( \mu \) thick sections, and stained with haematoxylin-eosin for histological examination.

**RESULTS**

**Plasma corticosterone after stress**

Corticosterone levels in the plasma of DI and control rats under various conditions are shown in Table 1. There was no significant difference in plasma corticosterone concentration between non-treated DI and control animals as indicated by almost identical values in non-treated rats of both groups. Plasma corticosterone 15 minutes after ether inhalation or injections of histamine, vasopressin, and acetylcholine rose to the same extent in the DI rats and the control animals. A smaller response was observed in DI rats following epinephrine injection, but the difference from the response in the control animals was statistically insignificant. Fifteen minutes after an intraperitoneal injection...
Table 1.
Plasma corticosterone of control and DI rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>µg/100 ml ± S.E. DI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated*</td>
<td>19.3 ± 1.87 (10)**</td>
<td>17.1 ± 1.91 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>Ether inhalation over 75 seconds</td>
<td>32.5 ± 1.45 (11)</td>
<td>33.9 ± 1.38 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>0.9 % saline, 0.2 ml</td>
<td>23.3 ± 2.15 (10)</td>
<td>14.2 ± 1.85 (11)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Epinephrine hydrochloride, 0.02 mg/100 mg</td>
<td>47.2 ± 4.27 (10)</td>
<td>36.3 ± 4.45 (11)</td>
<td>NS, 0.1 &gt; 0.05</td>
</tr>
<tr>
<td>Histamine dihydrochloride, 0.25 mg/100 g</td>
<td>43.7 ± 3.07 (10)</td>
<td>37.5 ± 2.97 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>Vasopressin 100 mU/100 g</td>
<td>38.2 ± 3.04 (10)</td>
<td>33.8 ± 5.38 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>Nembutal, 3.5 mg/100 g</td>
<td>23.0 ± 3.85 (10)</td>
<td>19.0 ± 3.91 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Nembutal and acetylcholine, 0.1 mg/100 g</td>
<td>35.7 ± 2.43 (10)</td>
<td>33.4 ± 2.61 (10)</td>
<td>NS</td>
</tr>
</tbody>
</table>

All samples injected 15 minutes before blood collection except Nembutal.

* In this experiment only, blood was collected from the trunk after decapitation without using ether anaesthesia.

** Number of rats per group.

of 0.2 ml of 0.9% saline, which may be considered a minor transient stress, plasma corticosterone levels in control rats were higher than those in DI rats ($P < 0.01$). However, there was no significant difference between non-treated values and those after saline injection in the control group and the DI group.

**Hypothalamic CRF**

CRF activities in the hypothalamic extracts obtained from DI and control animals are shown in Table 2. In this assay system a good dose-response relationship was obtained at 2 dose levels with an interval of 4. Calculated potency of CRF in the hypothalamic extract obtained from DI rats (per hypothalamus) was 47.2% of that of control animals, with 95% confidence limits of 10.3–104.5%. If CRF activities were expressed on weight basis, CRF in hypothalami of DI rats was 58.2% of that of control rats. The difference between CRF activities of these 2 groups was not statistically significant. If only the responses induced by the low dose were compared, CRF activity in DI hypothalamus was significantly less than the control ($P < 0.02$). Pressor assay of these 2 extracts showed that the pressor activity of hypothalamic extract equivalent to 0.7 hypothalami from the control rats was less than 3.2 mU of vasopressin. This dose does not influence the assay system employed. There was no measurable contamination of the extracts from control animals with
Table 2.
Hypothalamic CRF measured by plasma corticosterone in CPZ-M-N preparations.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hypothalamus</th>
<th>µg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 0.7</td>
<td>26.5 ± 3.64</td>
<td>(5)*</td>
</tr>
<tr>
<td>Control 0.175</td>
<td>17.7 ± 1.84</td>
<td>(5)</td>
</tr>
<tr>
<td>DI 0.7</td>
<td>22.3 ± 3.42</td>
<td>(5)</td>
</tr>
<tr>
<td>DI 0.175</td>
<td>10.8 ± 1.17</td>
<td>(5)</td>
</tr>
</tbody>
</table>

P value
- between control and DI: NS, > 0.05
- between doses: < 0.01
- lack of parallelism: NS

* Number of assay rats per group.

ACTH or ACTH-like substances, as indicated by lack of response in hypophysectomized rats (Table 3).

*Lack of CRF in the posterior pituitary lobe of the DI rats*

The acid extracts of the posterior lobe of control and DI rats, equivalent to 1/3 of the neural lobe, were tested for CRF in CPZ-M-N preparations. Plasma corticosterone concentrations in assay animals 15 minutes after an intravenous injection of the extract obtained from control rats was significantly higher than that following an injection of the extract obtained from DI rats (Table 3). But this difference may be explained in part by the presence of vasopressin in the posterior lobe of control rats since the dose administered contained 50 pressor mU. When 50 mU arginine vasopressin was given to assay animals, plasma corticosterone rose to 17.2 µg/100 ml.

The extracts obtained from DI rats which were lacking in vasopressin (Valtin et al. 1965) showed steroidogenic activity. Since the posterior lobe contains ACTH or ACTH-like activity (Itoh 1962; deWied et al. 1964), these extracts were tested for ACTH activity in hypophysectomized rats. As shown in Table 3, ACTH activity was present in the posterior pituitary extracts from control and DI rats in almost the same amount. These data, therefore, do not permit any definite conclusion as to the CRF content in the posterior pituitary glands of these rats.

Our previous study indicated that an intravenous injection of the acid extracts of the posterior pituitary lobes increased plasma ACTH in neurohypophysectomized rats to a much larger extent than equippressor doses of vasopressin (Arimura et al. 1965). We decided to test the posterior pituitary glands of DI rats for CRF using neurohypophysectomized rats. As shown in Table 3, CRF of the posterior pituitary lobe of DI rats was negligible in this assay system,
Table 3.
CRF and ACTH activities in the posterior pituitary lobes.

| CRF in the posterior pituitary lobes tested in CPZ-M-N preparations |
|---|---|---|
| Dose | Plasma corticosterone $\mu g/100$ ml | $p$ |
| Control | $1/3$ posterior lobe | $23.7 \pm 1.09$ (5)* | --- |
| DI | $1/3$ posterior lobe | $16.8 \pm 1.60$ (5) | $< 0.01$ |
| Saline | | $5.6 \pm 0.55$ (6) | |
| Vasopressin 50 mU | | $17.2 \pm 2.19$ (4) | |

| ACTH in the posterior pituitary lobes and the hypothalami tested in hypophysectomized rats |
|---|---|---|
| Dose | Plasma corticosterone $\mu g/100$ ml | $p$ |
| Control | $1/3$ posterior lobe | $28.9 \pm 2.54$ (5) | --- |
| DI | $1/3$ posterior lobe | $25.9 \pm 3.19$ (5) | NS |
| Control | $1/3$ hypothalamus | $3.9 \pm 0.20$ (7) | |

| CRF in the posterior pituitary lobes tested in neurohypophysectomized rats |
|---|---|---|
| Dose | Plasma ACTH** ng/min | $p$ |
| Control | 0.8 mg | $222 \pm 20.1$ (7) | --- |
| DI | 0.8 mg | $20 \pm 4.1$ (7) | $< 0.001$ |
| Saline | | $12 \pm 3.2$ (6) | |

* Number of rats per group.
** Plasma ACTH activity was expressed by corticosterone secretion rate in adrenal venous effluent in hypophysectomized assay rats.

while that of the control was considerably higher. Part of this activity may be ascribed to vasopressin.

Weight of the pituitary gland, the adrenal, and the reproductive organs; and morphology of the adrenal

Since there was significant difference in body weight between DI and control animals, the organ weight was expressed in g per 100 g body weight as well as their actual weight (Table 4). Some of the DI animals were 4 months old and some were 5 months old, while all control rats were 5 months old. However, the average body weight of the control rats at 4 months of age (357.5 g) was still significantly larger than that of the DI rats of the same age.
Table 4.
Body weight and weights of pituitary gland, adrenal, testes, and ventral prostate.

<table>
<thead>
<tr>
<th></th>
<th>Control rats</th>
<th>DI rats</th>
<th>P DI vs control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>408 ± 12.6 (10)*</td>
<td>235 ± 0.8 (10)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Anterior pituitary gland, mg</td>
<td>8.8 ± 0.29 (10)</td>
<td>5.0 ± 0.12 (10)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Anterior pituitary gland, mg/100 g</td>
<td>2.17 ± 0.128 (10)</td>
<td>2.11 ± 0.058 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Posterior pituitary gland, mg</td>
<td>1.7 ± 0.07 (10)</td>
<td>2.0 ± 0.11 (10)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Posterior pituitary gland, mg/100 g</td>
<td>0.42 ± 0.024 (10)</td>
<td>0.86 ± 0.025 (10)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Adrenal, mg</td>
<td>51.9 ± 1.85 (10)</td>
<td>31.1 ± 1.35 (10)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Adrenal, mg/100 g</td>
<td>12.7 ± 0.616 (10)</td>
<td>13.2 ± 0.59 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Testes, g</td>
<td>3.1 ± 0.08 (10)</td>
<td>2.1 ± 0.05 (10)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Testes, g/100 g</td>
<td>0.76 ± 0.082 (10)</td>
<td>0.89 ± 0.036 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Ventral prostate, g</td>
<td>0.35 ± 0.014 (10)</td>
<td>0.25 ± 0.013 (10)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ventral prostate, g/100 g</td>
<td>0.09 ± 0.016 (10)</td>
<td>0.11 ± 0.007 (10)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Number of rats per group.

or older (235.0 g). As reported by Valtin et al. (1965) the weight of the posterior pituitary lobe of DI rats was considerably greater than that of control animals. But the adenohypophyses, the testes, the ventral prostates, and the adrenals of DI and control animals were of the same weight when these weights were related to total body weight.

Histologic examination of the adrenals revealed that the zona fasciculata, zona glomerulosa, and zona reticularis were well developed in both DI and control animals. These findings suggest that the basal secretion of ACTH (as well as that of gonadotrophin) is well maintained in the DI rats. This is in good agreement with the data reported by McCann et al. (in press).

**DISCUSSION**

The data reported here show that plasma corticosterone rose to the same extent in response to ether, histamine, acetylcholine and vasopressin in both control and DI rats. Since the DI rats used in this experiment have been shown to lack vasopressin completely (Valtin et al. 1965), it follows that ACTH is released following stress even in the absence of vasopressin. These data could be interpreted as evidence that physiological CRF is not vasopressin. According to McCann et al. (in press) the plasma corticosterone levels of DI rats responded
to etherization and to mild restraint, but the increase was significantly less than in normal animals. In our experiments, however, plasma corticosterone rose to the same extent in both DI and control rats 15 minutes after etherization. Although our animals were etherized shortly before blood collection, the second etherization probably did not influence plasma corticosterone levels, since the difference in plasma corticosterone concentrations between the non-treated group which were not etherized for blood collection and the saline-injected group which were etherized for blood collection was not significant either in control or DI rats. However, after 0.9% saline injection intraperitoneally plasma corticosterone was significantly higher in control animals than in DI rats. A lower plasma corticosterone concentration was found in rats injected i.p. with saline than the level in non-treated DI rats. This does not imply that plasma corticosterone decreased after the saline injection. Since non-treated rats were transferred from the animal room to the laboratory before they were sacrificed, plasma corticosterone concentration in non-treated animals may not indicate true resting level of the steroid. It is possible that the stressful effect of saline injection might have been smaller than the stimulative effect resulting from transferring the animals to a strange environment. The true resting corticosterone concentration should be much lower than the non-treated values or any other low values shown in Table 3.

These data, in sum, imply that the pituitary adrenal axis of DI rats is activated to the same extent as that of normal animals, if the stress applied to the animals was intense. Transient and milder stress may induce a smaller response in DI rats as shown by the result with an intraperitoneal injection of saline, but this point was not exhaustively investigated.

A larger increase in plasma corticosterone, though not statistically significant, was also observed in control rats after epinephrine injection than in DI rats. If the intensity of any given stress can be evaluated by the extent of increase in plasma corticosterone, the stress of epinephrine injection may be considered to be intense. Therefore, different magnitudes of response between DI and control rats may be related not only to different intensities of stress, but also to different stresses. One could consider the possibility that vasopressin plays a secondary role in ACTH release under certain stressful circumstances. Similar findings were observed in neurohypophysectomized rats (Fisher & de Salvo 1959; de Wied et al. 1964; Arimura et al. 1965).

Hypothalamic CRF in DI rats assayed in CPZ-M-N rats was 47% of normal. Owing to the large standard error of the mean responses at high doses in the assay this difference does not reach statistical significance. However, it suggests that the amount of hypothalamic CRF is lower than normal in DI rats. This suggestion could be supported by our observation that administration of epinephrine or of saline intraperitoneally raised the level of plasma corticosterone less in DI rats than in normal rats (Table 1).

163
It is difficult to measure CRF in the posterior pituitary extracts of the rat, unless they are subjected to purification procedures, because they contain a large amount of vasopressin and ACTH or ACTH-like substances. As shown in Table 3, the acid extracts of the posterior pituitary lobe of control and DI rats possessed ACTH activity in the same amounts.

In CPZ-M-N preparations, the steroidogenic activity of the acid extracts of the posterior pituitary lobes of control rats may be ascribed to ACTH plus vasopressin and that of DI rats to ACTH alone. Consequently, a greater increase in plasma corticosterone was observed after the injection of the extract from control rats. Although the same amounts of posterior pituitary extracts seemed to induce greater steroidogenic responses in hypophysectomized, than in CPZ-M-N treated rats, this may be ascribed to different sensitivities of adrenal cortex between different batches of animals.

We could not test adrenal response to exogenous ACTH in DI rats. However, the fact that most of the stresses used induced similar increases in plasma corticosterone concentration both in DI and control rats which may suggest similar adrenal responsiveness to ACTH in these two groups of animals.

In order to test these extracts for CRF activity they were injected into neurohypophysectomized rats and the plasma ACTH activity of the recipients measured. It was considered unlikely that the small amount of ACTH in the material injected would contribute perceptively to this activity. We found that the extracts from normal rats raised plasma ACTH level significantly, whereas the extracts from DI rats did not. Whether the ACTH releasing activity of posterior pituitary extracts can be wholly ascribed to vasopressin was carefully studied in one of our previous experiments (Arimura et al. 1965). We suggested the presence of CRF in the rat’s neurohypophysis in addition to vasopressin. From the present data it may be concluded that CRF is absent in the posterior pituitary lobes of DI rats.

The absence of neurohypophysial CRF and a smaller amount of hypothalamic CRF in DI rats could reflect a partial defect of CRF synthesis. Or, it may be possible that chronic dehydration influences the neurohypophysial CRF, as it does oxytocin (Valtin et al. 1965). A limited number of DI rats did not permit us to study the effect of chronic treatment of these DI rats with vasopressin on CRF content of the hypothalamus and posterior pituitary gland. However, McCann et al. (in press) reported briefly that there was no difference in hypothalamic CRF activities between pitressin-treated DI rats and control rats.

ACKNOWLEDGEMENTS

We are indebted to Dr. Heinz Valtin, Dartmouth Medical School, for supplying us hereditary hypothalamic diabetes insipidus rats and control rats. We also wish to express our appreciation to Mrs. Catherine Guynes and Miss Joan Raymond for their

164
excellent technical assistance and to Dr. William Locke for his help in preparing the manuscript.

REFERENCES


Received on July 25th, 1966.