Adrenocorticotropic Action of Antidiuretic Hormone

By James G. Hilton, M.D.

By means of direct arterial perfusion of the adrenal glands of the hypophysectomized dog, it has been shown that synthetic lysine, arginine and acetyl arginine vasopressins stimulate the adrenal cortex directly to secrete hydrocortisone. Pressor activity and cortisol-stimulating activity were demonstrated to be independent of each other. Similar polypeptides, such as oxytocin, insulin, glucagon, and pressor amines, such as epinephrine and norepinephrine, did not show any cortisol-stimulating activity. ACTH was found consistently to increase the rate of secretion of aldosterone when perfused through the glands of hypophysectomized animals. The probable role of arginine vasopressin as an important factor in the stress reaction is considered along with its postulated ability to activate adrenal phosphorylase.

We have previously reported our preliminary experiments which show that synthetic vasopressin, when injected into the arterial circuit of the adrenal glands of the hypophysectomized dog, stimulates directly adrenal cortical secretion.1, 2 In this paper I should like to report the results of this work together with additional experiments designed to elucidate the mechanism of the adrenocorticotropic action of vasopressin. Associated with me in this work have been Dr. Louis F. Seian, who is a Fellow of this Association, Dr. Coenraad Westermann and Dr. Oscar Krueci.

Methods

Adult mongrel dogs were anesthetized by the intravenous administration of 0.5 mg. per Kg. body weight of sodium pentobarbital. When desired, hypophysectomy was done by the transbuccal approach of McClean.3

In all experiments, the adrenal glands were perfused using the in situ technic of Hilton et al.4 In this preparation, arterial heparinized blood obtained from donor dogs is pumped at a constant rate of 10 ml. per minute into the arterial circuit of the isolated adrenal glands of the recipient animal (fig. 1A). At the start of perfusion by the pump, the heart of the recipient animal is fibrillated so that the glands are perfused solely by donor blood for the duration of the experiment. Side arms on the tubing leading into the arterial circuit of the adrenal glands allow for the continuous recording of blood pressure within the isolated adrenal pouch preparation, as well as for the injection or infusion of various agents.4

The venous outflow from the adrenal veins was collected in iced graduated cylinders. In none of the experiments was any of the venous effluent from the adrenal glands allowed to recirculate or to return to the general circulation of the recipient (fig. 1B). The rate of secretion of hydrocortisone was calculated as the product of the rate of venous blood flow and the arteriovenous difference in the concentration of hydrocortisone.

After several control collections, the agents under study for their adrenocorticotropic activity were infused via a side arm in the arterial perfusing circuit and additional collections were made. At the end of each experiment, an injection of ACTH was given to verify adrenal viability and to compare the rates of secretion of hydrocortisone with those of the previous infusion periods.

The concentrations of hydrocortisone were determined by the method of Peterson et al.5 The concentrations of aldosterone in adrenal venous blood were measured by a double isotope dilution technic.*

Results

Experiments with Synthetic Lysine Vasopressin

Six experiments were done. They are summarized in table 1. Because of the variability in responsiveness of the adrenal glands to the standard dose of ACTH from 1 dog to the

*We are indebted to Dr. James O. Davis of the National Heart Institute for these determinations.
Figure 1
Diagram of adrenal arterial and venous perfusion pouches. In A the circuit is clamped off at 14 and blood from a donor animal is pumped into the aortic pouch via side arm 15. In B venous blood is not returned to the external jugular vein. (Reprinted by permission of the American Journal of Physiology.)

Table 1
Comparison of the Increment in the Rate of Secretion of Hydrocortisone (ΔF) Following Lysine Vasopressin with that Following ACTH

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Before ACTH</th>
<th>Lysine vasopressin dose</th>
<th>duration</th>
<th>ΔF after vasopressin</th>
<th>After ACTH* ΔF</th>
<th>Vasopressin responsiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F μg./min.</td>
<td>ΔF μg./min.</td>
<td></td>
<td></td>
<td>μg./min.</td>
<td></td>
</tr>
<tr>
<td>HYPOPHYSECTOMIZED</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.3</td>
<td>0.22 U./min. 10 min.</td>
<td>0.7</td>
<td></td>
<td>2.0</td>
<td>35%</td>
</tr>
<tr>
<td>2</td>
<td>0.3</td>
<td>0.3 U./min. 7 min.</td>
<td>1.6</td>
<td></td>
<td>3.5</td>
<td>46%</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>0.3 U./min. 7 min.</td>
<td>3.3</td>
<td></td>
<td>11.0</td>
<td>30%</td>
</tr>
<tr>
<td>NONHYPOPHYSECTOMIZED</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.6</td>
<td>0.22 U./min. 10 min.</td>
<td>6.2</td>
<td></td>
<td>8.1</td>
<td>77%</td>
</tr>
<tr>
<td>5</td>
<td>9.2</td>
<td>0.16 U./min. 7 min.</td>
<td>4.0</td>
<td></td>
<td>2.3</td>
<td>174%</td>
</tr>
<tr>
<td>6</td>
<td>6.0</td>
<td>0.2 U./min. 7 min.</td>
<td>2.4</td>
<td></td>
<td>1.9</td>
<td>126%</td>
</tr>
</tbody>
</table>

*One unit per minute for 7 minutes.
†ΔF after vasopressin
ΔF after ACTH × 100.

cortisol above the control rate of secretion gives a measure of the maximal responsiveness or sensitivity of the glands in each dog to the supramaximal dose of ACTH, i.e., 1 unit
Experiment showing effect of lysine vasopressin on the rate of secretion of hydrocortisone. Adrenal glands of a nonhypophysectomized dog are perfused with blood from a hypophysectomized donor.

Table 2
Effects of Graded Doses of Arginine Vasopressin on the Increment in the Rate of Secretion of Hydrocortisone ($\Delta F$) in Hypophysectomized Dogs

<table>
<thead>
<tr>
<th>No. experiments performed</th>
<th>Before ACTH av. F µg./min.</th>
<th>Arginine vasopressin av. $\Delta F$ µg./min.</th>
<th>After ACTH† av. $\Delta F$ µg./min.</th>
<th>Vasopressin‡ responsiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>&lt;0.1</td>
<td>0.001</td>
<td>0.4</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>&lt;0.1</td>
<td>0.01</td>
<td>1.6</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>&lt;0.1</td>
<td>0.1</td>
<td>2.5</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
<td>0.4</td>
<td>4.5</td>
<td>3.8</td>
</tr>
</tbody>
</table>

*All doses given over a 7-minute period.
†One unit per minute for 7 minutes.
‡ $\Delta F$ after vasopressin av. $\Delta F$ after ACTH $\times$ 100.

per minute for 7 minutes.* The increment in the secretion of cortisol following the administra-

*In our preparation, the secretion of hydrocortisone first becomes detectable following 1.0 mU. of ACTH and becomes maximal at 10 mU. All injections in this type of assay were given as single doses of 1 ml. each. There was no recirculation of the ACTH through the glands.21
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Effect of arginine vasopressin. Adrenal glands of a hypophysectomized dog are perfused with blood from a hypophysectomized donor.

Experiments with Synthetic Arginine Vasopressin

Figure 3 depicts a typical example of this group of experiments. Both recipient and donor animals had been completely hypophysectomized; consequently, control cortisol secretion rates were very low, averaging less than 0.2 μg./min. Following the injection of arginine vasopressin, the rate of secretion of cortisol rose to a peak of 4.2 μg./min. representing an increment of 4.0 μg./min. above control. Mean blood pressure rose by an average of 10 mm. Hg.

In an attempt to define a dosage-response relationship, 8 additional experiments were performed in completely hypophysectomized animals. These experiments are summarized in table 2, which is similar in design to table 1. There is a good correlation between the dose of vasopressin and the response of the glands.

Since in all experiments thus far the vasopressin had been injected only over a 7-minute...
Effect of 30 minutes of perfusion of arginine vasopressin on the rate of secretion of hydrocortisone in the hypophysectomized dog.

Table 3
Rate of Secretion of Hydrocortisone (F) and Aldosterone µg./min. Following Injections of Various Synthetic Vasopressins*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Vasopressin dosage over 7-minute period</th>
<th>F</th>
<th>Control aldosterone</th>
<th>After vasopressin F aldosterone</th>
<th>After ACTH F aldosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>lysine 0.3 U./min.</td>
<td>0.35</td>
<td>0</td>
<td>1.9</td>
<td>3.8</td>
</tr>
<tr>
<td>2</td>
<td>lysine 0.3 U./min.</td>
<td>0.6</td>
<td>0.005</td>
<td>3.5</td>
<td>0.008</td>
</tr>
<tr>
<td>3</td>
<td>acetyl arginine 14.3 µg./min.</td>
<td>0.6</td>
<td>0.01</td>
<td>6.5</td>
<td>0.045</td>
</tr>
<tr>
<td>4†</td>
<td>acetyl arginine 28.6 µg./min.</td>
<td>4.3</td>
<td>0.163</td>
<td>11.0</td>
<td>0.141</td>
</tr>
</tbody>
</table>

*ACTH was given at the end of each experiment at a rate of 1 U./min. for 7 minutes. All animals were hypophysectomized except as noted.
†Donor animal incompletely hypophysectomized at time of bleeding.

period, and since the stimulatory effect of vasopressin had more or less disappeared within 20 minutes after the end of the injection, the effect of a prolonged infusion of arginine vasopressin on the rate of secretion of cortisol was determined. Figure 4 illustrates the results which were obtained when such an experiment was performed on a hypophysectomized animal. Despite continuation of the vasopressin infusion (10 mU./min.) for 30 minutes, the rate of secretion of cortisol began to fall to control levels during the infusion.

Experiments Concerned with the Rate of Secretion of Aldosterone

Four experiments were performed and are summarized in table 3. It should be noted that in 3 of the experiments there was a definite increase in the rate of secretion of aldosterone following ACTH. In experiment 4 the donor animal was found to have a small remnant.
of pituitary tissue after hypophysectomy. Apparently this remnant was insufficient to produce a maximal secretion of cortisol in the perfused adrenal glands, since there was a further rise after the administration of vasopressin and ACTH, but was sufficient to maintain a sustained, high rate of aldosterone secretion.

Although lysine vasopressin (experiments 1 and 2) showed its usual stimulatory effect on the secretion of cortisol, it was without effect on the secretion of aldosterone. On the other hand, acetyl arginine vasopressin showed both hydrocortisone- and aldosterone-stimulating activity as may be seen in experiment 3, table 3. However, acetyl arginine vasopressin has no pressor activity, a phenomenon illustrated in figure 5. When arginine vasopressin was subsequently injected, the expected rise in mean blood pressure occurred without a rise in the secretion of cortisol. It is possible that the acetyl analogue blocked, in some manner, the usual stimulatory effect of arginine vasopressin on the secretion of hydrocortisone by the perfused adrenal glands without blocking its pressor activity on the blood vessels of the adrenal glands. Further investigations of this question of blocking action, as well as of the effects of arginine vasopressin itself on aldosterone secretion, are needed for definitive conclusions.

Experiments in Which Miscellaneous Compounds Were Studied

It was considered of interest to see whether other polypeptides, similar or dissimilar to vasopressin, might have cortisol-stimulating
activity. In 4 experiments (2 with hypophysectomized and 2 with nonhypophysectomized preparations), synthetic oxytocin, in a dosage ranging from 10 U./min. for 1 minute to 1.4 U./min. for 7 minutes, had no activity. At these high doses there was, however, pressor activity as seen in the experiment depicted in figure 6.

In 2 experiments with hypophysectomized and in 3 experiments with nonhypophysectomized animals, insulin, in a dosage of 1 U./min. to 80 U./min for 1 to 10 minutes, had no effect on either the mean blood pressure or the secretion of cortisol (fig. 7).

Glucagon, in 6 hypophysectomized and 14 nonhypophysectomized preparations, had no effect either on the rate of secretion of cortisol or on the mean blood pressure (fig. 7). Dosages used ranged from 0.01 mg./min. to 1.0 mg./min. for 1 to 38 minutes.

Finally, norepinephrine (4 μg./min. for 7 minutes) and epinephrine (2 μg./min. for 7 minutes) had no effect on the rate of secretion of cortisol. The former compound did, however, cause a rise in mean blood pressure of 15 mm. Hg in the perfused adrenal glands (fig. 8).

3′,5′-adenosine monophosphate (3′,5′-AMP) has been shown to have potent adrenocortico-
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Figure 8

Comparison of the effects of norepinephrine and ACTH on the rate of secretion of hydrocortisone. At a norepinephrine was injected at a rate of 0.1 µg./min. At b 1.0 U./min. of ACTH was administered. Animal hypophysectomized.

Figure 9

Comparison of the hydrocortisone-stimulatory activity of 3',5'-adenosinemonophosphate with that of arginine vasopressin and ACTH in the hypophysectomized dog.

tropic activity. Arginine vasopressin was injected into an adrenal gland preparation which was being perfused with 3',5'-AMP at 0.1 mg./min. The cortisol-stimulatory activity of 3',5'-AMP is illustrated in figure 9. Following each injection of arginine vasopressin (100 mU./min. for 7 minutes), there was a transient increment in the rate of secretion of cortisol of 2.1 µg./min. and 1.1 µg./min. respectively. Immediately following cessation of the 3',5'-AMP infusion, cortisol secretion fell to basal levels.

It is known from the experiments of Haynes that ACTH causes the accumulation of 3',5'-adenosinemonophosphate in incubates of beef adrenal cortex and that this, in turn, stimulates the production of adrenal phosphorylase. Haynes, Koritz and Péron have also shown that 3',5'-AMP added to the adrenal glands of the rat which have been incubated in vitro elicits an increase in the rate of production of corticosteroid which is equal to, or greater than, that produced by ACTH. The stimulation of the secretion of cortisol after perfusion of the intact adrenal glands of our preparation with 3',5'-AMP supports the
theory that corticosteroid synthesis is mediated by stimulation of the production of adrenal phosphorylase. Since the effect of vasopressin on the secretion of cortisol is relatively short-lived compared to that of 3',5'-AMP and ACTH, it is conceivable that vasopressin may act, in some manner, to activate adrenal phosphorylase rather than actually to stimulate its production.

Discussion

In 1958 Royce and Sayers demonstrated that vasopressin had an ACTH-like activity in the hypophysectomized rat. They suggested that vasopressin might directly stimulate the adrenal cortex but did not pursue this further. Instead, they proposed that it released ACTH from nonadenohypophyseal binding sites. It would appear that their first suggestion was correct. Our observations clearly demonstrate a direct cortisol-stimulating action of synthetic lysine, arginine and acetyl arginine vasopressins when they are perfused through the isolated adrenal glands. That this secretion is hydrocortisone was confirmed in 2 ways: (1) spectrophotometric analysis of the plasma Porter-Silber chromogens revealed maximal absorption at 410 m\(\mu\), similar to that of hydrocortisone alcohol, and (2) paper chromatography of methylene chloride extracts of plasma showed a motility identical to hydrocortisone alcohol (R.F. = 18).

Cortisol-stimulatory effects of arginine vasopressin, the naturally occurring form in dog and man, were noted with amounts as little as 7 mU. infused over a 7-minute period. Ginsburg and Brown have demonstrated in the peripheral blood of rats amounts of ADH ranging from 1 to 26 mU./ml. under conditions of severe osmotic stimulation or hemorrhage. Such amounts are well within the range which, in our experiments, produces maximal secretion of cortisol.

Recently, it has been shown that as little as 2 mU. of arginine vasopressin will cause a release of ACTH when injected directly into the third ventricle of the dog. Therefore, it is apparent that arginine vasopressin may have 3 major functions in salt and water homeostasis: (1) antidiuretic activity, (2) ACTH-releasing activity, and (3) direct stimulatory activity on the adrenal cortex.

We think it reasonable to ascribe an important role to arginine vasopressin in the "stress reaction." It is conceivable that the major role is played by the neurohypophysis, leaving to the adenohypophysis the regulation of nonstressful adrenal cortical homeostasis by means of ACTH.

Acknowledgment

Professor Vincent du Vigneaud's laboratory at Cornell University, School of Medicine, kindly supplied the synthetic lysine, arginine, and acetyl arginine vasopressins as well as the synthetic oxytocin used. Dr. Mary A. Root of the Lilly Research Laboratories generously donated crystalline glucagon.

References


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