Adrenoglomerulotropin

By Gordon Farrell, M.D.

The secretion of aldosterone by the zona glomerulosa of the adrenal cortex is controlled by a system which is not completely understood. Volume receptors are of importance although other receptors may be involved. The midbrain contains elements which influence the secretion of the steroid since lesions in this area of the brain stem reduce aldosterone output. A lipid factor, adrenoglomerulotropin, obtained from pineal extracts, selectively stimulates secretion of aldosterone; but a second factor, also from the pineal gland inhibits the output of aldosterone as well as of cortisol. It is suggested that the control of the secretion of aldosterone may be by an excitatory-inhibitory system involving the interplay of pituitary corticotropin, adrenoglomerulotropin and an anticorticotropin of pineal origin.

THREE questions are frequently asked about aldosterone. First, where is aldosterone synthesized; second, in what way is its secretion controlled; and finally, what is the role of the steroid in certain diseases? Considerable information has been brought to bear on these points during the relatively few years since aldosterone was discovered. Perhaps we can summarize some of the more recent developments.

With regard to the first question, almost everyone is agreed, now, that aldosterone is secreted by the outermost zone of the adrenal cortex, the zona glomerulosa. This zone undergoes selective hypertrophy in response to deprivation of sodium at a time when the secretion of aldosterone is markedly enhanced. This is illustrated in figure 1, from a recent experiment. Figure 1A is a photomicrograph of the adrenal gland of a dog which was given normal amounts of sodium. Figure 1B shows the effects of a 3-month period of deprivation of sodium. The rate of secretion of aldosterone in dogs in which the sodium intake is low (2 mEq./day) is approximately 3 times that in animals on a normal diet. It is of interest that the output of cortisol is frequently lower in dogs which are deprived of sodium than in control animals, indicating that the stimulation of the secretion of aldosterone is not mediated by an increased release of ACTH.

Additional and very convincing evidence that the zona glomerulosa is the locus of synthesis of aldosterone has recently been provided by the elegant experiments of Ayres, Gould, Simpson, and Tait,1 and of Stachenko and Giroud.2 Zona glomerulosa tissue synthesizes aldosterone in vitro, whereas very little is elaborated by tissue from the inner zones. Interestingly enough, ACTH (which stimulates steroidogenesis when added to incubates of tissue from the zona fasciculata and the zona reticularis) does not appear to affect the zona glomerulosa in vitro.2 Taken together, these observations appear to settle the long dispute touched off by the original publications of Deane and Greep some years before aldosterone was discovered. These authors postulated that the zona glomerulosa secretes an electrolyte-active steroid independently of the rest of the adrenal gland,3,4 and their speculations have apparently been proven correct.

The second question, with regard to the modus operandi of the system for the control of the secretion of aldosterone, is not so readily answered. The stimuli which alter aldosterone output are many.5 However, alterations in the balance of sodium and in the volume of body fluids stand out as especially important. The restriction of sodium is a

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This work was carried out during the tenure of an Established Investigatorship of the American Heart Association. Work supported by grants from the U. S. Public Health Service and the Cleveland Area Heart Society.

Circulation, Volume XXI, May 1950

1099
constant and potent stimulator of the secretion of aldosterone in all species which have been studied, although the manner in which the organism detects the depletion or repletion of sodium is still unknown. Sodium-sensitive chemoreceptors somewhere within the vascular system are a possibility, but thus far none have been described. The subject of volume receptors for the control of the secretion of aldosterone has received considerably more study. The location of the receptors is still in question; the atria may very well contain them as well as the carotid arteries. This subject will be considered by subsequent speakers in this symposium.

Whatever the nature of the receptor systems may be, it seems clear that an effector system must exist by which the zona glomerulosa is stimulated to increase the secretion of aldosterone in response to changes in the internal environment. It is this effector system which I should like to consider briefly now. The participation of nerve fibers can probably be discounted since denervation of the adrenal glands has no effect on the output of aldosterone. Direct action on the gland by electrolytes of the blood has been suggested by some authors as a possible stimulus, but the modest effects on steroidogenesis induced by alterations in the sodium and potassium content of the adrenal perfusate are probably too small to account for the changes in aldosterone output observed under various experimental conditions. Furthermore, in many instances, profound changes in the rate of secretion of the steroid occur without any change in the concentrations of electrolytes in the blood.

The possibility that the zona glomerulosa is subject to the influence of a tropic factor which is specific for this zone was entertained in our laboratory a few years ago, and indirect

Figure 1

Adrenal gland of the dog. A. Control. From above downward are zona glomerulosa, zona fasciculata and zona reticularis. B. Adrenal gland of a dog on a low-sodium diet for 3 months. Note the hypertrophy of the zona glomerulosa.
evidence for such a factor was obtained. The concept has since received support from other laboratories. Although the existence of this hormone was originally proposed on the basis of admittedly indirect evidence, we feel that presently available data definitely establish that the hormone exists, and that it can be obtained from extracts of the diencephalon. This hormone was first demonstrated in the course of assays of neutral saline extracts of various areas of beef diencephalon. The assay animal was the mideollicular, decerebrate dog in which the brain above the level of the cut was removed together with the pituitary and pineal glands. The concentrations of steroids were determined by analysis of adrenal venous blood which was collected during the infusion of test solutions. Extracts of whole diencephalon stimulated the secretion of aldosterone (table 1). When the diencephalon was divided into anterior and posterior parts, activity was found primarily in the posterior portion when this structure included the pineal gland. When the pineal gland was removed and assayed separately, most of the activity was found to be present in the pineal gland (table 1). An acetone extract of pineal glands also yielded activity. To our surprise, when the residue from the acetone extract of pineal glands was partitioned between hexane and water, the activity was recovered from the hexane fraction indicating a definite degree of lipid solubility (table 1). We originally proposed the name glomerulotropin for this hormone, but reservations of renal physiologists who feel that the name implies an effect on the glomerulus of the kidney lead us to suggest that it be called adrenoglomerulotropin.

We have recently carried out a number of procedures designed to purify adrenoglomerulotropin. Chromatography of the residue from acetone or alcohol extracts of pineal gland on a florosil column, using hexane as the mobile phase, results in a many-fold purification. The active fractions from florosil columns have been subjected to paper chromatography. In the system propylene glycol–hexane, the activity runs with or just behind the solvent front, indicating a polarity much less than that of any of the corticosteroids. In the system water-chloroform-ethanol (3:5:5) on silicatreated paper, in which the developing phase is aqueous ethanol, the active material runs

| Table 1 | Steroidogenic Properties of Extracts of Beef Diencephalon or Pineal |
| --- | --- | --- |
| Experiment | No. of experiments | Aldosterone µg./100 Kg. Body wt./hr. | Cortisol µg./Kg. body wt./hr. |
| Intact controls | 9 | 31.2 ± 3.7 | 32.8 ± 3.9* |
| Decerebrate controls† | 8 | 7.86 ± 0.87 | 2.19 ± 0.49 |
| Decerebrate controls | 6 | 8.5 ± 0.95 | 1.72 ± 0.38 |
| Decerebrate, infused with saline extracts of whole diencephalon, including pineal | 7 | 24.21 ± 3.2‡ | 1.93 ± 0.55 |
| Decerebrate, infused with saline extracts of pineal alone | 6 | 30.3 ± 2.9‡ | 2.17 ± 0.51 |
| Decerebrate, infused with acetone extracts of pineal | 4 | 25.0 ± 1.45‡ | 3.98 ± 1.03 |
| Decerebrate, infused with hexane fraction from water-hexane partitioning of residue from acetone extracts | 4 | 34.6 ± 4.2‡ | 3.69 ± 0.66 |
| Decerebrate, infused with aqueous partitioning | 3 | 10.92 ± 3.2 | 9.93 ± 3.0 |

*Mean and standard error. †Animals used 4 to 6 hours postdecerebration. ‡Statistically significant (P < .01) when compared with the corresponding controls.
with an R. F.* of about 0.9, suggesting the presence of 1 or more groups contributing some degree of water solubility. The active fractions from paper chromatograms contain very small amounts of solid material. Although precise quantitation is not possible, we can say that an effective dose infused into an assay dog over a 2-hour period is certainly less than 100 µg. Thus far we have not succeeded in isolating sufficient quantities of the material to permit chemical characterization. The substance does not appear to have a specific ultraviolet absorption curve, at least not in the amounts isolated. A weak reaction for phenols is given in the active region of the chromatograms; however, we have no assurance that this reaction is not due to a contaminant. Further characterization obviously must await the preparation of larger amounts of the hormone.

In the course of our efforts to purify adrenoglomerulotropin, we had noted that the potency appeared to increase as fractions containing adrenoglomerulotropin were purified on column or paper chromatograms. We wondered whether the crude extracts contained an inhibitor which was later separated from adrenoglomerulotropin. In screening some of our recent chromatograms with bio-assay, we found that eluates of 1 region of the chromatograms (fraction I, table 2), when infused into the decerebrate assay dog, did indeed reduce the secretion of the steroid below the basal level. This eluate has also been assayed for inhibitory activity in intact dogs. Solutions of the suspected fraction were administered intravenously 1 hour prior to cannulation of the adrenal vein and during a 2-hour period of collection of adrenal venous blood. There appears to be no question that the material actively inhibits steroidogenesis (table 2). It is readily separated from adrenoglomerulotropin on chromatograms since it has an R. F. of about 0.3 in the system water-chloroform-ethanol (3:5:5) on silicone-treated paper.

The inhibitor appears to be of pineal origin since extracts of equal amounts of cerebral cortex do not inhibit steroidogenesis. Its chemical nature is also unknown except for the fact that it appears to be a lipid. Curiously enough, although it inhibits the secretion of cortisol (table 2), the reduction in the secretion of aldosterone is even more striking. Although interpretation of these results must be tempered with caution, one is tempted to

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*Table 2
A Factor from Pineal Extracts Which Inhibits Steroid Secretion

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of experiments</th>
<th>Aldosterone µg./100 Kg. body wt./hr.</th>
<th>Cortisol µg./Kg. body wt./hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact animals, infused with vehicle (saline) or extract of cerebral cortex</td>
<td>8</td>
<td>36.0 ±3.2*</td>
<td>35.9 ±3.3</td>
</tr>
<tr>
<td>Intact animals, infused with fraction I from pineal</td>
<td>5</td>
<td>15.8 ±1.5 (P &lt; .001)</td>
<td>24.6 ±2.7 (P &lt; .05)</td>
</tr>
<tr>
<td>Decerebrate animals, infused with vehicle†</td>
<td>5</td>
<td>15.1 ±1.5</td>
<td></td>
</tr>
<tr>
<td>Decerebrate, infused with fraction I from pineal</td>
<td>8</td>
<td>6.8 ±0.4 (P &lt; .001)</td>
<td></td>
</tr>
<tr>
<td>Decerebrate, infused with adrenoglomerulotropin fraction from pineal</td>
<td>5</td>
<td>29.3 ±3.0</td>
<td></td>
</tr>
</tbody>
</table>

*Mean and standard error.
†Animals used for assay 3 hours after decerebration. The rate of secretion of aldosterone was thus somewhat higher than in dogs permitted to rest for longer periods postoperatively, cf., table 1, experimental groups 2, 3.

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*R.F. = the rate of movement of the band of the substance under scrutiny divided by the rate of movement of the advancing front of the liquid phase.
speculate that the inhibitor is a normal secretory product of the pineal gland and that it participates in the regulation of neuroendocrine. The material (which may be thought of as an anticorticotropin) may compete with pituitary corticotropin and with adrenoglo- merulotropin to restrain adrenal activity. The quantitative relationship between the 3 factors in any given situation may determine the final rate of steroid secretion. I should like to emphasize that this is, at best, a working hypothesis and only with time and additional study will it be possible to evaluate the relative biologic importance of these materials in the regulation of the secretion of aldosterone and cortisol.

The isolation from the epiphysis cerebri of factors which play a part in the control of the secretion of steroids naturally raises the question of the consequences of pinealectomy. Since adrenoglomerulotropin is found in pineal extracts, one might imagine that removal of the gland would result in cessation of the secretion of aldosterone. This is not the case. It is true that in the first few hours following pinealectomy, a decrease in the secretion of aldosterone was observed (table 3). However, 1 week postoperatively, the secretion rate of the steroid was found to be at control levels; moreover, even though the rate of secretion was slightly lower 3 weeks post-operatively than it was 1 week after operation, it is apparent that the pinealectomized dog is quite capable of synthesizing aldosterone. Pinealectomized dogs also respond, in a perfectly normal fashion, to deprivation of sodium, showing hypertrophy of the zona glomerulosa and a markedly increased output of aldosterone. Indeed, in 2 pinealectomized dogs recently studied on a low-sodium regimen for a period of 3 months, the rate of secretion of aldosterone (as well as of cortisol) was found to be nearly twice that in sham-operated dogs on the same diet (table 3). It would appear, at least in this experimental situation, that the long-term consequences of pinealectomy are those which might be anticipated from removal of inhibitory, rather than stimulatory, influences.

Some of the confusion which seems to confront the investigator at this stage of the problem may be related to our tendency to oversimplify. It is quite possible that we are dealing not with a single gland but with 2 glands. Another glandular structure in this area of the brain is the subcommissural organ, a structure which, though known for many years, has only recently attracted the attention of physiologists. Information on the functions of the subcommissural organ is still very limited. However, there is evidence that it is important in the control of water bal-

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Table 3

Steroid Secretion Rates in Pinealectomized Dogs

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of animals</th>
<th>Aldosterone</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µg./100 Kg. body wt./hr.</td>
<td>µg./Kg. body wt./hr.</td>
</tr>
<tr>
<td>Intact animals</td>
<td>9</td>
<td>31.2 ±3.7*</td>
<td>32.8 ±3.9</td>
</tr>
<tr>
<td>4 hours postoperative sham</td>
<td>3</td>
<td>36.5 ±8.5</td>
<td>34.9 ±4.8</td>
</tr>
<tr>
<td>1 week postoperative sham</td>
<td>3</td>
<td>14.3 ±1.42</td>
<td>31.2 ±2.5</td>
</tr>
<tr>
<td>3 weeks postoperative sham</td>
<td>5</td>
<td>34.6 ±5.7</td>
<td>39.3 ±8.1</td>
</tr>
<tr>
<td>16 weeks postoperative (last 12 weeks on low-sodium diet)</td>
<td>2</td>
<td>37.9 ±4.6</td>
<td>38.9 ±3.9</td>
</tr>
<tr>
<td>sham</td>
<td>2</td>
<td>24.1 ±2.9</td>
<td>39.4 ±3.1</td>
</tr>
<tr>
<td>pinealectomy</td>
<td>6</td>
<td>36.8 ±4.2</td>
<td>54.4 ±4.5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>37.9 ±4.6</td>
<td>38.9 ±3.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>88, 105</td>
<td>13.3, 16</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>193, 158</td>
<td>30.8, 33.4</td>
</tr>
</tbody>
</table>

*Mean and standard error.
Tayl or, in our laboratory, has been able to relate the subcommissural gland, or the area around it, to the control of the secretion of aldosterone. Taylor placed high frequency coagulation lesions in various areas of the brain stem in cats and determined, by analyses of adrenal venous blood, the rates of secretion of aldosterone. She found that lesions involving the central gray substance of the midbrain, at the level where the cerebral aqueduct opens anteriorly into the third ventricle, reduce the output of aldosterone. Effective lesions need not involve the pineal gland, but the subcommissural organ was disrupted in most of the experiments in which a decreased output of aldosterone was found, suggesting that this structure might be the source of stimulatory influences. On the other hand, it is possible that the effective lesions interrupt nerve pathways destined for other areas of the brain which are concerned with steroid regulation. It is anticipated that studies currently under way will permit a decision on this point.

I think it should be clear from the data which we have presented that there are many more questions about the regulation of the secretion of aldosterone than there are answers. One topic, mentioned at the beginning, remains for discussion: the possible role of abnormalities in the secretion of aldosterone in various diseases. Increased output of aldosterone has been implicated in a number of diseases, including cardiac failure, nephrosis, cirrhosis, and possibly essential hypertension. In only 1 syndrome, however, has a clear-cut relationship been established between the secretion of the steroid and the development of a clinical disorder. This is, of course, in the Conn syndrome, in which an abnormally high rate of secretion of aldosterone by an adrenal adenoma or by a hyperplastic adrenal gland is clearly the cause for the hypertension, the abnormal concentrations of electrolytes in the blood, and the renal pathology. Whether hypersecretion of the steroid complicates or plays an etiologic role in other diseases must, at this time, be considered an open question. An important recent discovery made in the laboratory of Sayers should probably be mentioned at this time. Sayers and his collaborators, using the isolated heart-lung preparation of the rat, have been able to show that aldosterone has a direct digitalis-like action on the myocardium. This is shown especially well in increased work capacity of the heart-lung preparation from adrenalectomized animals. This observation raises the question of whether aldosterone may serve to support myocardial function in stressful situations. If this proves to be the case, the increased secretion of aldosterone in certain diseases may represent one of the means by which the organism attempts to compensate for the cardiovascular consequences of the disease.

References


Fragments of Life

About the middle of the last century the younger physiologists broke away from the vitalistic traditions which had been handed down to them, and set about to investigate living organisms piece by piece, precisely as they would investigate the working of a complex mechanism. This method seemed to them to promise success, and was popularized by such masters of clear and forceful expression as Huxley. It is still the orthodox method of physiology, but the old confidence in it has steadily diminished in proportion as exact experimental investigation has shown that the various activities of a living organism cannot be interpreted in isolation from one another, since organic regulation dominates them.—J. S. Haldane. *Respiration*. New Haven, Yale University Press, 1922, p. 32.
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Circulation. 1960;21:1009-1015
doi: 10.1161/01.CIR.21.5.1009

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